Universidade Federal do Rio Grande
Instituto de Ciências Biológicas
Pós-graduação em Biologia de
Ambientes Aquáticos Continentais

Estratégias de desenvolvimento de duas espécies arbóreas palustres, *Allophylus edulis* e *Citronella gongonha*, em condições de inundação

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Tese apresentada ao Programa de Pós-graduação em Biologia de Ambientes Aquáticos Continentais como requisito parcial para a obtenção do título de Doutora em Biologia de Ambientes Aquáticos Continentais.

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“Quanto mais amor temos,
mais fácil fazemos a nossa passagem pelo mundo”
Immanuel Kant

Aos meus pais Similda e Miguel,
Ao meu marido Daniel,
À minha filha Sara
dedico.

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> "Eu transbordo de júbilo no meio de todas as minhas tribulações"
>
> (Paulo de Tarso)

RESUMO

As florestas paludosas são áreas úmidas que permanecem com o solo inundado ou encharcado por longos períodos. No entanto, é comum nesses ambientes a existência de variações microtopográficas que determinam a ocorrência de microambientes inundados e não inundados. Essa característica pode contribuir para a coexistência de espécies com diferentes níveis de tolerância a saturação hídrica do solo, tornando esses ecossistemas mais complexos e difíceis de restaurar. Nesse sentido, o objetivo geral dessa tese foi estudar os efeitos da inundação no desenvolvimento de duas espécies arbóreas palustres: *Citronella gongonha* e *Allophylus edulis*, descrevendo algumas das estratégias adotadas para colonizar com sucesso as florestas paludosas. Ambas as espécies são abundantes em formações paludosas no sul do Brasil, no entanto alguns aspectos da sua distribuição espacial indicam que elas contrastam na tolerância ao estresse por inundação. Os resultados obtidos, através da avaliação da germinação das sementes, bem como de parâmetros de crescimento, bioquímicos e/ou da avaliação da fluorescência da clorofila *a* em plantas jovens das espécies, durante diferentes períodos de inundação, permitem prever o microambiente preferido das espécies. O capítulo 1 mostra que *C. gongonha* apresenta sementes com baixa tolerância a inundação. No entanto, as plantas dessa espécie exibem adaptações que permitem tolerar longos períodos de inundação do solo. Entre as adaptações destacam-se crescimento hipertrófico do caule através da expansão do aerênquima e desenvolvimento de lenticelas, raízes adventícias, acúmulo e uso de carboidratos de reserva. O capítulo 2 mostra que tanto as sementes quanto as plantas jovens de *A. edulis* toleram curtos períodos de inundação, mas inundações prolongadas inviabilizam as sementes e danificam o aparato fotossintético, levando à morte das plantas. As estratégias adaptativas observadas para *A. edulis* foram aumento da taxa de germinação e do índice de velocidade de germinação das sementes após um curto período de inundação (15 dias). Além disso, houve inibição do crescimento e dissipação de energia por processos não fotoquímicos em plantas inundadas. Os resultados dos capítulos 1 e 2 dessa tese ajudam a explicar as variações espaciais na distribuição das espécies estudadas em ambientes inundados por longos períodos, bem como a sua distribuição ao longo do gradiente de inundação em ambientes temporariamente inundados.

Palavras-chave: hipóxia; germinação de sementes; raízes adventícias; carboidratos de reserva; fluorescência da clorofila *a*; estratégias adaptativas.

ABSTRACT

Swamp forests are wetlands that remain with flooded or waterlogged soil for long periods. However microtopographic variations occur leading to the occurrence of flooded and non-flooded microenvironments. This characteristic may contribute to the coexistence of species with different levels of tolerance to soil water saturation, making these ecosystems more complex and difficult to restore. The general objective of this thesis was to study the effects caused by flooding on the development of two swamps trees: *Citronella gongonha* e *Allophylus edulis*, describing some of the strategies adopted by species to successfully colonize the swamp forests. Both species are abundant in swamp in southern Brazil; however some aspects of their spatial distribution indicate that they contrast in flood stress tolerance. The results obtained through the evaluation of seed germination, growth parameters, biochemical and / or chlorophyll *a* fluorescence evaluation in young plants during different flooding periods; allow predicting the preferred microenvironment of the species. Chapter 1 shows that *C. gongonha* has seeds with low flood tolerance. However, plants of this species exhibit adaptations that allow them to tolerate long periods of soil flooding. Adaptations include stem hypertrophic growth through aerenchyma expansion, and development of lenticels, adventitious roots, accumulation and use of reserve carbohydrates. Chapter 2 shows that both seeds and *A. edulis* young plants tolerate short periods of flooding, but prolonged flooding makes the seeds unfeasible and damages the photosynthetic apparatus, leading to plant death. The adaptive strategies observed for *A. edulis* were increase in germination rate and seed germination speed index after short flooding period (15 days), growth inhibition and energy dissipation by non-photochemical processes in flooded plants. The results of chapters 1 and 2 of this thesis help to explain the spatial variations in the distribution of the studied species in flooded environments for long periods, as well as their distribution along the flood gradient in temporarily flooded environments.

Key-words: hypoxia; seed germination; adventitious roots; reserve carbohydrates; chlorophyll fluorescence *a*; adaptive strategies.

APRESENTAÇÃO

As estratégias ecofisiológicas desenvolvidas por espécies arbóreas palustres sob as condições de inundação é o principal tema abordado nessa tese. Foram estudadas duas espécies, *Citronella gongonha* (Mart.) R.A.Howard e *Allophylus edulis* (A.St.-Hil. et al.) Hieron. ex Niederl., típicas de formações florestais paludosas no sul do Brasil. A tese está estruturada em uma introdução geral, seguida de dois capítulos que tratam dos resultados alcançados, considerações finais e perspectivas que surgiram com o estudo. Na introdução apresento meu tema de estudo e as observações que nortearam a escolha das referidas espécies. Adicionalmente, faço uma abordagem sobre os parâmetros utilizados para inferir sobre a tolerância das espécies aos eventos de inundação do solo. Através desta introdução, espero que o leitor compreenda a complementação existente entre os dois capítulos gerados, mesmo que algumas das ferramentas metodológicas para avaliar a tolerância das espécies à inundação do solo sejam distintas. O artigo do primeiro capítulo tem como título "Effects of flood duration on seed germination, growth and ecophysiology of the swamp tree *Citronella gongonha* from the southern neotropics" e foi submetido ao periódico Flora. O artigo do segundo capítulo tem como título "Seed germination, growth and chlorophyll a fluorescence in young plants of *Allophylus edulis* in different periods of flooding" e está publicado no periódico Acta Physiologiae Plantarum.

Nas considerações finais faço um apanhado sobre as conclusões extraídas nos artigos de cada capítulo, relacionando os resultados encontrados com o padrão de distribuição das espécies em florestas paludosas. Além disso, com base nesses resultados abordo algumas questões para conservação e recomendações para o plantio das espécies estudadas em iniciativas voltadas a restauração de ambientes inundados. Cada capítulo foi redigido de acordo com as normas dos periódicos para os quais os manuscritos foram submetidos.

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INTRODUÇÃO GERAL

O termo inundação ou alagamento refere-se ao excesso de água no solo, o tornando encharcado ou ainda formando lâmina d´água. Durante a inundação, o ar presente nos poros do solo é substituído por água, provocando a diminuição ou desaparecimento de O_2, acúmulo de CO_2 e formação de compostos tóxicos às plantas (DREW, 1997; KOZLOWSKI, 1997). O Brasil possui uma grande extensão territorial de ambientes sujeitos a inundações temporária ou permanente, dentre estes se destacam as florestas paludosas que desempenham importante papel ecológico na manutenção dos recursos hídricos e na conservação da biodiversidade. As florestas paludosas brasileiras praticamente desapareceram devido a práticas intensas de desmatamento (SCARANO, 2006). De forma geral, as florestas inundáveis se encontram ameaçadas por diversas atividades de origem antrópica (SILVA et al., 2012).

Estudos realizados em remanescentes de florestas paludosas no domínio da Mata Atlântica indicam que esse tipo de vegetação é extremamente frágil sendo a resiliência, desse ecossistema, baixa em comparação com outros tipos de florestas brasileiras, incluindo as florestas inundáveis da Amazônia (SCARANO, 2006). As iniciativas de revegetação de florestas paludosas têm encontrado grandes dificuldades e os esforços muitas vezes resultam em pouco êxito (SCARANO; RIOS; ESTEVES, 1998; ZAMITH; SCARANO, 2010).

Uma das projeções das mudanças climáticas para Brasil é o aumento dos eventos climáticos extremos com períodos de secas e chuvas intensas o que possivelmente afetará o padrão hidrológico em florestas inundáveis (MARENGO; DIAS, 2006; IPCC, 2019). Através desse aumento da intensidade e volume de chuvas, a inundação tende a ser amplificada em nível espacial ou temporal nas florestas inundáveis. Mudanças no padrão do regime hídrico de áreas úmidas podem afetar vários processos entre eles a germinação de sementes, a sobrevivência e o crescimento de plantas. Como consequência disso pode ocorrer mudanças na composição florística e estrutura da comunidade (JUNK et al., 2013). Diante deste cenário, é importante entender os efeitos da inundação para espécies florestais, bem como as estratégias de germinação de sementes e sobrevivência de plantas em condições de estresse.

Ecofisiologia de espécies arbóreas tolerantes a inundação

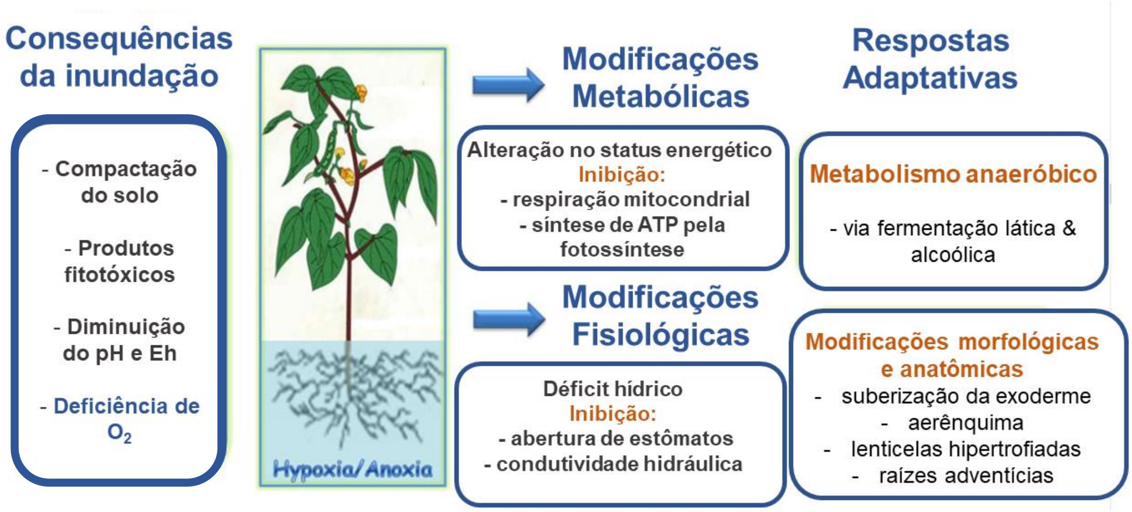
Os estudos ecofisiológicos das plantas visam fornecer evidências de efeitos aleatórios ou mecanicistas para questões ecológicas sobre a sobrevivência, distribuição, abundância e interações das plantas com outros organismos (LAMBERS; CHAPIN III; PONS, 2008). Estudos com esse viés podem ajudar a entender os processos associados ao padrão de distribuição das espécies em florestas sujeitas a inundação (BLOM; VOESENEK, 1996) fornecendo base para ações de conservação e restauração. No entanto, os dados sobre a ecofisiologia e adaptações de árvores em florestas inundáveis em todo o mundo ainda são escassos (PAROLIN; WITTIMAN, 2010). Nesse contexto, podem ser incluídas as florestas paludosas do sul da América do sul, para as quais existem poucos estudos que descrevem o comportamento ecofisiológico das espécies (ZÚÑIGA-FEEST et al., 2017).

Em florestas inundáveis as plantas podem ser submetidas a diversas condições de oxigenação do solo, que podem ser condições normais (normoxia), reduzidas (hipóxia) ou ausência total (anóxia) de O_2. As condições de normoxia prevalecem quando o suprimento de O_2 não limita a fosforilação oxidativa. A hipóxia quando a pressão parcial de O_2 é baixa o suficiente para limitar a produção de ATP pelas mitocôndrias, enquanto a anóxia é atingida quando a produção mitocondrial de ATP é insignificante em comparação com a gerada pela glicólise e fermentação (DREW, 1997).

As condições hipóxicas e anóxicas exercem forte efeito na sobrevivência e crescimento das plantas (KOZLOWSKI, 1997). Tais condições podem causar déficit hídrico, diminuição da assimilação fotossintética entre outras alterações (KOZLOWSKI, 1997; PAROLIN et al., 2004). A tolerância à inundação envolve mecanismos com múltiplos fatores, que incluem respostas morfológicas e anatômicas (COLMER, 2003; ZHANG et al., 2017); mecanismos para remoção de espécies reativas de oxigênio; metabolismo de carboidratos e adequações fotossintéticas (Figura 1) (GRAVATT; KIRBY 1998; PARENT et al., 2008; IRFAN et al. 2010; PAROLIN; WITTMANN, 2010; VOESENEK; BAILEY-SERRES, 2013).

A tolerância à inundação pode variar de poucas horas a muitos dias, conforme a espécie, os órgãos afetados e o estágio de desenvolvimento (KOZLOWSKI, 1997; VARTAPERIAN; JACKSON, 1997; KREUZWIESER; RENNENBERG, 2014). Nesse sentido, as características dos ecossistemas como a profundidade da coluna d'ʻágua e a duração dos eventos de inundação podem atuar como um filtro ambiental selecionando espécies e, portanto, influenciando a composição florística do local.

Figura 1. Modificações físico-químicas no solo durante a inundação que determinam respostas adaptativas em plantas



Fonte: Adaptado de PARENT et al., 2008

Aspectos da vegetação arbórea em florestas paludosas do sul do Brasil

O sul do Brasil é caracterizado por vários ecossistemas de áreas úmidas, dentre eles as formações florestais paludosas onde a inundação do solo pode durar cerca de sete meses a cada ano (DUARTE, 2012). Essas florestas, durante o período inundado apresentam profundidade da lâmina d‟água entre 20 e 40 cm, o que determina a submersão do sistema radicular de muitas árvores jovens e adultas (RICARDO, 2010; DUARTE, 2012). Conforme dito anteriormente, longos períodos de inundação resultam em condições de hipóxia ou até mesmo anóxia nos espaços radiculares.

A ocorrência de uma espécie em uma área predominantemente inundável é um forte indício da sua tolerância, no entanto, não é uma informação conclusiva pelo de fato existirem alguns fatores que podem beneficiar o estabelecimento das plantas com menor tolerância a inundação do solo. As interações positivas entre as plantas (SCARANO, 2002) e a presença de microambientes protegidos da inundação (SHIMAMURA; MOMOSE, 2005; FREUND et al. 2018) são fatores que possivelmente atuam em florestas paludosas influenciando os processos de regeneração e coexistência de espécies.

Uma característica importante de florestas paludosas é a ocorrência de variações microtopográficas, as quais criam uma variedade de condições hidrológicas contribuindo para o aumento da heterogeneidade espacial nesses ecossistemas (IVANAUSKAS; RODRIGUES;

NAVE, 1997; SHIMAMURA; MOMOSE, 2006; TEIXEIRA; ASSIS, 2009). No sul do Brasil é possível notar que as variações microtopográficas exercem influência sobre a estrutura e a composição florística das florestas paludosas. O interior dessas florestas é caracterizado pela presença de microambientes onde o substrato é permanentemente saturado de água, outros em que há formação de lâmina da água de alguns centímetros e de locais não inundados devido a pequenas elevações no solo (microrrelevos) (DUARTE, 2012).

Figura 2. Interior de uma formação florestal paludosa localizada em Rio Grande, RS, Brasil. Na imagem é possível observar microambientes inundados e microambientes não inundados.

Fonte: A autora.

Figura 3. Interior de uma formação florestal paludosa localizada em Rio Grande, RS, Brasil. Na imagem é possível observar adensamento de plantas lenhosas sobre um microrrelevo protegido da inundação do solo.

Fonte: A autora

As figuras 2 e 3 ilustram a presença de microambientes inundados e não inundados no interior de uma floresta paludosa localizada no munícipio de Rio Grande, RS, Brasil. A presença de pequenas microelevações no solo (microrrelevos) é marcante nesse ambiente. A área desses microrrelevos varia entre 0,27 a 10,88 m^2 (média 4,60 ± 2,69 m^2) e nível topográfico entre 9 e 47cm (média 24,71 ± 8,05 cm) sendo assim, o estresse por inundação é atenuado nesses microambientes. A abundância de plantas lenhosas é maior nos microrrelevos do que nas áreas inundadas (Figura 3). Sendo *Citronella gongonha* (Mart.) R.A.Howard*,* *Erythrina crista-galli* L. e *Ficus cestrifolia* Schott *ex* Spreng as espécies com maiores valores de importância (VI) nos microrrelevos (DUARTE et al., dados não publicados). Em termos fitossociológicos o VI corresponde a soma da frequência, densidade e área basal relativa entre as espécies de uma determinada área amostrada (MUELLER-DOMBOIS; ELLENBERG, 1974).

As espécies *C. gongonha, E. crista-galli* e *F. cestrifolia* que são frequentemente representados por indivíduos adultos ocupando uma posição mais centralizada nesses microambientes (Figura 3 e 5). Também ocorrem sobre os microrrelevos outras 26 espécies arbóreas. Algumas dessas espécies são representadas por poucos indivíduos adultos, porém elevada abundância de plantas em estágio jovem (DUARTE et al. dados não publicados). A exemplo disso, pode ser citada a espécie *Allophylus edulis* (A.St.-Hil. et al.) Hieron. ex Niederl (Figura 6).

A distribuição agregada de plantas, em ambiente com elevado estresse ambiental pode indicar interação positiva entre plantas, um processo que também é chamado de facilitação (CALLAWAY; WALKER, 1997). Nesse processo são denominadas facilitadoras (*nurse plant*) as plantas que melhoram o desempenho fisiológico, a sobrevivência e / ou crescimento de plantas vizinhas através da modificação do ambiente (CALLAWAY et al. 1995; BRUNO; STACHOWICZ; BERTNESS, 2003). Em áreas úmidas algumas espécies tolerantes a inundações podem aliviar a anóxia do solo liberando O_2 para a rizosfera e isso pode permitir que as plantas vizinhas tenham um melhor desempenho de sobrevivência ou crescimento (LUO et al., 2010; ZHANG; SHAO, 2013). Além disso, o sistema radicular de algumas árvores pode contribuir para modificação da microtopografia do solo em florestas paludosas (SHIMAMURA; MOMOSE, 2005; SHIMAMURA; MOMOSE; KOBAYASHI, 2006). Os indivíduos de *C. gongonha* e *E. crista galli* desenvolvem raízes adventícias e *F. cestrifolia* tem raízes tabulares, esse tipo de raízes mais próximas a superfície do solo podem contribuir para o acúmulo de matéria orgânica e sedimentos no entorno das árvores criando microrrelevos ou aumentando o tamanho de microrrelevos pré-existentes. Essas alterações em nível de microtopografia podem tornar as condições favoráveis para o desenvolvimento inicial de algumas plantas.

Espécies selecionadas

Conforme exposto anteriormente, as variações espaciais nas condições hidrológicas do solo podem permitir a coexistência de espécies com diferentes níveis de tolerância a inundação. Nesse sentido, foram selecionadas duas espécies típicas de florestas paludosas: *C. gongonha* e *A. edulis* para estudar o comportamento ecofisiológico durante diferentes períodos de inundação. As sementes para a realização dos experimentos foram obtidas a partir de frutos maduros coletados em árvores de uma floresta paludosa localizada em Rio Grande, RS, Brasil. Dados fitossociológicos obtidos nessa floresta (RICARDO, 2010) bem como observações de campo sobre a distribuição de *C. gongonha* e *A. edulis* sugerem que elas possivelmente contrastam quanto à tolerância aos eventos de inundação, utilizando-se de diferentes estratégias para se desenvolver em florestas paludosas.

Citronella gongonha

Citronella gongonha (Mart.) R.A.Howard (Figura 4) é uma espécie arbórea da família Cardiopteridaceae, popularmente conhecida como congonha, laranjeira-do-banhado, erva-de-anta, falso-mate entre outros nomes (LORENZI, 2009). É chamada de falso mate, pois existe registro do uso das folhas aromáticas desta planta como substituto da erva-mate (SAUERESSIG, 2014). Apresenta frutos do tipo drupa, roxo-escuros, com polpa suculenta, sabor adocicado e muito apreciados pela avifauna (LORENZI, 2009). Essa espécie é categorizada como pioneira ou secundária inicial (LORENZI, 2009), sendo encontrada em formações inundáveis (SILVA et al. 2012) na Argentina, Brasil, Uruguai e Paraguai (VIANI; VIEIRA, 2007). No Brasil ocorre de Mato Grosso e Minas Gerais até o Rio Grande do Sul, nos domínios fitogeográficos do Cerrado e Mata Atlântica (STEFANO, 2020).

Figura 4. Individuo adulto de *Citronella gongonha* com frutos maduros.

Fonte: A autora.

C. gongonha é encontrada preferencialmente em florestas paludosas (SILVA et al., 2007), onde os eventos de inundação podem durar longos períodos. A espécie apresenta elevada abundância em áreas da floresta paludosa onde a profundidade da água é maior (RICARDO, 2010), no entanto a distribuição de indivíduos jovens e adultos é visualmente maior sobre microelevações do solo (Figura 5). As plantas de *C. gongonha* desenvolvem um sistema de raiz adventício que propiciam o acúmulo de matéria orgânica e sedimentos contribuindo consequentemente para a elevação do substrato em torno da árvore em longo prazo (KELLER; VELAZCO; KRAUCZUK, 2016). Assim, a associação de indivíduos arbóreos adultos de *C. gongonha* a microelevações pode estar relacionada a seu papel na formação e expansão desses microambientes. No entanto, a abundância, visualmente maior, de indivíduos jovens da espécie sobre as microelevações sugere que o desenvolvimento inicial da espécie é prejudicado pela inundação prolongada.

Figura 5. Em (a) indivíduo adulto de *Citronella gongonha* estabelecido em um microrrelevo em uma formação florestal paludosa localizada em Rio Grande, RS, Brasil. Em (b) e (c) detalhe da superfície do substrato no microrrelevo evidenciando as raízes. A seta amarela aponta para o tronco e as vermelhas para raízes adventícias de *C. gongonha.*

Fonte: A autora.

Com base nesse contexto, foram formuladas as seguintes hipóteses (i) as sementes de *C. gongonha* não germinam e perdem a viabilidade quando inundadas por um longo período;

no entanto, (ii) plantas jovens da espécie sobrevivem e mantêm o crescimento sob condições prolongadas de inundação, utilizando carboidratos de reserva e desenvolvendo adaptações morfológicas.

Allophylus edulis

Allophylus edulis (A.St.-Hil. et al.) Hieron. ex Niederl (Figura 6) é uma espécie arbórea que pertencente à família Sapindaceae conhecida popularmente como chal-chal, fruta-de-passarinho, vacum, baga-de-morcego, fruta-de-pombo entre vários outros nomes (LORENZI, 1992; SAUERESSIG, 2014). Os frutos são coca, globosa, indeiscente, de consistência carnácea com coloração avermelhada (ABREU et al.; 2005). Os frutos têm sabor adocicado e são utilizados para produzir uma bebida vinosa conhecida como "chicha" (SAUERESSIG, 2014). Além disso, a espécie tem importância econômica pela madeira de boa qualidade; sendo indicada para arborização urbana e para a recuperação de ecossistemas degradados (LORENZI, 1992; SAUERESSIG, 2014). Devido a características como altas taxas de germinação (ABREU et al.; 2005), crescimento rápido, fornecimento de flores melíferas e frutos atrativos para a fauna pode acelerar o processo de restauração florestal (TURCHETTO et al., 2017).

A área de distribuição geográfica de *A. edulis* compreende Paraguai, Uruguai e Argentina (COELHO, 2014). Em território brasileiro, a espécie apresenta ampla distribuição geográfica ocorrendo nos domínios fitogeográficos da Amazônia, Caatinga, Cerrado, Mata Atlântica e Pantanal (COELHO, 2018), onde coloniza áreas abertas (SAUERESSIG, 2014) e formações florestais que apresentam inundação periódica ou permanente (SILVA et al., 2012). *A. edulis*, tem sido classificada como uma espécie seletiva hidrófita devido a ocorrência recorrente em solos úmidos de planícies aluviais, início de encostas e beira de rios (LORENZI, 1992). Estudos fitossociológicos evidenciam maior abundância dessa espécie em áreas inundadas temporalmente do que em florestas paludosas, onde a inundação ocorre em caráter quase permanentemente (SLVA et al., 2007). Nas florestas paludosas do sul do Brasil, os indivíduos jovens e adultos dessa espécie encontram-se estabelecidos principalmente sobre microrrelevos (Figura 7). Com base nesse contexto, foi formulada a hipótese de que *A. edulis* não tolera longos períodos de inundação durante os estágios iniciais de seu desenvolvimento. Sendo, portanto, necessárias condições não inundadas para a germinação de sementes, bem como a sobrevivência e o crescimento de plantas jovens.

Figura 6. Indivíduo adulto de *Allophylus edulis* com frutos em processo de amadurecimento.

Fonte: A autora.

Figura 7. Indivíduo jovem de *Allophylus edulis* sobre um microrrelevo em uma formação florestal paludosa localizada em Rio Grande, RS, Brasil.

Fonte: A autora.

Parâmetros avaliados

O padrão de distribuição de uma espécie em ambientes palustres é determinado em grande parte pelo nível de tolerância das sementes e das plantas a saturação hídrica do solo. Vários parâmetros podem ser usados para definir a tolerância de uma espécie vegetal a inundação do solo e a resposta muitas vezes está associada ao estágio do seu ciclo de vida. Geralmente as árvores adultas toleram a inundação do solo melhor do que as plantas jovens da mesma espécie (HUENNEKE; SHARITZ, 1990; SIEBEL; BLOM, 1998; PAZ et al., 2017). É possível também, que as plantas jovens de uma determinada espécie tenham adaptações para enfrentar com sucesso a inundação, enquanto as sementes sejam sensíveis e tenham rápida perda da viabilidade sob condições de estresse (PAZ et al. 2017), e o inverso da situação também pode ocorrer.

Diante disso, buscando abranger duas fases do ciclo de vida da planta, nessa tese foram utilizados como parâmetros a germinação de sementes e o crescimento de plantas jovens das duas espécies selecionadas para o estudo. As avaliações dos parâmetros foram feitas sob as condições de inundação por diferentes períodos. Adicionalmente utilizamos a técnica da fluorescência da clorofila *a* para avaliar o desempenho fotossintético de plantas jovens de *A. edulis* em diferentes períodos de inundação. E alguns parâmetros bioquímicos, como carboidratos de reserva, para avaliar o comportamento de plantas jovens de *C. gongonha* sob os diferentes períodos de inundação.

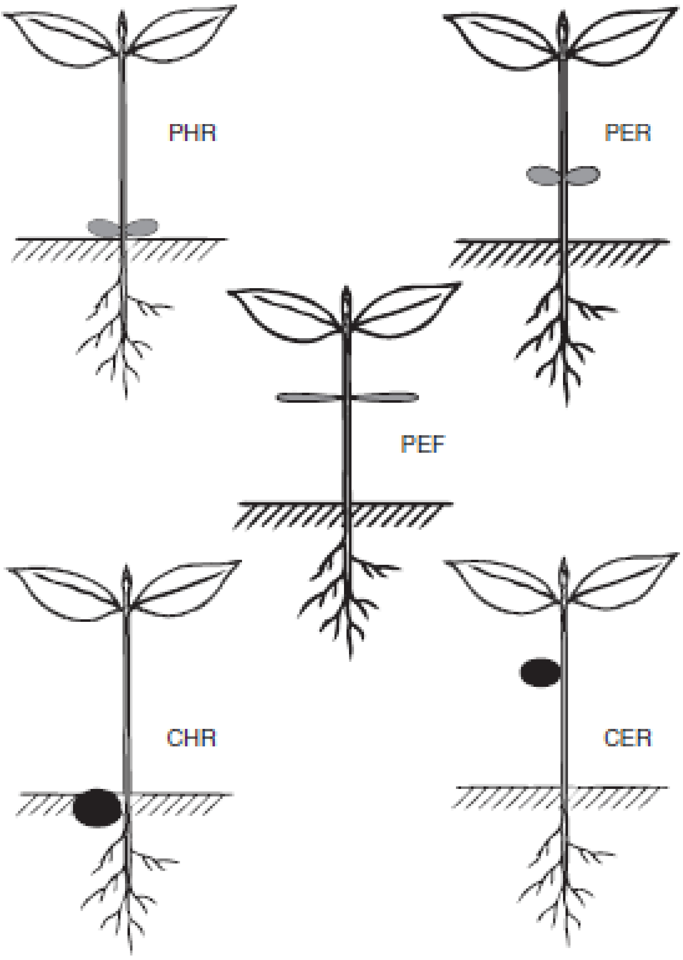
Germinação de sementes e desenvolvimento de plântulas

Durante a germinação de sementes, ocorre uma sequência de reações bioquímicas onde as substâncias de reserva são quebradas e mobilizadas ao eixo embrionário (BUCKERIDGE et al., 2004). A água é essencial para que ocorra esse processo, uma vez que a ativação do metabolismo é iniciada com a embebição (FERREIRA; BORGHETTI, 2004). No entanto, o excesso de água pode ser prejudicial, por reduzir a disponibilidade de O_2 requerida para que ocorram reações metabólicas importantes na semente, especialmente a respiração, sendo necessário para a degradação das substâncias de reserva e suprimento de energia para o desenvolvimento embrionário (KOZLOWSKI, 1997). Ainda que, nos primeiros momentos, a respiração seja anaeróbica, posteriormente, passa a ser absolutamente dependente de oxigênio, mesmo antes que a radícula rompa o tegumento (LARCHER, 2006). Dessa forma, a maioria das sementes de plantas terrestres que possui alta taxa de germinação no solo, não germina na água e pode rapidamente perder a viabilidade sob tais condições (PAROLIN, 2001).

Embora existam alguns casos, que a inundação possa estimular a germinação de sementes (FERREIRA et al., 2006; WITTMANN et al., 2007), na maioria das vezes a inundação apresenta um efeito negativo inibindo ou retardando a germinação de sementes (KOZLOWSKI, 1997; OKAMOTO; JOLY, 2000). Em florestas inundáveis em que a época de frutificação está sincronizada com período de inundação, como é o caso da Amazônia, as sementes e plântulas têm adaptações para tolerar as condições de inundação (PAROLIN, 2001a; PAROLIN, 2001b; PAROLIN et al. 2004). Dentre as adaptações das sementes podem ser destacadas as características morfológicas que melhoram flutuabilidade das sementes (PAROLIN, 2001b; PAROLIN et al. 2004), dormência mecânica imposta pelo tegumento (FERREIRA, 2005; LUCAS et al. 2012) e dormência fisiológica (SCARANO; PEREIRA; RÔÇAS, 2003). Além disso, os carboidratos de reserva estruturais podem diminuir a velocidade de embebição durante as condições de inundação e os carboidratos de reserva não-estruturais podem aumentar a velocidade de germinação quando as condições se tornam favoráveis (FERREIRA et al., 2009).

O desenvolvimento das plântulas é outra etapa crítica para a colonização de um ambiente inundável. Os aspectos relacionados ao tamanho, a capacidade fotossintética ou ao acúmulo de reservas nutritivas podem ser determinantes para o sucesso de uma planta em estágio jovem. O tipo funcional da plântula fornece importantes pistas sobre as estratégias de sobrevivência das plantas em ambientes inundáveis (PAROLIN, 2001a; PAROLIN, 2003; CONSERVA et al., 2018). O tipo funcional da plântula refere-se à morfologia da plântula em relação à função e posição do cotilédone. Essa classificação foi criada com base em espécies lenhosas e tem sido usada, principalmente, em florestas tropicais para caracterizar estratégias de regeneração das plantas (PEREZ-HARGUINDEGUY et al., 2016). As cinco categorias de plântulas (Figura 8) resultam da combinação de diferentes possibilidades em relação à exposição do cotilédone (fanerocotilar ou criptocotilar), posição (epigeal ou hipogeal) e função (armazenamento ou foliáceo).

Figura 8. Tipos funcionais das plântulas (folhas em branco, cotilédones em cinza, sementes em preto). Morfologia funcional das plântulas: PHR = Fanero Hipogeal com cotilédones de reserva (armazenador); PER = Fanero Epigeal com cotilédones de reserva; PFE = Fanero Epigeal com cotilédones foliáceos; CHR = Cripto Hipogeal com cotilédones de Reserva; e CER =Cripto Epigeal com cotilédones de reserva.



Fonte: PEREZ-HARGUINDEGUY et al., 2016

Alguns estudos, em florestas de várzea, indicam que as plântulas do tipo PEF (Fanero Epigeal com cotilédones foliáceos) prevalecem em locais inundados por longos períodos (PAROLIN, 2001a; PAROLIN, 2001b; CONSERVA et al, 2018). Dentre as características que podem conferir vantagem ecológica as plântulas PEF destacam-se a rápida germinação e emergência de cotilédones fotossintetizantes os quais podem ser resistentes a inundações prolongadas (CONSERVA et al, 2018). Já em florestas onde a inundação ocorre em menor escala temporal muitas espécies têm sementes de germinação mais lenta, sem uma fina sincronia com os eventos de inundação, e tendem a ter cotilédones de armazenamento (PAROLIN, 2001a; CONSERVA et al, 2018).

Crescimento e alterações morfológicas

Tanto as árvores tolerantes (OLIVEIRA; JOLY, 2010; LARRÉ et al. 2013) como as intolerantes podem ter prejuízo no crescimento e produção de biomassa mediante as condições de inundação da raiz (KOZLOWSKI, 1997). Isso acontece devido a uma soma de fatores associados as condições de hipóxia ou anóxia do solo, como menor absorção de água e nutrientes minerais, alterações nas trocas gasosas foliares, no balanço hormonal, queda na taxa fotossintética e na translocação de fotoassimilados (KOZLOWSKI, 1997; KOZLOWSKI; KRAMER; PALLARDY, 2001; LARCHER, 2006; TEWARI; MISHRA, 2018).

Alguns autores têm associado as alterações no crescimento e desenvolvimento das plantas inundadas a estratégias ecológicas que possibilitam a sua sobrevivência sob condições de estresse. Dentre as estratégias observadas destaca-se o rápido alongamento do caule, como estratégia para evitar a submersão total da planta (PAROLIN, 2001). Há também as espécies que reduzem ou inibem o crescimento, a fim de reduzir o consumo de energia (VOESENEK; BAILEY-SERRES, 2013). Também pode ocorrer a queda de folhas ou a redução da área foliar para reduzir a superfície transpiracional e evitar a perda de água (PAROLIN, 2004).

Quando os efeitos da inundação do solo se tornam muito severos as plantas podem desenvolver lenticelas hipertrofiadas, raízes adventícias e aerênquima com canais de ar para a parte aérea que permitem a difusão de O_2 para as novas raízes (COLMER, 2003). Essas modificações morfológicas são desenvolvidas por muitas espécies arbóreas consideradas tolerantes após alguns dias de inundação (PAROLIN et al. 2004; MEDINA et al. 2009; ALVES et al. 2013, LARRÉ et al. 2013) possibilitando a recuperação de atividades fisiológicas inicialmente prejudicadas pelo estresse (MIELKE et al., 2005; MEDINA et al. 2009; LARRÉ et al., 2013). Em alguns casos essas estruturas são responsáveis pela retomada de crescimento das plantas (KOZLOWSKI, 1997; PAROLIN et al., 2004).

Fluorescência da clorofila a

A fotossíntese é um processo fisiológico fundamental do metabolismo das plantas através do qual as plantas convertem a energia luminosa em energia química (ATP e NADPH), utilizada para a síntese de carboidratos que são fundamentais para a manutenção dos vegetais (TAIZ; ZEIGER, 2006). A fotossíntese ocorre nos cloroplastos e compreende duas etapas interdependentes e simultâneas. Uma é a etapa fotoquímica que envolve reações de absorção e conversão da energia luminosa, e ocorre nos tilacóides. A outra é a etapa bioquímica que envolve as reações de carboxilação e ocorre no estroma (TAIZ; ZEIGER, 2006).

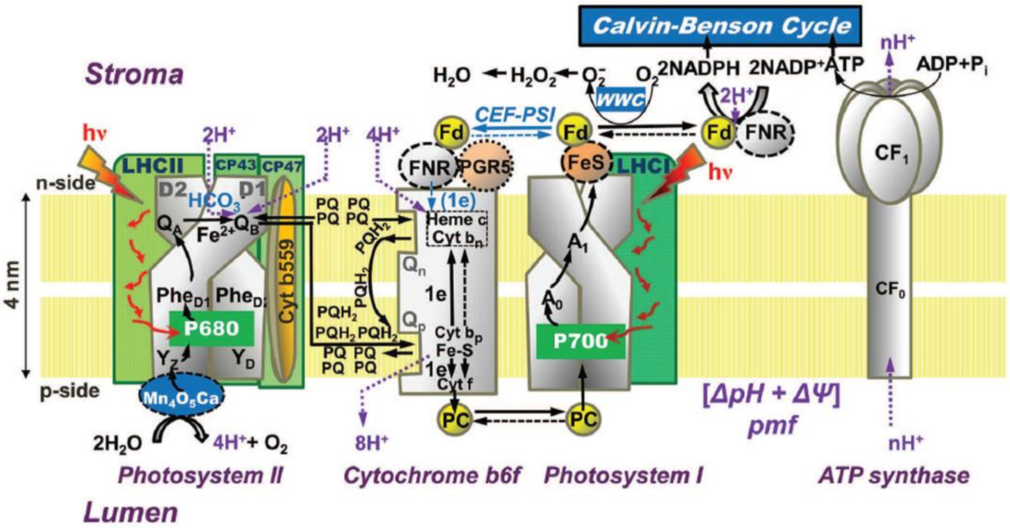
Quando a luz incide sobre clorofila está molécula altera seu estado eletrônico, passando da condição basal para a excitada. Esta condição é temporária e a clorofila tem três maneiras de dissipar a energia absorvida: (1) dissipação fotoquímica (fotossíntese); (2) dissipação como calor, ou (3) re-emissão de luz (fluorescência da clorofila *a*). Esses três processos ocorrem de maneira competitiva, e sob condições de estresse, a fotoquímica diminui enquanto a dissipação de calor e a emissão de fluorescência aumentam (CAMPOSTRINI et al., 2001).

Para que a etapa fotoquímica aconteça, dois fotossistemas (FS) trabalham em série, para oxidar a água em O_2, reduzir $NADP^+$ (nicotinamida adenina dinucleotídeo fosfato) e produzir ATP (adenosina trifosfato) que serão usados no ciclo de Calvin-Benson para fixação de CO_2 ou em outros processos de assimilação. Cada FS é composto por um complexo de coleta de luz (complexo antena) que possui pigmentos e proteínas; e por um centro de reação (CR). O CR contém uma molécula especial de clorofila *a* que têm o pico de absorção de luz na faixa de 680 nm (FSII) e 700nm (FSI). Conforme, ilustra o diagrama da figura 9, a etapa fotoquímica é caracterizada por uma cascata de fluxo de energia, onde podem ser definidos vários eventos biofísicos (STIRBET et al., 2013).

A fotossíntese é particularmente sensível às restrições ambientais tornando as medidas fotossintéticas um importante componente dos estudos de estresse em plantas (KALAJI et al., 2012; KALAJI et al., 2016; STIRBET et al., 2018). O estudo da fluorescência da clorofila *a*, é um método rápido e não invasivo, que fornece dados precisos sobre a eficiência fotoquímica que podem ser utilizados para monitorar mudanças no estado fisiológico do sistema fotossintético (KALAJI et al., 2016; STIRBET et al., 2018).

A técnica consiste em iluminar uma amostra fotossintética após ela ter sido adaptada ao escuro, a fim de avaliar a fluorescência da clorofila *a*. A emissão da fluorescência da clorofila *a* decorre de aumento polifásico ou transiente do processo a partir da fluorescência inicial, F0, até a intensidade máxima, FM (ou Fp – 300 ms). Esta cinética, exibe a sequência de passos, definidos como O, J, I e P, os quais refletem o sucessivo preenchimento do pool dos aceptores de elétrons do FSII (LAZÁR, 2006; KALAJI et al., 2016; STIRBET et al., 2018).

Figura 9. Diagrama da etapa fotoquímica da fotossíntese, ilustrando uma membrana de tilacóide com os quatro principais complexos de proteínas, que são usados para a produção de ATP e NADPH. Da esquerda para a direita: Fotossistema II, citocromo (Cyt) b6f, Fotossistema I e ATP sintase. O ATP e o NADPH produzidos durante a fase fotoquímica da fotossíntese são usados no ciclo de Calvin Benson para fixar o CO_2 na produção de açúcares.



Fonte: STIRBET et al., 2013

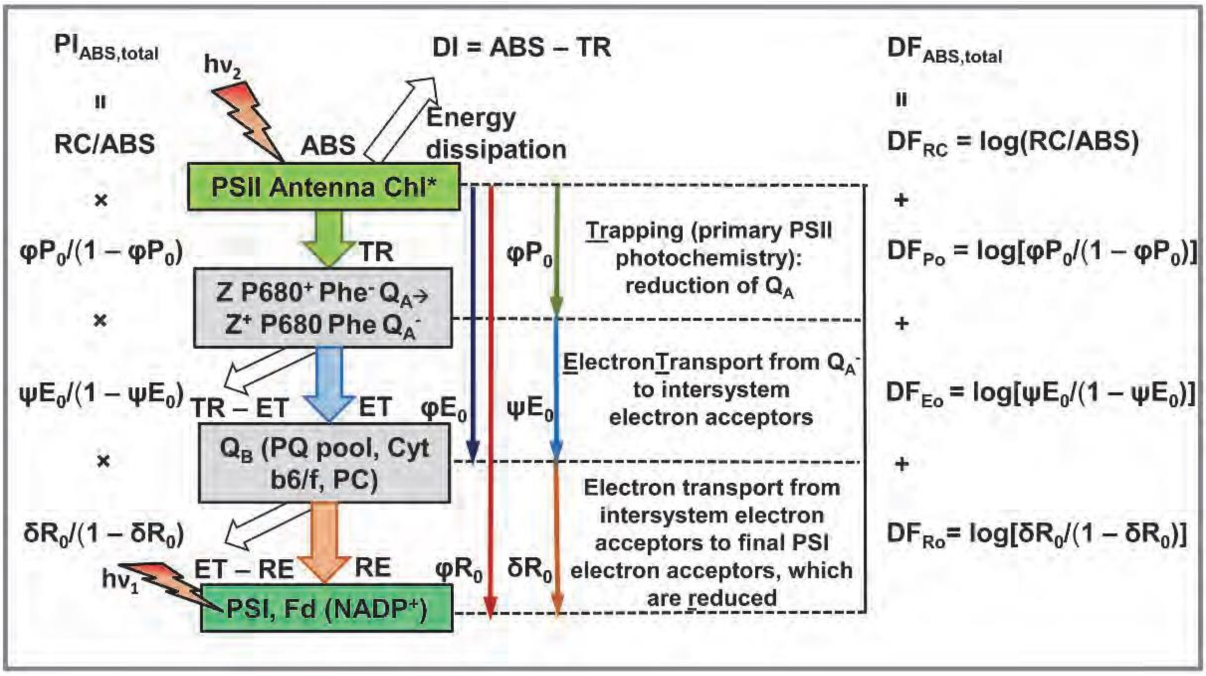
A partir das análises do aumento da fluorescência transiente OJIP, STRASSER; STRASSER (1995) propuseram um teste que foi intitulado Teste JIP. O Teste-JIP é uma análise multiparamétrica do rápido aumento da fluorescência OJIP, onde O é a intensidade de fluorescência inicial, J e I são intensidades medidas a 2 e 30 ms, respectivamente, e P é a intensidade máxima da fluorescência da clorofila (STIRBET et al., 2018). O uso dos parâmetros calculados pelo Teste-JIP permite a quantificação da eficiência de absorção e aproveitamento da energia luminosa através da cadeia de transporte de elétrons (CTE) da fotossíntese. Além de avaliar a funcionalidade do FSII, que reflete a taxa de transporte de elétrons no interior da membrana dos tilacóides e, subsequente funcionamento da ferredoxina-NADP oxirredutase e ciclo de Calvin (KALAJI et al., 2016; STIRBET et al., 2018).

O Teste JIP é baseado na Teoria de Fluxo de Energia em Biomembranas (STRASSER, 1981). Essa teoria pode ser aplicada para qualquer amostra fotossintética em membranas biológicas. O diagrama, na figura 10, mostra a relação entre vários parâmetros do teste JIP, onde ABS refere-se ao fluxo de fótons absorvidos pelos pigmentos antena. Parte dessa energia absorvida é dissipada (DI) como calor e fluorescência, outra parte é capturada ("trapping" - TR) pelo RC e convertida em força redox, permitindo a redução do aceptor primário de elétrons de Q_A para Q_A^-. Quando o aceptor de elétrons é reoxidado tem início o transporte de elétrons ("electron transport" - ET) para o intersistema, ou seja, plastoquinonas, citocromo b6/f e plastocianina. Se não houver alterações na CTE haverá a redução do aceptor final de elétrons (ferredoxina) no lado do aceptor de elétrons do FSI (STRASSER et al. 2004; STIRBET et al., 2018).

Alguns estudos mostram que a inundação pode provocar alterações na CTE diminuindo o desempenho fotossintético (LARRÉ et al., 2013; MARTINAZZO et al., 2013). O comprometimento da fase fotoquímica pode ter como consequência a formação de baixo poder redutor (NADPH2), tendo como resultado, a formação de espécies reativas de oxigênio (EROs), que causam prejuízos para o metabolismo da planta por aumentarem a oxidação de proteínas e lipídeos (TEWARI; MISHRA, 2018).

Mesmo espécies que apresentam alta taxa de sobrevivência e ampla distribuição nos habitats propensos a inundações demonstraram queda no desempenho fotossintético e crescimento sob condições de inundação no solo (MIELKE et al. 2003; JING et al., 2009; LARRÉ et al., 2013). Para evitar danos irreversíveis no FSII, as espécies mais tolerantes, acionam o sistema de enzimas antioxidantes para remover as EROs (ALVES et al., 2013; LARRÉ et al. 2016) e também rearranjam o aparato fotossintético, para evitar a produção de EROs, através da dissipação de energia por processos não fotoquímicos (MIELKE et al. 2003; RENGIFO; TEZARA; HERRERA, 2005; JING et al., 2009; LARRÉ et al., 2013).

Figura 10. Diagrama mostrando vários parâmetros do teste JIP com os processos sequenciais que ocorrem durante a indução da fotossíntese. ABS é o fluxo de fótons absorvido pela antena das unidades do FSII; TR é a parte do ABS aprisionado pelos centros de reação do FSII reduzindo a plastoquinona primária do FSII; DI é a parte do ABS dissipado na antena FSII por outros processos que não o aprisionamento de exciton; ET é para o fluxo de energia associado ao transporte de elétrons de Q_A para receptores de elétrons entre sistemas; RE é para o fluxo de energia associado ao transporte de elétrons dos aceptores de elétrons de QA para FSI final; RC é para o número total de centros de reação do FSII; φP0 (= TR0 / ABS), φE0 (= ET0 / ABS), φR0 (= RE0 / ABS), ψE0 (= ET0 / TR0) e δR0 (= RE0 / ET0) são parâmetros, que são proxies propostos para as proporções dos respectivos fluxos de energia; PIABS, total é o índice de desempenho total com base na absorção; DFABS, ou, log (PIABS, total) (= DFRC + DFPo + DFEo + DFRo) é a força motriz total na base de absorção; Z é o doador secundário de PSII (Tyr-161 de D1); QB é a plastoquinona secundária (aceitadora de elétrons) de PSII; P680 é o principal doador de elétrons do PSII; Phe é feofitina, o principal receptor de elétrons do PSII; Cyt b6/f é citocromo b6/f; PC é plastocianina; e Fd é ferredoxina.



Fonte: STIRBET et al., (2018)

Carboidratos de reserva

Os microambientes, nas florestas paludosa, que são expostos a longos períodos de inundação apresentam uma redução de O_2 na rizosfera. Nas plantas sob deficiência de O_2, o metabolismo aeróbico é substituído pelo anaeróbico levando à paralisação do Ciclo de Krebs e da cadeia transportadora de elétrons mitocondrial, tornando a glicólise e o metabolismo fermentativo o principal meio de obtenção de ATP pelas células vegetais (TEWARI; MISHRA, 2018). A fermentação possui baixo rendimento energético, sendo a ineficiência do processo compensada pela aceleração da glicólise para satisfazer as exigências por ATP do metabolismo vegetal (SOUSA; SODEK, 2002; BAILEY-SERRES; VOESENEK, 2008). Assim, em condições de inundação é essencial a disponibilidade de carboidratos prontamente fermentáveis (BAILEY-SERRES; VOESENEK, 2008; FERREIRA et al., 2009; TEWARI; MISHRA, 2018).

De modo geral, as plantas que apresentam maiores reservas de carboidratos nas raízes e um mecanismo metabólico eficiente associado à sua mobilização via metabolismo fermentativo, apresentam maior tolerância para enfrentar o período de privação de O_2 (DAT et al., 2006).As inundações tendem a reduzir a translocação de produtos fotossintéticos das folhas para as raízes, o que pode provocar acúmulo de fotoassimilados nas folhas, na forma de amido (KOZLOWSKI, 1997; RENGIFO; TEZARA; HERRERA, 2005; DAT et al., 2006). Assim, o acúmulo de carboidratos, como o amido, no sistema radicular no período que antecede as inundações é uma importante adaptação para as espécies sob inundação (CRAWFORD, 1996; DAT et al., 2006).

Objetivos

Objetivo Geral

Avaliar os efeitos da inundação no desenvolvimento de duas espécies arbóreas palustres: *Citronella gongonha* e *Allophylus edulis*, descrevendo algumas das estratégias adotadas pelas espécies para colonizar com sucesso as florestas paludosas.

Objetivos Específicos

- Avaliar a resposta germinativa das sementes das espécies selecionadas sob distintas condições de inundação;
- Investigar se o crescimento e a morfologia das plantas jovens das espécies são influenciados pelas condições de inundação da raiz;
- Avaliar a resposta de plantas jovens de *C. gongonha* durante diferentes períodos de inundação da raiz, usando parâmetros bioquímicos.
- Avaliar o desempenho fotossintético de plantas jovens de *A. edulis* inundadas na raiz, através de parâmetros da fluorescência da clorofila *a*;

Referências Bibliográficas

ABREU, D.C.A., et al. Caracterização morfológica de frutos, sementes e germinação de *Allophylus edulis* (St.-Hil.) Radlk.(Sapindaceae). **Revista Brasileira de Sementes**, v.27, p.59-66, 2005.

ALVES, J.D. et al. Antioxidative responses and morpho-anatomical adaptations to waterlogging in *Sesbania virgata*. **Trees**, v. 27, n. 3, p. 717-728, 2013.

BAILEY-SERRES, J.; VOESENEK, L.A.C.J. Flooding Stress: Acclimations and Genetic Diversity. **Annual Review of Plant Biology**, v. 59, p. 313-339, 2008.

BLOM, C.W.P.M.; VOESENEK, L.A.C.J. Flooding: the survival strategies of plants. **Trends in Ecology & Evolution**, v. 11, n. 7, p. 290-295, 1996

BORGHETTI, F.; FERREIRA, A. G. Germinação: do básico ao aplicado. **Porto Alegre: Artmed**, p. 209-222, 2004.

BRUNO, J. F.; STACHOWICZ J. J.; BERTNESS, M. D. Inclusion of facilitation into ecological theory. **Trends in Ecology & Evolution**, 18, p. 119-125, 2003.

BUCKERIDGE M.S. et al. Acúmulo de reservas. In: Ferreira AG, Borghetii F (Orgs) **Germinação: do básico ao aplicado**. Porto Alegre: Artmed, 2004. pp 31-50.

CALLAWAY, R. M. Positive interactions among plants. **Botanical Review**, 61, p. 306-349, 1995.

CALLAWAY, R. M.; WALKER, L. R. Competition and facilitation: a synthetic approach to interactions in plant communities. **Ecology**, Cidade, 78, p. 1958-1965, 1997.

CAMPOSTRINI, E. Fluorescência da clorofila a: considerações teóricas e aplicações práticas. **Rio de Janeiro: UFNF**, 2001.

COELHO, R. L. G. **Estudos sistemáticos das espécies neotropicais de *Allophylus* L. (Sapindaceae)**. Tese (Doutorado em Biologia Vegetal), Universidade Estadual de Campinas, São Paulo, 449 p. 2014.

COELHO, R.L.G. *Allophylus* in **Flora do Brasil 2020 em construção.** Jardim Botânico do Rio de Janeiro.Disponível em: <http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB20873>. Acesso em: 08 Jul. 2018.

COLMER, T. D. Long- distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. **Plant, Cell & Environment**, v. 26, n. 1, p. 17-36, 2003.

CONSERVA, A. et al. Germinative behaviour of ten tree species in white-water floodplain forests in central Amazonia. **Folia geobotanica**, v. 53, n. 1, p. 89-101, 2018.

CRAWFORD, R.M.M. Whole plant adaptations to fluctuating water tables. **Folia Geobotanica**, v. 31, n. 1, p. 7-24, 1996.

DAT, J. et al. Hypoxia stress: current understanding and perspectives. **Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues**, v. 3, p. 664-674, 2006.
DREW, M.C. Oxygen deficiency and root metabolism: Injury and Acclimation Under Hypoxia and Anoxia. **Annual Review of Plant Physiology and Plant Molecular Biology**. v.48, p.223–250, 1997.

DUARTE, C. I. **Influência da variação hidrológica e da luminosidade na composição e estrutura do componente herbáceo em uma floresta paludosa no extremo sul do Brasil**. Dissertação (Mestrado em Biologia de Ambientes Aquáticos Continentais). Universidade Federal do Rio Grande, Rio Grande, 36p. 2012.

FERREIRA C.S.; PIEDADE M.T.F.; BONATES L.C. Germinação de sementes e sobrevivência de plântulas de *Himatanthus sucuuba* (Spruce) Wood. em resposta ao alagamento, nas várzeas da Amazônia Central1. **Acta Amazonica**, v. 36, n. 4, 2006.

FERREIRA, A. G.; BORGHETTI, F. **Germinação: do básico ao aplicado**. Porto Alegre: Artmed, 2004.

FERREIRA, C.S. et al. The role of carbohydrates in seed germination and seedling establishment of *Himatanthus sucuuba*, an Amazonian tree with populations adapted to flooded and non-flooded conditions. **Annals of Botany**, v. 104, n. 6, p. 1111-1119, 2009.

FREUND, C.A. et al. Microtopographic specialization and flexibility in tropical peat swamp forest tree species. **Biotropica**, v. 50, n. 2, p. 208-214, 2018.

GARWOOD, N. C. Functional morphology of tropical tree seedlings. **The ecology of tropical forest tree seedlings**, 1996.

GRAVATT, D.A.; KIRBY, C.J. Patterns of photosynthesis and starch allocation in seedlings of four bottomland hardwood tree species subjected to flooding. **Tree physiology**, v. 18, n. 6, p. 411-417, 1998.

HUENNEKE, L. F.; SHARITZ, R.R. Substrate heterogeneity and regeneration of a swamp tree, Nyssa aquatica. **American Journal of Botany**, v. 77, n. 3, p. 413-419, 1990.

IPCC (Intergovernmental Panel on Climate Change). **Climate Change and Land**: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems, 2019. Disponível em: https://www.ipcc.ch/site/assets/uploads/2019/08/4.SPM_Approved_Microsite_FINAL.pdf. Acesso em 03 de janeiro de 2020.

IRFAN, M. et al. Physiological and biochemical changes in plants under waterlogging. **Protoplasma**, v. 241, n. 1-4, p. 3-17, 2010.

IVANAUSKAS, N. M.; RODRIGUES, R. R.; NAVE, A. G. Aspectos ecológicos de um trecho de floresta de brejo em Itatinga, SP: florística, fitossociologia e seletividade de espécies. **Brazilian Journal of Botany**, 20, p. 139-153, 1997.

JING, Y. X. et al. gas exchange, chlorophyll fluorescence and growth responses of Melaleuca alternifolia seedlings to flooding and subsequent recovery. **Photosynthetica**, v. 47, n. 4, p. 595-601, 2009.

JUNK, W.J. et al. Current state of knowledge regarding the world‟s wetlands and their future under global climate change: a synthesis. **Aquatic sciences**, v. 75, n. 1, p. 151-167, 2013.

KALAJI, H.M. et al. Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. **Acta physiologiae plantarum**, v. 38, p. 102, 2016.

KALAJI, H.M. et al. Fluorescence parameters as early indicators of light stress in barley. **Journal of Photochemistry and Photobiology B: Biology**, v. 112, p. 1-6, 2012.

KELLER, H.A.; VELAZCO, S.J.E; KRAUCZUK, E.R. Regeneración de plantas leñosas bajo arbustos aislados en un sector de los esteros del Iberá, Corrientes, Argentina, implicancias etnoecológicas. **Bonplandia**, v. 25, n. 2, p. 103-114, 2016

KOZLOWSKI, T. T. Responses of woody plants to flooding and salinity. **Tree Physiology,** v. 17, 490–490, 1997.

KOZLOWSKI, T. T.; KRAMER, P. L.; PALLARDY, S. G. **The physiological ecology of woody plants**. Academic Press, 657 p. 1991.

KREUZWIESER, J.; RENNENBERG, H. Molecular and physiological responses of trees to waterlogging stress. **Plant, cell & environment**, v. 37, n. 10, p. 2245-2259, 2014.

LAMBERS, H.; CHAPIN III, F.S.; PONS, T. L. **Plant physiological ecology**. Springer Science & Business Media. 623p. 2008.

LARCHER, W. **Ecofisiologia vegetal**. Editora RiMa, São Carlos. 531p. 2006.

LARRÉ, C.F. et al. Atividade antioxidante e metabolismo fermentativo na planta Erythrina crista-galli L. em condições de inundação. **Semina: Ciências Agrárias**, v. 37, n. 2, p. 567-580, 2016.

LAZÁR, D. The Polyphasic chlorophyll a fluorescence rise measured under high intensity of exciting light. **Functional Plant Biology**. v. 33, p. 9-30, 2006.

LORENZI, H. **Árvores brasileiras**: **manual de identificação e cultivo de plantas arbóreas nativas do Brasil**. Edição: 3. ed. 384 p. Ano de publicação:2009.

LUCAS, C.M. et al. Effects of short-term and prolonged saturation on seed germination of Amazonian floodplain forest species. **Aquatic botany**, v. 99, p. 49-55, 2012.

LUO, W. et al. Competition and facilitation in three marsh plants in response to a water-level gradient. **Wetlands**, v. 30, n. 3, p. 525-530, 2010.

MARENGO, J. A.; DIAS, P. S. Mudanças climáticas globais e seus impactos nos recursos hídricos. **Águas doces no Brasil: capital ecológico, uso e conservação**, v. 3, p. 63-109, 2006.

MARTINAZZO, E.G. et al. Atividade fotossintética em plantas de ameixeira submetidas ao déficit hídrico e ao alagamento. **Ciência Rural**, v. 43, n. 1, 2013.

MIELKE, M. S. et al. Some photosynthetic and growth responses of Annona glabra L. seedlings to soil flooding. **Acta Botanica Brasilica**, v. 19, n. 4, p. 905-911, 2005.
MIELKE, Marcelo S. et al. Leaf gas exchange, chlorophyll fluorescence and growth responses of *Genipa americana* seedlings to soil flooding. **Environmental and experimental botany**, v. 50, n. 3, p. 221-231, 2003.

MUELLER-DOMBOIS, D.; ELLENBERG, H. **Aims and methods of vegetation ecology**. New York: Willey and Sons, 1974. 574 p.

OKAMOTO, J. JOLY, C.A. Ecophysiology and respiratory metabolism during the germination of *Inga sessilis* (Vell.) Mart. (Mimosaceae) seeds subjected to hypoxia and anoxia. **Brazilian Journal of Botany**, v. 23, n. 1, p. 51-57, 2000.

OLIVEIRA, V.C.; JOLY, C. Flooding tolerance of *Calophyllum brasiliense* Camb. (Clusiaceae): morphological, physiological and growth responses.**Trees**, v. 24, n. 1, p. 185-193, 2010.

PARENT, C. et al. An overview of plant responses to soil waterlogging. **Plant stress**, v. 2, n. 1, p. 20-27, 2008.

PAROLIN, P. de et al. Central Amazonian floodplain forests: tree adaptations in a pulsing system. **The Botanical Review**, v. 70, n. 3, p. 357-380, 2004.

PAROLIN, P. Fugitive and possessive establishment strategies in Amazonian floodplain pioneers. **Flora**, London, v. 198, n. 6, p. 436-443, 2003.

PAROLIN, P. Morphological and physiological adjustments to waterlogging and drought in seedlings of Amazonian floodplain trees. **Oecologia**, v. 128, n. 3, p. 326-335, 2001a.

PAROLIN, P. Seed germination and early establishment of 12 tree species from nutrient-rich and nutrient-poor Central Amazonian floodplains. **Aquatic Botany**, v.70 89-103, 2001b.

PAZ, A. A. et al. Induced flooding as environmental filter for riparian tree species. **Environmental and Experimental Botany**, v. 139, p. 31-38, 2017.

PEREZ-HARGUINDEGUY, N. et al. Corrigendum to: new handbook for standardised measurement of plant functional traits worldwide. **Australian Journal of botany**, v. 64, n. 8, p. 715-716, 2016.

PEZESHKI, S. R. Wetland plant responses to soil flooding. **Environmental and Experimental Botany**, v. 46, p. 299-312, 2001.

RENGIFO, E.; TEZARA, W.; HERRERA, A. Water relations, chlorophyll a fluorescence, and contents of saccharides in tree species of a tropical forest in response to flood. **Photosynthetica**, v. 43, n. 2, p. 203-210, 2005.

RICARDO C. R. **Estrutura da comunidade arbórea e suas relações com variáveis ambientais na mata de restinga paludosa da Estrada velha, Rio Grande, Rio Grande do Sul.** Dissertação (Mestrado em Biologia de Ambientes Aquáticos Continentais), Universidade Federal do Rio Grande, Rio Grande, 45p. 2010.

SAUERESSIG, D. **Plantas do Brasil - Árvores** Nativas Vol.1. 1. ed. Irati: Editora Plantas do Brasil, 2014. v. 1. 324p.

SCARANO, F. R. Plant community structure and function in a swamp forest within the Atlantic rain forest complex: a synthesis. **Rodriguésia**, v. 57, n. 3, p. 491-502, 2006.

SCARANO, F. R. Structure, function and floristic relationships of plant communities in stressful habitats marginal to the Brazilian Atlantic rainforest. **Annals of Botany**, 90, p. 517-524, 2002.

SCARANO, F. R.; PEREIRA, T. S.; RÔÇAS, G. Seed germination during floatation and seedling growth of *Carapa guianensis*, a tree from flood-prone forests of the Amazon. **Plant Ecology**, v. 168, n. 2, p. 291-296, 2003.

SCARANO, F.R.; RIOS, R.I.; ESTEVES, F.A. Tree species richness, diversity and flooding regime: case studies of recuperation after anthropic impact in Brazilian flood-prone forests. **International Journal of Ecology and Environmental Sciences**, v. 24, p. 223-235, 1998.

SHIMAMURA, T.; MOMOSE, K. Organic matter dynamics control plant species coexistence in a tropical peat swamp forest. **Proceedings of the Royal Society B: Biological Sciences**, v. 272, n. 1571, p. 1503-1510, 2005.

SHIMAMURA, T.; MOMOSE, K.; KOBAYASHI, S. A comparison of sites suitable for the seedling establishment of two co- occurring species, *Swintonia* glauca and *Stemonurus scorpioides*, in a tropical peat swamp forest. **Ecological Research**, v. 21, n. 5, p. 759-767, 2006.

SIEBEL, H. N.; BLOM, C. W. P. M. Effects of irregular flooding on the establishment of tree species. **Acta Botanica Neerlandica**, v. 47, n. 2, p. 231-240, 1998.

SILVA, A. C. et al. Florestas Inundáveis: ecologia, florística e adaptações das espécies. 1. ed. Lavras: Editora da UFLA, 2012. 170p.

SILVA, A. C.et al. Comparação florística de florestas inundáveis das regiões Sudeste e Sul do Brasil. **Brazilian Journal of Botany**, v. 30, n. 2, p. 257-269, 2007.

SOUSA, C. A. F.; SODEK, Ladaslav. The metabolic response of plants to oxygen deficiency. **Brazilian Journal of Plant Physiology**, v. 14, n. 2, p. 83-94, 2002.

STEFANO, R.D. *Citronella in* **Flora do Brasil 2020 em construção.** Jardim Botânico do Rio de Janeiro.Disponível em: <http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB6678>. Acesso em: 08 jan. 2020.

STIRBET, A. et al. Chlorophyll a fluorescence induction: can just a one-second measurement be used to quantify abiotic stress responses? **Photosynthetica**, v. 56, n. 1, p. 86-104, 2018.

STIRBET, A. et al. Modeling chlorophyll a fluorescence transient: relation to photosynthesis. **Biochemistry (Moscow)**, v. 79, n. 4, p. 291-323, 2014.

STRASSER, B.J; STRASSER, R.J. Measuring fast fluorescence transients to address environmental questions: The JIP-test. In: MATHIS, P. (Ed) **Photosynthesis**: from Light to Biosphere, Montpellier, France, p. 977– 980, 1995.

STRASSER, R. J., The grouping model of plant photosynthesis: heterogeneity of photosynthetic units in thylakoids. In: Photosynthesis II, Structure and Molecular Organization of the Photosynthetic Apparatus (Akoyunoglou G, ed.). **Philadelphia: Balaban International Science Services**, p.727-737, 1981.

STRASSER, R. J.; TSIMILLI-MICHAEL, M.; SRIVASTAVA, A. **Chlorophyll a Fluorescence:** A Signature of Photosynthesis, Analysis of the chorophyll a Fluorescence Transient. Chapter 12, Spring. Printed in The Netherlands, v. 14, p. 321-362, 2004.

TAIZ, L.; ZEIGER, E. **Fisiologia Vegetal**. Artmed, Porto Alegre, 721p. 2006.

TEIXEIRA, A. P.; ASSIS, M. A. Relação entre heterogeneidade ambiental e distribuição de espécies em uma floresta paludosa no Município de Cristais Paulista, SP, Brasil. **Acta Botanica Brasílica**, 23, p. 843-853, 2009.

TURCHETTO, F. et al. Phytosociology as a tool for forest restoration: a study case in the extreme South of Atlantic Forest Biome. **Biodiversity and Conservation**, v. 26, n. 6, p. 1463-1480, 2017.

VARTAPERIAN, B.B.; JACKSON, M.B. Plant adaptations to anaerobic stress. **Annals of Botany**, v.79 (A), n.1, p.3-20, 1997.

VIANI, R.A.G.; VIEIRA, A. O. S. Flora arbórea da bacia do rio Tibagi (Paraná, Brasil): Celastrales sensu Cronquist. **Acta Botanica Brasilica**, 2007.

VOESENEK, L.A.C.J., BAILEY-SERRES, J., 2013. Flooding tolerance: O2 sensing and survival strategies. **Current Opinion in Plant Biology**.16, 647-653.

WITTMANN, A. Germination in four low-várzea tree species of Central Amazonia.**Aquatic Botany**, v. 86, n. 3, p. 197-203, 2007.

YU, B. et al. Morphological, physiological, and biochemical responses of *Populus euphratica* to soil flooding. **Photosynthetica**, v. 53, n. 1, p. 110-117, 2015.

ZHANG, L.; SHAO, H. Direct plant–plant facilitation in coastal wetlands: a review. **Estuarine, Coastal and Shelf Science**, v. 119, p. 1-6, 2013.

ZHANG, Q. et al. Benefits of flooding-induced aquatic adventitious roots depend on the duration of submergence: linking plant performance to root functioning. **Annals of botany**, v. 120, n. 1, p. 171-180, 2017.

ZÚÑIGA-FEEST, A. et al. Physiological and morphological responses to permanent and intermittent waterlogging in seedlings of four evergreen trees of temperate swamp forests. **Tree Physiology**, v. 37, n. 6, p. 779-789, 2017.

CAPÍTULO I

Efeitos da duração da inundação na germinação de sementes, crescimento e ecofisiologia da árvore de pântano *Citronella gongonha* do sul da região neotropical

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Highlights

- Germination was impaired as seed flooding time increased.
- All plants survived 120 days of flood and post-flood period.
- Plants have developed morphophysiological strategies to tolerate the flooding.
- Hydrological conditions are determinants for the initial development this species

Effects of flood duration on seed germination, growth and ecophysiology of the swamp tree *Citronella gongonha* from the southern neotropics

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Abstract

Citronella gongonha is an abundant tree species in flood-prone areas of southern South America. In swamp forests, the distribution of young and adult individuals of the species occurs mainly on hummocks. In this study, we address some aspects of the ecology and physiology of *C. gongonha* that contribute to the knowledge of its distribution in swamp forests. We evaluated seed germination and growth parameters, as well as chlorophyll, malondialdehyde, hydrogen peroxide, nitrate and carbohydrate levels in young *C. gongonha* plants during different periods of flooding. Germination did not occur under flooding conditions, and seeds persisted only up to 30 days of flooding, losing germination potential as submersion time increased. However, young plants survived a long period of flooding (until 120 days) by reducing growth, developing morphological adaptations and using carbohydrate pools. These results help to explain the distribution pattern of *C. gongonha* in swamp forests, predominantly on hummocks where soil flooding is attenuated.

Keywords: adaptive strategies, swamp forest, *adventitious root,* non-structural carbohydrates, lenticels.

1. Introduction

Swamp forests are fragile and naturally fragmented ecosystems due to their occurrence restricted to hydromorphic soils (Teixeira et al., 2008; Zamith and Scarano, 2010). The microtopographic variation in these forests determines a variety of hydrological conditions through fluctuating water levels (Huenneke and Sharitz, 1990; Shimamura and Momose, 2005; Teixeira et al., 2008, 2011; Freund et al., 2018). The elevated areas („hummocks‟) are less susceptible to inundation, whereas water collects in low-lying depressions („hollows‟) (Freund et al., 2018). The high environmental heterogeneity in swamp forests may allow the coexistence of tree species with different levels soil flooding tolerance (Shimamura and Momose, 2005; Teixeira et al., 2008, 2011; Freund et al., 2018).

The extreme south of Brazil is characterized by a wide territory of wetlands that also include swamp forests. These forests have a high density of tree species such as *Citronella gongonha*, *Erythrina crista-galli*, *Sebastiania brasiliensis* and *Ficus cestrifolia* (Duarte et al, unpublished data; Waechter and Jarenkow, 1998). As observed in other swampy forests, the topography enables a mosaic of hydrological conditions; it is possible to distinguish non-flooded and flooded microenvironments in a few meters scale. The abundance of woody species is visually greater on hummocks where water depth and the duration of soil flooding is less. Low-lying areas remain flooded for up to seven months of the year with a water depth of about 30 cm. Few woody plants occur in hollows, but herbaceous hydrophytes such as *Lemna valdiviana*, *Spirodela intermedia*, *Leersia hexandra* and *Luziola peruviana* are frequent in these microenvironments (Duarte et al, unpublished data).

The flooding causes a reduction in oxygen availability and electrochemical changes in the soil that results in higher concentrations of toxic elements to plants (Kozlowski, 1997; Colmer, 2003). Consequently, plant cells are exposed to internal oxygen variations, carbon dioxide and reactive oxygen species (Voesenek and Bailey-Serres, 2013, 2015); in flooded areas, seed viability and plant survival depend on metabolic, physiological and morphological adaptations (Perata et al., 2011; Voesenek and Bailey-Serres, 2013, 2015). One of the most critical factors for woody plant survival during soil flooding is the availability of energy resources (Voesenek and Bailey-Serres, 2013); the ability to store and use reserve carbohydrates can be a key factor for plant survival and growth during soil flooding (Irfan et al., 2010; Perata et al., 2011; Béjaoui et al., 2016). Morphological adaptations such as adventitious roots, aerenchyma and hypertrophied lenticels are also important because they facilitate oxygen circulation to the roots, enable oxidation of the rhizosphere and contribute to the release of toxic products (Kozlowski, 1997; Colmer, 2003; Zhang et al., 2017).

Positive interactions result from neighboring plants that protect themselves from stressful conditions; these are important community forces in physically stressful ecosystems (Bertness and Leonard, 1997). In environments with flooded soil, some plants have improved the conditions of the anoxic substrate by increasing the oxygen levels of the rhizosphere or increasing the soil level (Fogel et al., 2004; Zhang and Shao, 2013). According to Wittman and Parolin (2005), sediment deposition in the Amazon floodplain has a close relationship with the root system of some pioneer tree species such as *Salix* spp., which produce roots above ground in places flooded for long periods. Although the primary function of these adventitious roots is to improve the oxygenation conditions for the plant, they also contribute to the deposition of sediments, leading to a topographic increase in the sites associated with the root system (Wittman et al. 2004; Wittman and Parolin, 2005). These geomorphological changes make the conditions favorable to the establishment of other tree species (Wittman et al. 2004).

In swamp forests, the root system of some flood-tolerant species can also contribute to the soil sedimentation process by creating a mound around the tree (Shimamura and Momose, 2005). In this context, tree species with different levels of flooding tolerance may have distributions associated with hummocks in the swampy forests of southern Brazil. The individuals of species with greater tolerance to flooding would contribute to the formation and expansion of hummocks. This microenvironment decreases soil flooding by favoring survival and growth, as well as the physiological performance of plants with greater restriction to soil-water saturation. Due to the complex nature of ecological relationships that determine the patterns of plant distribution in swamp forests, it is important to know the response of seeds and plants of different species to flooding. This information contributes to the knowledge about the dynamics of these ecosystems, as well as to the planning of successful restoration efforts (Zamith and Scarano, 2010; Freund et al., 2018).

Citronella gongonha (Mart.) R.A. Howard belongs to the Cardiopteridaceae family and is a pioneer or secondary species in Argentina, Brazil, Uruguay and Paraguay (Viani and Vieira, 2007). In the swampy forests of southern Brazil, young and adult individuals of this species are predominantly associated with hummocks in areas where the soil is flooded for long periods (Fig. 1). According to Keller et al. (2016), *C. gongonha* develops an adventitious root system that, in combination with the roots of other plants, allows the accumulation of organic matter, providing suspended load retention and long-term elevation of the substrate around the tree. Accordingly, the predominance of adult *C. gongonha* trees on hummocks may be associated with their role in the formation and expansion of these microenvironments.

However, the greater abundance of young individuals of the species on hummocks, when compared to hollows, suggests that their early development may be hampered by soil flooding.

Despite many phytosociological studies that have reported the presence of *C. gongonha* in flood-prone forests (Silva et al., 2007; Teixeira et al., 2008, 2011) the ecophysiological response of this species to flooding is still unknown. Thus, in this work, we evaluated the germination of seeds and the growth of young plants of *C. gongonha* under soil flooding conditions for different periods. We also evaluated some biochemical parameters in leaves and roots to describe the physiological performance of plants during soil flooding. Given the predominance of young and adult *C. gongonha* individuals on hummocks in the swampy forests, we hypothesize that the level of tolerance of this species to soil flooding depends on the stage of its life cycle, with seeds being sensitive to flooding, and plants being tolerant of stress.

2. Material and methods

2.1. Characteristics of the site and trees selected for fruit collection

C. gongonha presents drupe fruits that, when ripe, have a dark purple color. The seeds have an elongated shape, measuring about 1.3 cm × 0.66 cm. The collection of the fruits of *C. gongonha* was performed in December 2016 in a swamp forest located in the municipality of Rio Grande, RS, Brazil (32º 07' S; 52º 09' W). The climate in this region has four well-defined seasons. According to the Provisional Climatological Norms from 1991 to 2010, the municipality of Rio Grande has a large thermal amplitude between summer and winter, with the highest average temperature in January (23 °C) and lowest in July (12 °C). Precipitation is well distributed over the months, but evaporation follows a pattern similar to that of temperatures, that is, with great contrasts between summer and winter. Evaporation values are highest in December (140 mm) and January (140 mm) and lowest in July (55 mm) (Reboita and Kruche, 2018). The Köppen climate classification for the region is Cfa, humid subtropical (Alvares et al., 2013).

The swamp forest where the fruits of *C. gongonha* were collected is characterized by two hydrological phases, one flooded and the other dry (Duarte et al, unpublished data). During the flooded phase, the forest interior presents a mosaic of flooded and non-flooded microenvironments (Fig. 1A). The soil-flooded microenvironments have a water depth of approximately 30 cm. This hydrological phase lasts approximately seven months and comprises the months with the lowest averages of temperature and evaporation (May to November). When there is an increase in the values of these two climatic factors, in late spring, the water levels in the soil tend to fall. The dry phase occurs in the summer when the temperature and evaporation values are high. The soil, in this hydrological phase, normally does not have a water depth.

The fruits of *C. gongonha* were collected from eight trees established in different hummocks (Fig. 1A) at least 5 meters apart. After collection, the fruits were taken to the Plant Physiology Laboratory at the Federal University of Rio Grande where they were pulped to obtain seeds. Seeds were disinfected by submersion in 1% commercial sodium hypochlorite water solution for two minutes and washed in distilled water. The seeds were used soon after collection in the germination tests (Sections 2.2 and 2.3) and to obtain plants for Experiment 3 (Section 2.4).

2.2. Seed germination in flooded and non-flooded conditions (Experiment 1)

This experiment consisted of two treatments: non-flooded (NFL) and flooded (FL), using ten Gerbox® boxes (11 cm × 11 cm × 3 cm) with 20 seeds each, totaling 200 seeds per treatment. In the NFL treatment, the sowing of the seeds was done in a paper moistened with 20 mL of distilled water; in the FL treatment, sowing was done in 150 mL of distilled water (approximately 2 cm of water depth). The water of each recipient was changed weekly to avoid toxin accumulation.

The germination tests were carried out for 60 days in a BOD germination chamber at 25 ºC and a photoperiod of 12 hours. Seeds with changes in color, presence of fungi, susceptible to breakage and with cracks in the integument were considered deteriorated and, therefore, removed during the evaluations. We measured total germination, percentage of germination, time to reach 50% of germination, percentage of seedlings formed and total length (cm) (Oliveira et al., 2015). Seedlings of *C. gongonha* were classified to functional type according to the position (length of hypocotyls), exposure (cotyledons covered or uncovered by seed coat) and functionality of cotyledons (Garwood, 1996; Perez-Harguindeguy et al., 2016).

2.3. Seed germination after different flooding periods (experiment 2)

To simulate soil forest flood receding and to examine the effect of different durations of seed submersion on germination, simultaneously, tests were performed in Gerbox® boxes, using a layer of 2 cm of medium-textured, washed and sterilized sand. The germination tests followed the same criteria of germination, temperature and photoperiod as in Experiment 1 (Section 2.2).

Experiment 2 consisted of four tests with ten samples of 20 seeds, totaling 200 seeds per test. The seeds were submitted to four temporal levels of flooding. In the control treatment (time 0), the seeds were sown directly on the non-flooded sand and moistened with 20 mL of distilled water. In the other tests, to ensure submersion, the seeds were initially semi-buried in a sandy substrate and submitted to 15, 30 and 60 days of flooding, characterizing different stress levels by flooding time. To this end, the Gerbox® boxes were filled with 150 mL of distilled water and maintained at a water depth about of 2 cm. At the end of each flooding period, the seeds were removed from the flooded conditions (stress relief) and transferred to Gerbox® boxes with the same conditions of the control treatment, to evaluate the germination parameters.

2.4. Development of young plants under flooding conditions (experiment 3)

2.4.1. Plant material and growth conditions

The seedlings for this experiment were obtained from the germination of *C. gongonha* seeds in 5-L plastic pots containing organic soil obtained from the Rio Grande Federal University through composting by windrow system. All pots were filled with soil from the same production lot. An experiment to evaluate the effects of soil flooding on *Citronella gongonha* plants was performed when the plants were 23.62 ± 0.37 height shoot; 5 ± 0.25 leaves and 3.88± 0.07 cm stem diameter. For that, the plants were selected at random and submitted to two treatments: (i) non-flooded plants (NFL) that were irrigated daily to keep the substrate moist and (ii) flooded plants in the root zone (FL), where the pots were placed in 10-L buckets to maintain a water depth of about 3 cm above the substrate. The water of each bucket was changed weekly to avoid toxin accumulation.

The plants were kept in an area covered with a black shade net that partially diminished the incident solar radiation on the plants (50% shading). The flood experiment started in September and ended in December 2017. NFL and FL plants had several parameters evaluated at 30, 60, 90 and 120 days of the experiment. Global daily solar radiation (mean ± SD), during 1 to 30 days, 31 to 60 days, 61 to 90 days and 91 to 120 days of soil flooding were, respectively, 1005.4 ± 627.7 kJ/m^{-2}, 2007.6 ± 836.6 kJ/m^{-2}, 1961.7 ± 870.9 kJ/m^{-2} and 1874.0 ± 828.09 kJ/m^{-2}. The air temperature for the same periods were 18.1 ± 0.9 ºC, 18.4 ± 1.7 ºC, 19.4 ± 2.3 ºC and 22.3 ± 1.9 ºC, respectively. Daily values of global solar radiation and air temperature were obtained by Rio Grande Automatic Meteorological Station, RS, Brazil (32° 01' S, 52° 06' W) approximately 2 km from the flooding experiment and provided by National Institute of Meteorology (INMET).

2.4.2. Growth performance and morphological changes

Plant growth was evaluated by total leaf area, number of leaves, height, stem basal diameter and dry mass of the aerial part and roots. Evaluations were performed at 30, 60, 90 and 120 days on 10 plants per treatment.

Plant growth was evaluated by shoot height, stem diameter, number of leaves, total leaf area, dry mass of the shoot and roots. Evaluations were performed at 30, 60, 90 and 120 days on 10 plants per treatment. Each plant evaluated had height measured from the base of the stem, just above the soil surface, to its apex with a ruler (cm). Stem basal diameter (mm) was measured 2 cm above the ground with a digital caliper. The total number of mature leaves per plant was counted and ten of these mature leaves were scanned. Leaf images were used to estimate the leaf area through the Image J software (Rasband, 2016); the height was measured from the base of the stem, just above the soil surface, to its apex with a ruler (cm).

Stem basal diameter (mm) was measured 2 cm above the ground with a digital caliper. To estimate the dry mass, the plants were separated into the aerial components and root system, individually stored in paper bags, oven-dried at 70 °C for 48 h and weighed on an analytical balance. Additionally, every five days, possible morphological changes, such as the development of hypertrophic lenticels and adventitious roots, were observed and recorded.

At the end of the experiment, ten plants that survived the soil flood treatment for 120 days were selected to assess soil post-flooding survival (PFL). For this, the pots with the plants were removed from the buckets containing water (FL) and left under NFL conditions to evaluate survival after 120 days.

2.4.3. Analysis of physiological parameters

The physiological parameters analyzed included photosynthetic pigments of leaves obtained from five plants of each treatment (NFL and FL) at 30, 60, 90 and 120 days of the experiment. The hydrogen peroxide (H_2O_2), malondialdehyde (MDA), nitrate (NO_3^-) and non-structural carbohydrates (total soluble sugars, sucrose and starch) contents were also quantified in leaves and roots of five plants of each treatment at 30, 60 and 90 days of experiment.

The photosynthetic pigments evaluated were chlorophyll *a* (Chl_{a})*,* chlorophyll *b* (Chl_{b}) and total chlorophyll (Chl_{a+b}). The leaves were wrapped in aluminum foil and transported on ice to the laboratory where a sample of each leaf was withdrawn and weighed. Subsequently, the samples were macerated with 80% (v/v) acetone (Arnon, 1949) and filtered using Whatmann n1 filter paper, obtaining the extract from chloroplast pigments. The absorbance readings were obtained from the ketone extracts at the wavelengths of 646 and 663 nm. From the absorbance and dilution factors, the pigment contents were quantified according to the equations described by Wellburn (1994).

To quantify H_2O_2 and MDA content, approximately 250 mg of fresh mass (FM) of leaves and roots were macerated in liquid N_2 with added 20% polyvinylpolypyrrolidone (PVPP) and 2 mL of a solution containing 0.1% trichloroacetic acid (TCA). The material was homogenized and centrifuged (20 min, 12,000 × *g*), and the resulting supernatant was transferred to 2-mL microcentrifuge tubes. H_2O_2 content was determined according to Velikova et al. (2000). In test tubes containing 10 mM potassium phosphate buffer pH 7.0 and 1 M Kl, an aliquot of the supernatant was added, followed by incubation at 30 °C for 10 minutes. Readings were taken by a spectrophotometer at 390 nm. The H_2O_2 content was given on a standard curve prepared with known H_2O_2 concentrations (0-100 µmol mL^{-1}) and expressed in µmol H_2O_2 g^{-1} FM.

Lipid peroxidation was determined by the quantification of thiobarbituric acid-reactive species (TBARS) as described by Cakmak and Horst (1991). The reaction medium was composed of 0.5% (w/v) thiobarbituric acid (TBA) and 10% (w/v) TCA; an aliquot of the supernatant was added and then incubated at 90 °C for 20 minutes. The reaction was stopped by rapid cooling on ice for 10 minutes immediately after removal from incubation. The measurements were performed in a spectrophotometer at 535 nm and 600 nm. TBA forms reddish colored complexes with low-molecular-weight aldehydes such as malondialdehyde (MDA), which is a secondary product of the peroxidation process. The concentration of the MDA/TBA complex was calculated by the following equation: $[\mathrm{MDA}] = (\mathrm{A535} - \mathrm{A600}) / (\,.\, b)$, where is the extinction coefficient = 155×10^{-3} M^{-1} cm^{-1} and b is the optical length = 1. Peroxidation was expressed as µmol MDA g^{-1} FM.

Plant root and leaf extracts were obtained according to the methodology adapted from Bieleski and Turner (1966) for the evaluation of non-structural carbohydrates and nitrate content. Plant root and leaf extracts were obtained according to the methodology adapted from Bieleski and Turner (1966) for the evaluation of non-structural carbohydrates and nitrate content. The samples with approximately 250 mg were macerated with liquid N_2 and homogenized with 10 mL of methanol: chloroform: water (12: 5: 3, v/v/v). After 24 hours, the extracts were centrifuged for 30 min at 600 × *g*. For each 4.0 mL of the obtained supernatant, 1.0 mL of chloroform and 1.5 mL of water were added. The extract was centrifuged again (30 min, 600 × *g*) and the upper phase was transferred to the water bath at 38ºC reducing the sample to half of the initial volume. Then, the total soluble sugars (TSS) (Graham and Smydzuk, 1965), sucrose (Van Handel, 1968) and starch (McCready et al., 1950) were determined in appropriately diluted root and leaf extracts. For NO_3^- dosage 50 µL of plant extracts duly diluted in pure water were mixed with 200 µL of salicylic reagent (5% salicylic acid in concentrated H_2SO_4) and then vortexed. After 20 minutes at 25 ºC, 4.75 mL of 2N NaOH were slowly added to the tubes. The quantification of nitrate was carried out at 410 nm according to Cataldo et al. (1975); KNO_3 (0-500 nmol NO_3^-) was used as standard.

2.5. Statistical analysis

The effect of the different flood periods on the germination of seeds parameters in experiment 2 (Section 2.3) was evaluated with a one-way ANOVA. Subsequently, we applied a Tukey post hoc test, at a significance level of 0.05 to compare all treatment levels.

The effect of soil flooding conditions (NFL and FL) and time (30, 60, 90 and 120 days) on plant growth parameters (Section 2.4.1) and chlorophyll levels (Section 2.4.3) were tested with a two-way ANOVA. The same test was used to assess the effect of soil flood conditions (NFL and FL) and time (30, 60 and 90 days) on the biochemical parameters in leaves and roots of plants (Section 2.4.3). Two-way ANOVA was followed by a Tukey post-hoc test, at a level of significance of 0.05. We applied the Box-Cox transformation to the height, number of leaves, nitrate (root), TSS (leaves) and starch (root) data to obtain normality and homoscedasticity. In addition, we apply the logarithmic transformation to the Chl $_b$, Chl $_{(a+b)}$ and H_2O_2 data and to transform the dry mass data we use square root. All other data presented normal and homoscedastic distribution.

3. Results

3.1. Seed germination in flooded and non-flooded conditions

There was no seed germination in the FL treatment; under NFL conditions there was 52.5% seed germination, of which 47% formed seedlings by the end of the evaluation (Table 1, Fig. 4A). The functional type of *C. gongonha* seedlings was phanerocotylar epigeal with foliaceous cotyledons (PEF) (Fig. 4A and B).

3.2. Seed germination after different flooding periods

The germination percentage decreased as the time the seeds were flooded increased (Table 1). The germination evaluation after the seeds were removed from the flooding treatment for 15 and 30 days was terminated at 60 days of follow-up when the non-germinated seeds showed signs of deterioration. No seed germinated after 60 days of flooding (Table 1). Follow-up of flooded seeds germination for 60 days was done up to 40 days after stress relief. The evaluation was terminated at this time due to the state of seed deterioration (Fig. 2). The 15-day and 30-day flood treatments caused a delay in the start of the germination of viable seeds. However, the seeds that underwent these treatments had germination with greater synchronization than the seeds from the non-flooded treatment (time 0), reducing the time required to germinate 50% of the viable seeds (Fig. 2).

3.3.Growth and morphological evaluation

All plants survived until the end of the experiment regardless of the treatment (FL, NFL and PFL). After 10 days of flooding, 100% of the plants flooded at the root had hypertrophic lenticels. At 30 days, this structure was quite developed and adventitious roots were also observed (Fig. 4C). Cortical cracks showed evidence of aerenchyma expansion near the base of the stem (Fig. 4F), which resulted in hypertrophic growth in FL plants (Fig. 3C). At 120 days, all FL plants had adventitious roots; many of these roots were arch-shaped (Fig. 4H). The biomass of the underground roots was affected by soil flooding (Fig. 4G), therefore the increase in the total dry mass of roots is due to the formation of adventitious roots as the time of soil flooding increases (Fig.3F).

In addition to the typical symptoms of morphological response to flooding, FL plants exhibited some stress symptoms such as significant reductions in height, number of leaves and shoot dry mass (Table 2 and Fig. 3). During the PFL period, the hypertrophied lenticels had acquired a dehydrated aspect and some adventitious roots became lignified (Fig. 4I).

3.4. Physiological performance

The concentrations of Chl $_a$ and Chl $_{a+b}$ were lower in FL plants compared to NFL, but only at 120 days of the experiment (Table 2 and Fig. 5). Table 3 shows the effects of time and soil flooding conditions on the concentration of the biochemical parameters evaluated. Leaf H_2O_2 and MDA concentrations were higher in FL plants than in NFL plants during the three periods evaluated (Fig. 6A and B). In roots, the H_2O_2 levels were also higher in FL plants during the three periods evaluated (Fig. 6C), but MDA concentrations differed between FL and NFL plants only at 60 days of the experiment (Fig. 6D). We found no differences for NO_3^- concentration in FL and NFL leaves (Fig. 7A). However, NO_3^- concentration was lower in FL plant roots by approximately 45%, 55% and 34% of NFL at 30, 60 and 90 days, respectively (Fig. 7B).

The total soluble sugar (TSS) concentration in leaves was higher in FL plants compared to NFL plants from 60 days of the experiment (Fig. 8A). Leaf sucrose concentrations were lower in FL plants at 30 and 60 days; however, at 90 days, there was a significant increase in sucrose concentration in the leaves of FL plants, reversing the pattern observed in the two previous evaluations (Fig. 8B). Leaf starch concentration was higher in FL plants at 30 days; however, at 90 days, the leaves of the FL plants had lower starch concentration compared to NFL plants (Fig. 8C).

In the roots, we noticed higher concentrations of TSS in FL plants at 30 and 60 days of flooding (Fig. 8D). FL plants had higher sucrose concentration in root tissues than NFL plants during the three periods evaluated (Fig. 8E). The starch concentrations were higher in the roots of NFL plants at 60 and 90 days of the experiment (Fig. 8F).

4. Discussion

Our results show that seeds and young plants of *C. gongonha* respond differently to flooding. The seeds are sensitive to flooding, however, the young plants of the species show high tolerance to root flooding. Even if the adult plant of a particular species is soil flood-tolerant, its seeds and seedlings may not be tolerant (Huenneke and Sharitz, 1990; Paz et al., 2017). In the case of *C. gongonha*, even a short period of flooding can be detrimental to the seed, since after 15 days of submersion in water the seed germination percentage decreased by 50% compared to control treatment (time 0). After 60 days of submersion, the seeds lost viability. These results corroborate our hypothesis that *C. gongonha* seeds have low flood tolerance and help to explain the greater visual abundance of young individuals of this species on hummocks in the swamp forests. The fruiting of *C. gongonha* occurs from November to January (Lorenzi, 2009). This period coincides with the period when water levels drop and swamp forests in the extreme south of Brazil enter the dry hydrological phase. Given that seeds of this species lose germination potential as flooding time increases, the preferred sites for germination should be non-flooded microenvironments or those where the water level drops most rapidly.

Flooding delayed the germination of *C. gongonha* seeds; however, when the seeds were removed from the stress conditions, the time required to germinate the viable seeds was reduced. Rapid germination is a characteristic of species whose strategy is to establish in the environment as quickly as possible by taking advantage of favorable environmental conditions (Borghetti and Ferreira, 2004). Several tree species from flooded areas start germination soon after seed dispersal, probably because the appropriate conditions for seedling establishment in these environments last for only a short time (Parolin et al., 2003; Wittmann et al., 2006; Lucas et al., 2012; Conserva et al., 2018; Duarte et al., 2020).

In general, the period immediately after germination is the most vulnerable stage of the plant life cycle (Leck et al., 2008). The variations in seedling morphology may provide important clues about the regeneration strategy of the species (Ibarra-Manríquezet et al., 2001; Perez-Harguindeguy et al., 2016). Seedlings of *C. gongonha* are classified as PEF; the phanerocotylar epigeal germination is related to the rapid expansion and release of cotyledons from above-ground seed envelopes. Foliaceous cotyledons have limited energy reserves and therefore have morphofunctional adaptations for the first photosynthetic activity of the plant (Perez-Harguindeguy et al., 2016). These characteristics help PEF seedlings maximize the non-flooded period for seedling growth (Parolin et al., 2003; Conserva et al., 2018). Rapid growth and accumulation of energy reserves prior to flooding is important to cope with stress, as long flooding periods can cause photosynthetic performance to decline (Gravatt and Kirby, 1998; Larré et al., 2013; Santos Junior et al., 2015; Duarte et al., 2020).

The young *C. gongonha* plants survived the root flooding treatment for 120 days. This is considered a long time under stress for most plants (Oliveira and Joly, 2010; Zanandrea et al., 2010; de Melo et al., 2015; Yu et al., 2015). The plants were also tolerant to reoxygenation of the soil, as they were all alive 120 days after being removed from flooding conditions. Our results indicate that to withstand the long period of flooding, plants develop morphological changes, decrease growth and use reserve carbohydrates, which also confirms our hypothesis.

At 10 days of flooding, all plants had hypertrophied lenticels. Although the physiological role of these structures is not yet clear, it is believed that they are involved in the diffusion of oxygen to the roots as well as in the release of compounds produced as byproducts of anaerobic metabolism (Patel et al., 2014). There was the expansion of adventitious roots, but the biomass of the underground roots decreased (Fig. 4G). Long periods of soil flooding tend to reduce the biomass of underground roots. Adventitious roots contribute to maintaining the supply of water and nutrients when the functioning of the underground root system is compromised. (Malik et al., 2001; Colmer, 2003; Zhang et al., 2017). Adventitious roots originate from the stem and maintain contact with surface waters that are richer in oxygen (Colmer, 2003). Species whose stem and root tissues have higher porosity tend to be more tolerant to soil flooding (Armstrong, 1979; Colmer, 2003). The intercellular gaps in the aerenchyma increase the porosity of adventitious root tissue, and thus contribute significantly to gas diffusion inside and outside the organs surrounded by water (Armstrong, 1979; Colmer, 2003; Voesenek and Bailey-Serres, 2013). Through adventitious roots and aerenchyma tissue, oxygen diffuses into the underground roots, maintaining aerobic respiration and basic root activities (Armstrong, 1979; Zhang et al., 2017).

The combination of arc-shaped adventitious roots with high porosity tissue and the presence of many thin and short lateral roots in *C. gongonha* plants (Fig. 4F and H) may play a role in rhizosphere oxidation. According to some studies these morphological characteristics contribute to the oxidation of the rhizosphere by protecting the roots against toxins derived from flooded soil (Colmer, 2003; Parolin et al., 2004). In addition to ecophysiological adaptation, flooding-resistant species may play a role in the survival of less resistant species in the same plant community (Ernst, 1990). The root morphology developed above the soil surface by *C. gongonha* also favors the accumulation of organic matter and sediment retention around the tree, which can contribute to the elevation of the substrate over time (Keller et al., 2016). Some tree species in areas where the soil is flooded for long periods can modify microtopography by forming and / or accentuating hummocks through their root system (Shimamura and Momose, 2005; Wittmann and Parolin, 2005; Shimamura et al., 2006). In these places the relief from stressful flood conditions occurs, allowing the establishment of species with greater restrictions on soil water saturation (Shimamura and Momose, 2005; Keller et al., 2016).

Although the plants developed morphological adaptations and withstood a long period of flooding, our results also show evidence of impaired growth of plants under flooding conditions. Retarded growth is a common response to flooding, even for tolerant species (Kozlowski, 1997; Yu et al., 2015; Infante-Mata et al., 2019). In many species, plant survival occurs by reducing metabolism and saving energy through diminishing total biomass (Voesenek and Bailey-Serres, 2013; Voesenek and Bailey-Serres, 2015). In addition, the decrease in dry mass may be caused by a malfunction of the photosynthetic apparatus, reduction of photosynthetic pigment contents and concomitant nutritional imbalance (Liu et al., 2015).

Many authors have provided data on chlorophyll content reduction in leaves of flooded plants (Jackson et al., 1991; Malik et al., 2001; Parolin et al., 2004; Santos Junior et al., 2015). In this study, we found a reduction in chlorophyll content only at 120 days of soil flooding. The leaves of the FL plants also maintained NO_3^- levels similar to those of the NFL plants. However, there were lower NO_3^- concentrations in the roots of FL plants during the three evaluated periods. As mentioned earlier, adventitious roots help to maintain endogenous oxygenation in plants under soil flooding conditions. These roots also improve oxygenation in the rhizosphere, which possibly contributes to the absorption, reduction and assimilation of NO_3^-. However, as flooding time increases, the more basal roots are possibly exposed to a less oxygenated environment. Under hypoxic conditions many plants experience a reduction in endogenous NO_3^- (Puiatti and Sodek, 1999; Brandão and Sodek, 2009; Cardoso et al., 2017).

After NO_3^- is absorbed from the medium there are three major metabolic destinations for this ion in the root cells: (i) it can be stored in vacuole, (ii) it can be reduced and assimilated, forming amino acids or (iii) it can be transported through the xylem for reduction and assimilation in the shoots (Brandão and Sodek, 2009). In general, the leaves are one of the main sites of NO_3^- assimilation, but depending on the species, the roots can also make an important contribution to the process (Smirnoff and Stewart, 1985). However, under low oxygen conditions, the reduction and assimilation of NO_3^- in the roots can be severely restricted. This is because in some species, hypoxic conditions determine a decrease in the activity of nitrate reductase, the enzyme responsible for the initial step in reducing and assimilating nitrate (Brandão and Sodek, 2009). In addition, the activity of nitrite reductase, the second enzyme of the assimilation pathway, depends on the presence of O_2 (Botrel et al., 1996). Thus, it is possible that NO_3^- was absorbed in the roots of FL plants in our experiment and transported to the aerial part where conditions were more favorable for its assimilation to occur. This would justify the maintenance of chlorophyll levels in FL plants, because nitrogen is part of the chemical structure of chlorophyll.

The cell membrane damage reflects the extent of lipid peroxidation caused by reactive oxygen species (Halliwell and Gutteridge, 2015). Flood stress can cause lipid peroxidation of the plasma membrane, affecting its permeability and pattern of ion leakage (Peng et al., 2013; Yu et al., 2015; Peng et al., 2017). Malondialdehyde (MDA) is a cytotoxic product of lipid peroxidation and an indicator of tissue damage under stress conditions (Halliwell and Gutteridge, 2015). Thus, increased MDA content means increased oxidative stress. In our experiments, the MDA concentration in leaves and roots was higher in FL plants than in NFL plants; therefore, soil flooding was detrimental to *C. gongonha* plants. However, MDA levels remained relatively stable on the leaves throughout the flood period (Fig. 6B). In addition, in the roots after 90 days of flooding, the MDA values were similar to the NFL treatment (Fig. 6D), although the H_2O_2 levels increased over the period of soil flooding (Fig. 6C). This indicates a metabolic change due to improvements in oxygenation conditions through the development of adventitious roots and aerenchyma.

Plants can reduce flooding stress damage and maintain growth by triggering reserve carbohydrates (Irfan et al., 2010; Béjaoui et al., 2016). We found an initial increase in leaf starch content in the FL plants. Starch accumulation has been reported in leaves of various flooded plants (Irfan et al., 2010) and may be associated with reduced phloem transport, perhaps because of a decline in root metabolism (Gravatt and Kirby, 1998). We also found that as soil flooding time increased, starch levels decreased and total soluble sugars and leaf sucrose increased, indicating that starch reserves were mobilized.

High levels of soluble carbohydrates (TSS and sucrose) were found in flooded roots during the three evaluated periods. This result is associated with the fact that the starch contents in the flooded roots remained stable and shows that the translocation of assimilates from the aerial part to the roots was not affected. Continuous supply of fermentable sugars to the roots is considered critical for the long term survival of various plants under soil flooding conditions (Gravatt and Kirby, 1998; Zanandrea et al., 2010; Marcílio et al., 2019). Although the FL plants did not increase aerial mass, there was growth of adventitious roots. The soluble carbohydrates were possibly directed to the development of adventitious roots and the maintenance of anaerobic metabolism. In addition, the increased levels of soluble sugars may provide protection for flooded plants because they improve the osmotic potential of cells and maintain cell turgor pressure (Irfan et al., 2010; Tewari and Mishra, 2018). Therefore, the increased concentration of TSS in the roots of flooded plants can be considered a form of stress tolerance (Araki et al., 2012). The maintenance of low starch concentrations in leaves and high starch concentrations in root tissues are also important characteristics that confer flood tolerance (Gravatt and Kirby, 1998).

5. Conclusion

The success of *Citronella gongonha* in colonizing swampy forests in southern Brazil is due to the high tolerance of young plants to soil flooding. To tolerate a long period of flooding of the root zone, plants invest in hypertrophied lenticels and adventitious roots. It is possible that these morphological changes have improved nitrogen absorption and assimilation due to the increased production of energy triggered by aerobic metabolism. In addition, maintaining the levels of photosynthetic pigments during the long period of soil flooding may have contributed to a better yield of photosynthesis during stress, making available the sugars necessary to reduce oxidative damage in tissues during the flooding of the root zone

Our data showed 100% plant survival at 120 days of post-flood recovery. The maintenance of reserve carbohydrate levels during soil flooding may have contributed to plant recovery. The plants maintain the adventitious roots in an arc shape in the post-flood period, which can improve the physiological performance and growth of these plants in a future flood event. In addition, because these roots are above the surface of the soil, they may be associated with the formation and expansion of hummocks in the Brazilian swampy forests. Unlike plants, *Citronella gongonha* seeds are sensitive to flooding. The germination of seeds of this species is inhibited by floods, and the duration of stress affects the germination potential of the seed, making them unviable after 60 days of flooding. These results indicate that the germination of seeds and the regeneration process of this species correlate with the hydrological pattern of the southern Brazilian swampy forests.

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Tables

Table 1. Average germination percentage, number of days until half of the viable seeds germinate (Days to 50%), and percentage of seedlings formed and total length of *Citronella gongonha* seedlings. Experiment 1: seeds subjected to the non-flooded and flooded regimes; Experiment 2: germination after relief from stress at 15, 30 and 60 days (n = 10, mean ± SE)

Treatment	Germination (%)	Days to 50%	Seedlings (%)	Length of seedlings (cm)
Experiment 1				
Non-flooded	52.5 ± 3.81	34	47 ± 4.09	8.59 ± 1.05
Flooded	0.00	-	-	-
Experiment 2				
0 days	48.5 ± 7.83^{a}	35	45.5 ± 2.41^{a}	8.73 ± 1.07^{a}
15 days	24.0 ± 3.48^{b}	34	21.0 ± 2.76^{b}	7.25 ± 0.50^{a}
30 days	18.0± 2.70^{b}	28	15.5 ± 2.52^{b}	8.80 ± 1.49^{a}
60 days	0.00	-	-	-

Means followed by equal letters in the columns did not differ ($p > 0.05$) by Tukey's test

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Table 2. Results of the two-way ANOVA on the influence of the time (30, 60, 90 or 120 days) and soil flooding conditions (non-flooded plants or plants flooded at the root) on growth parameters (shoot height, number of leaves, stem diameter, leaf area, shoot and root dry mass) and in the levels of chlorophyll *a* (Chl $_a$), chlorophyll *b* (Chl $_b$) and total chlorophyll (Chl $_{(a+b)}$) of *Citronella gongonha*.

Model term	d.f	F	*p*-value
Growth			
Shoot height			
Time	3	8.1	<0.001
Soil flooding conditions	1	407.3	<0.001
Time × soil flooding conditions	3	20.6	<0.001
Number of leaves			
Time	3	8.18	<0.001
Soil flooding conditions	1	79.32	<0.001
Time × soil flooding conditions	3	13.33	<0.001
Stem diameter			
Time	3	95.74	<0.001
Soil flooding conditions	1	58.26	<0.001
Time × soil flooding conditions	3	2.97	0.046
Leaf area			
Time	3	7.76	<0.001
Soil flooding conditions	1	87.17	<0.001
Time × soil flooding conditions	3	5.02	<0.001
Shoot dry mass			
Time	3	172.5	<0.001
Soil flooding conditions	1	126.6	<0.001
Time × soil flooding conditions	3	1.9	0.14
Root dry mass			
Time	3	42.7	<0.001
Soil flooding conditions	1	112.1	<0.001
Time × soil flooding conditions	3	5	<0.01
Chlorophyll			
Chl $_a$			
Time	3	2.93	0.049
Soil flooding conditions	1	32.37	<0.001
Time × soil flooding conditions	3	2.24	0.1
Chl $_b$			
Time	3	41.06	<0.001
Soil flooding conditions	1	13.03	<0.01
Time × soil flooding conditions	3	1.2	0.037
Chl $_{(a+b)}$			
Time	3	22.56	<0.001
Soil flooding conditions	1	26.25	<0.001
Time × soil flooding conditions	3	1.04	0.39

Table 3. Results of the two-way ANOVA on the influence of the time (30, 60 or 90 days) and soil flooding conditions (non-flooded plants or flooded at the root) on the concentration of hydrogen peroxide (H_2O_2), malondialdehyde (MDA), nitrate, soluble sugars (TSS), sucrose and starch in the leaves and roots of *Citronella gongonha*.

Model term	Leaf			Root		
	d.f	F	*p*-value	d.f	F	*p*-value
H_2O_2						
Time	2	97.6	<0.001	2	22.8	<0.001
Soil flooding conditions	1	335.7	<0.001	1	453.9	<0.001
Time × soil flooding conditions	2	6.4	<0.01	2	2.8	0.08
MDA						
Time	2	7.2	<0.01	2	10.91	<0.001
Soil flooding conditions	1	182.6	<0.001	1	30.37	<0.001
Time × soil flooding conditions	2	0.7	0.49	2	4.84	0.02
Nitrate						
Time	2	9.13	<0.001	2	5.1	0.01
Soil flooding conditions	1	0.07	0.79	1	103.4	<0.001
Time × soil flooding conditions	2	3.86	0.04	2	2.3	0.12
TSS						
Time	2	32.37	<0.001	2	91.48	<0.001
Soil flooding conditions	1	84.35	<0.001	1	37.5	<0.001
Time × soil flooding conditions	2	5.84	<0.01	2	3.29	0.055
Sucrose						
Time	2	102.3	<0.001	2	1.2	0.31
Soil flooding conditions	1	25.1	<0.001	1	128.1	<0.001
Time × soil flooding conditions	2	50.8	<0.001	2	2.4	0.11
Starch						
Time	2	12.28	<0.001	2	10.3	<0.001
Soil flooding conditions	1	10.78	<0.01	1	130.8	<0.0001
Time × soil flooding conditions	2	52.63	<0.0001	2	50.6	<0.0001

Figures

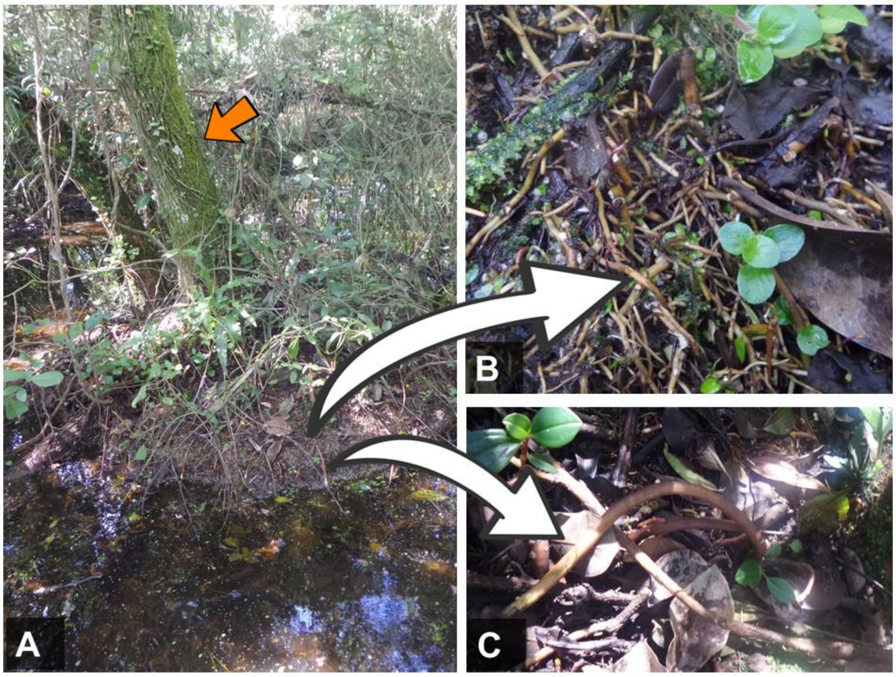

Fig. 1. Characteristic aspects of the trees and the swamp forest where the fruits of *Citronella gongonha* were collected. From these fruits, seeds used in the three experiments of this study were obtained. **(A)** *C. gonhonha* adult tree (orange arrow) with hummock associated root system. **(B)** and **(C)** detail of the soil surface in hummock showing the roots of *C. gongonha*.

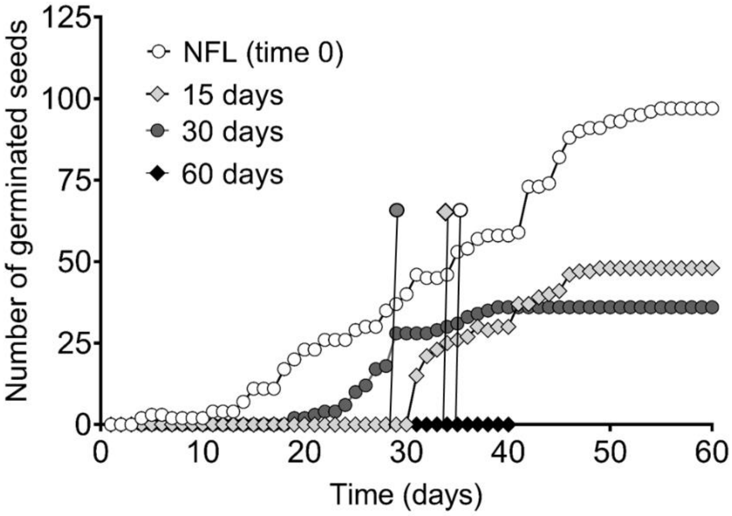

Fig. 2. Germination of *Citronella gongonha* seeds under non-flooded (NFL) and after being removed from flooded conditions for 15, 30 and 60 days. Vertical bars represent time for 50% germination of viable seeds for each treatment.

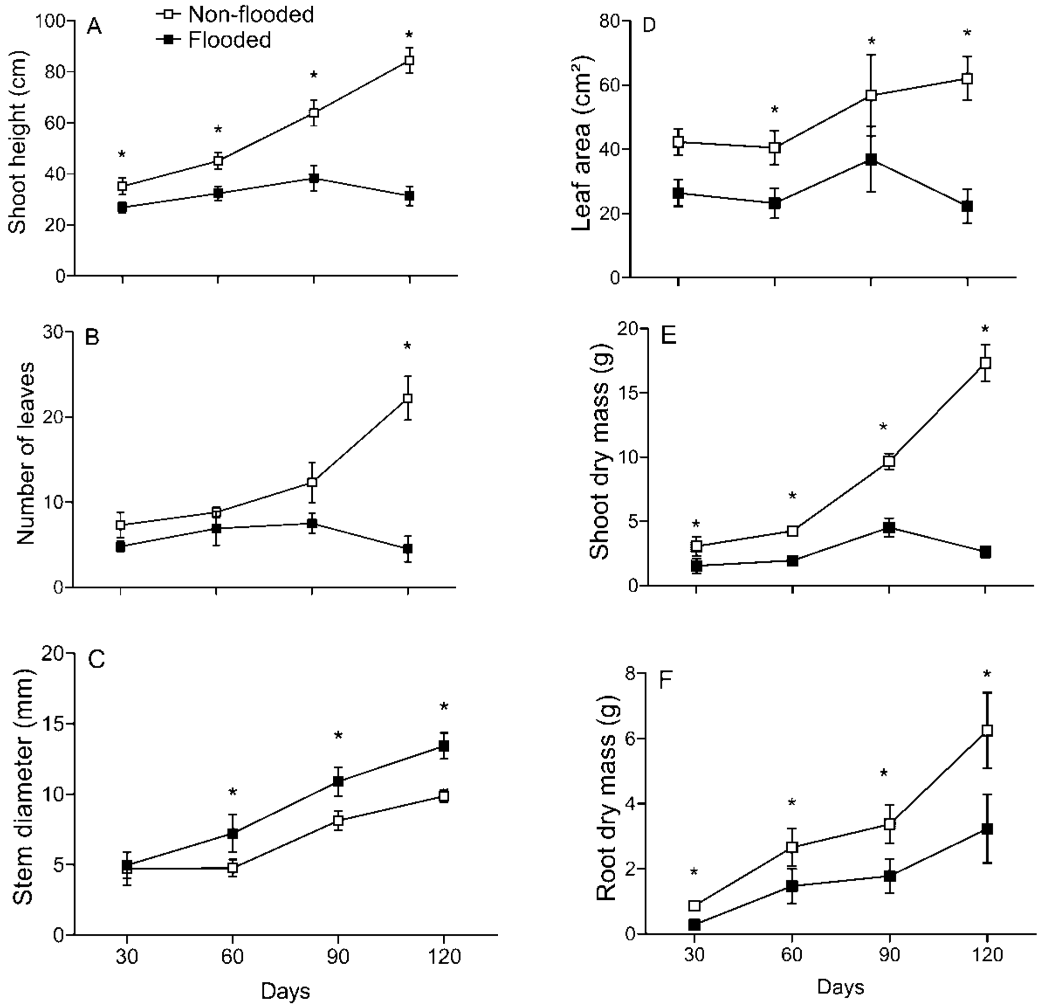

Fig. 3. Mean (±SE, n=10) values for shoot height, number of leaves, stem diameter, leaf area, shoot dry mass and root dry mass of young *Citronella gongonha* plants during four periods of evaluation. Asterisk indicates significant difference ($p < 0.05$) between non-flooded plants and flooded plants in the root zone by Tukey's test.

Fig. 4. Morphological aspects of germinated seeds, non-flooded plants (NFL), flooded plants in the root zone (FL) and post-flooding plants (PFL) of *Citronella gongonha*. **(A)** seed germination and seedling development the type phanerocotylar epigeal with foliaceous cotyledons (PEF); **(B)** seedling emergence used in experiment 3; **(C)** FL plant at 30 days; **(D)** FL plant at 60 days; **(E)** FL plant at 90 days; **(F)** detail of base of the stem in FL plant at 90 days; **(G)** FL and NFL plants at 90 days; **(H)** FL plant at 120 days and **(I)** PFL plant. Aerenchyma (AE); adventitious roots (AR); foliate cotyledons (FC); hypertrophied lenticels (HL); primary leaf (PL); primary roots (PR).

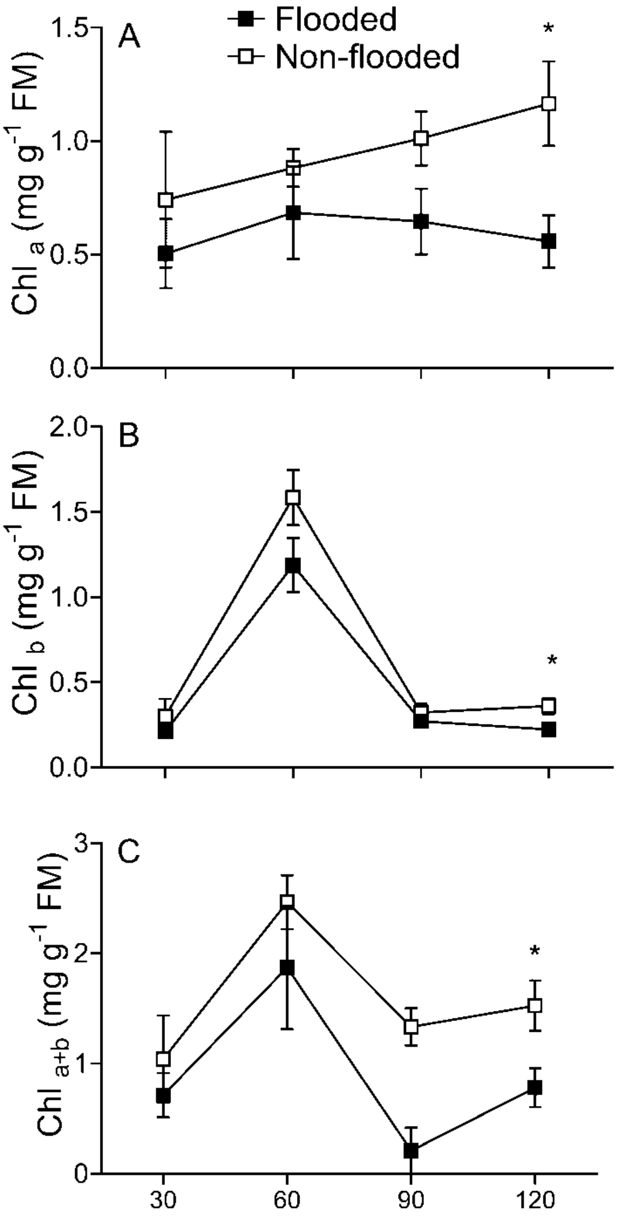

Fig. 5. Chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b) and total chlorophyll (Chl_{a+b}) concentration in leaves of young *Citronella gongonha* plants during four periods of evaluation. Asterisk indicates significant difference ($p < 0.05$) between non-flooded plants and flooded plants in the root zone by Tukey's test.

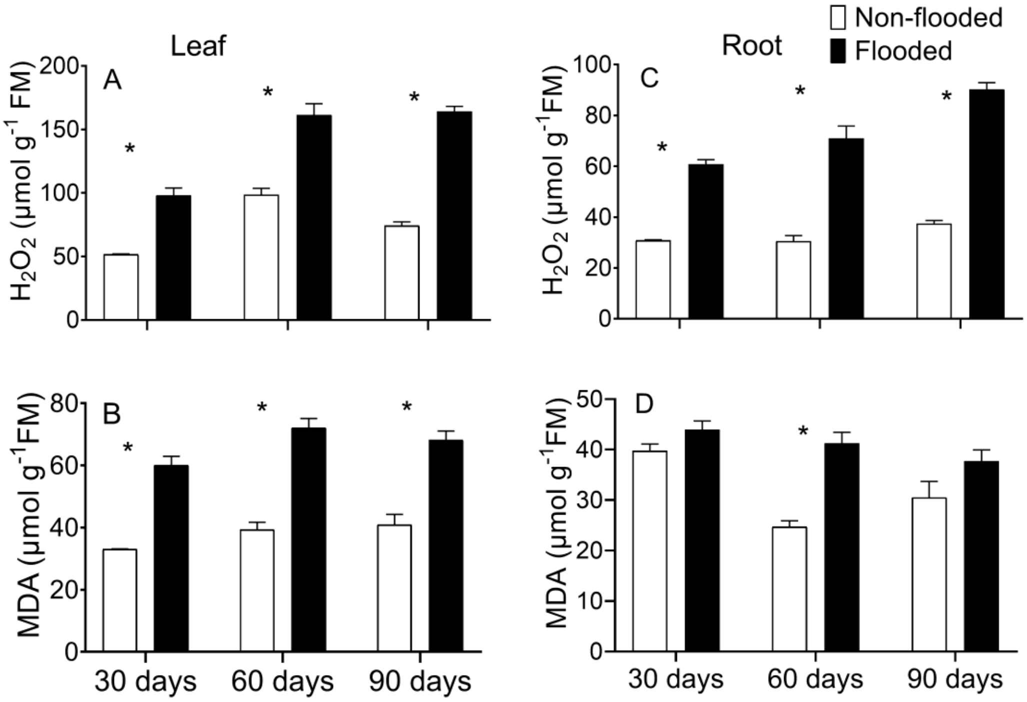

Fig. 6. Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) concentration (±SE, n=5) in the leaves (A and B) and roots (C and D) of young *Citronella gongonha* plants during three periods of evaluation. Asterisk indicates significant difference ($p < 0.05$) between non-flooded plants and flooded plants in the root zone by Tukey's test.

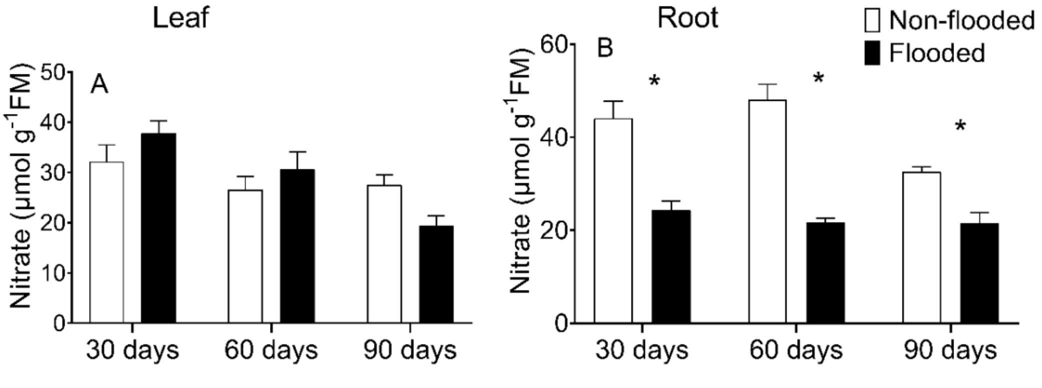

Fig. 7. Nitrate concentration (±SE, n=5) in the leaves and roots of young *Citronella gongonha* plants during three periods of evaluation. Asterisk indicates significant difference ($p < 0.05$) between non-flooded plants and flooded plants in the root zone by Tukey's test.

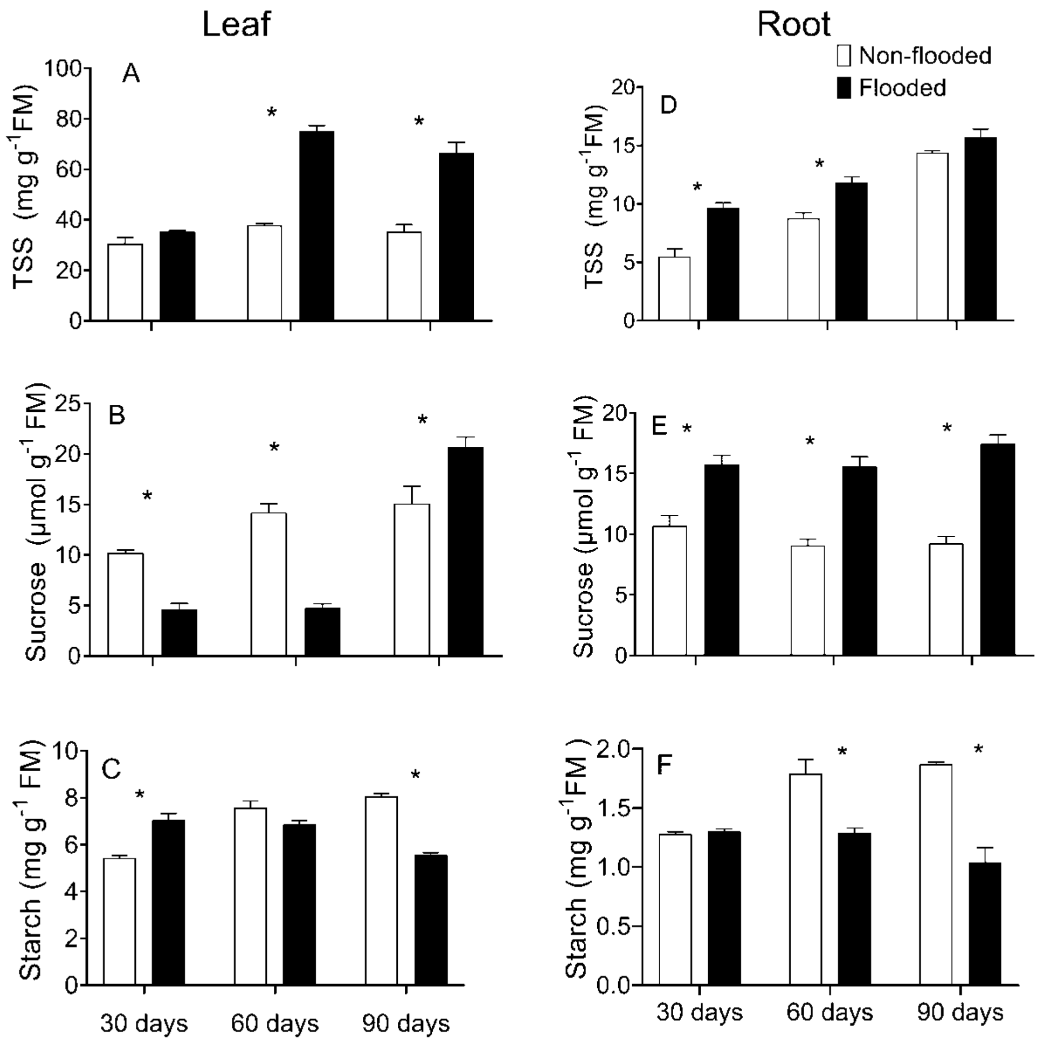

Fig. 8. Total soluble sugars (TSS), sucrose and starch concentration (±SE, n=5) in the leaf (A, B and C) and roots (D, E and F) of flooded and non-flooded young plants of *Citronella gongonha* during three periods of evaluation. Asterisk indicates significant difference ($p < 0.05$) between non-flooded plants and flooded plants in the root zone by Tukey's test.

References

Alvares, C.A., Stape, J.L., Sentelhas P.C., Moraes G., Leonardo J., Sparovek G., 2013. Köppen's climate classification map for Brazil. Meteorol Z. 22, 711-728. https://doi.org/10.1127/0941-2948/2013/0507.

Armstrong, W., 1979. Aeration in higher plants. Adv. Bot. Res. 7, 225-332. https://doi.org/10.1016/S0065-2296(08)60089-0.

Araki, H., Hossain, M.A.,Takahashi, T., 2012. Waterlogging and hypoxia have permanent effects on wheat root growth and respiration. J. Agron. Crop Sci. 198, 264-275. https://doi.org/10.1111/j.1439-037X.2012.00510.x.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24, 1-15. https://doi.org/10.1104/pp.24.1.1.

Béjaoui, Z., Mguis, K., Abassi, M., Albouchi, A., Lamhamedi, M. S., 2016. Involvement of carbohydrates in response to preconditioning flooding in two clones of *Populus deltoides* Marsh. × *P. nigra* L.J. Plant Growth Regul. 35, 492-503. https://doi.org/10.1007/s00344-015-9555-0.

Bertness, M.D., Leonard, G.H., 1997. The role of positive interactions in communities: lessons from intertidal habitats. Ecology 78, 1976-1989. https://doi.org/10.1890/0012-9658(1997)078[1976:TROPII}2.0.CO;2.

Bieleski, R.L.,Turner, A., 1966 Separation and estimation of amino acids in crude plant extracts by thin-layer electrophoresis and chromatography. Anal. Biochem. 17, 278- 293. https://doi.org/10.1016/0003-2697(66)90206-5.

Borghetti, F., Ferreira, A.G., 2004. Interpretação de resultados de germinação. In: Borghetti, F., Ferreira, A.G. (Eds.), Germinação: do básico ao aplicado. Artmed, Porto Alegre, pp. 209-222.

Botrel, A., Magné, C., Kaiser, W.M.,1996. Nitrate reduction, nitrite reduction and ammonium assimilation in barley roots in response to anoxia. Plant Physiol. Biochem. 34, 645-652.

Brandão, A.D., Sodek, L., 2009. Nitrate uptake and metabolism by roots of soybean plants under oxygen deficiency. Brazilian J. Plant Physiol. 21, 13-23. http://dx.doi.org/10.1590/S1677-04202009000100003.

Cakmak, I., Horst, W.J., 1991. Effects of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). Physiol. Plant. 83, 463-468. https://doi.org/10.1111/j.1399-3054.1991.tb00121.x.

Cardoso, K.P., Palheta, J.G., de Sousa, J.D., Nascimento, V.R., Nogueira, G.A.D.S., Machado, L.C., ... & dos Santos Filho, B.G., 2017. Physiological and biochemical metabolism in jatoba plants (*'Hymenaea courbaril'* L.) affected by water stress and flooding. Aust. J. Crop Sci. 11, 844-852. doi: 10.21475/ajcs.17.11.07. pne498.

Cataldo, D.A., Haroon, M., Schrader, L. E., Youngs, V.L., 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun. Soil Sci. Plant Anal. 6,71-81. https://doi.org/10.1080/00103627509366547.

Colmer, T.D., 2003. Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. Plant Cell Environ. 26,17-36. https://doi.org/10.1046/j.1365-3040.2003.00846.x.

Conserva, A., Camargo, J.L.C., De Santana, D.G., Piedade, M.T.F., 2018. Germinative behaviour of ten tree species in white-water floodplain forests in central Amazonia. Folia Geobot. 53, 89-101. https://doi.org/10.1007/s12224-017-9284-1.

de Melo, R.B., Franco, A.C., Silva, C.O., Piedade, M.T.F., Ferreira, C.S., 2015. Seed germination and seedling development in response to submergence in tree species of the Central Amazonian floodplains. AoB Plants 7, plv041. https://doi.org/10.1093/aobpla/plv041.

Duarte, C.I., Martinazzo, E.G., Bacarin, M.A., 2020. Seed germination, growth and chlorophyll a fluorescence in young plants of *Allophylus edulis* in different periods of flooding. Acta Physiol Plant 42, 80. https://doi.org/10.1007/s11738-020-03063-7.

Ernst, W.H.O., 1990. Ecophysiology of plants in waterlogged and flooded environments. Aquat. Bot.38, 73-90. https://doi.org/10.1016/0304-3770(90)90099-7.

Freund, C.A., Harsanto, F.A., Purwanto, A., Takahashi, H., Harrison, M.E., 2018. Microtopographic specialization and flexibility in tropical peat swamp forest tree species. Biotropica 50, 208-214. https://doi.org/10.1111/btp.12512.

Fogel, B.N., Crain, C.M., Bertness, M.D., 2004. Community level engineering effects of *Triglochin maritima* (seaside arrowgrass) in a salt marsh in northern New England, USA. J. Ecol. 92, 589-597. https://doi.org/10.1111/j.0022-0477.2004.00903.x.

Garwood, N.C., 1996. Functional morphology of tropical tree seedlings. In: Swaine, M.D. (Ed.), The Ecology of Tropical Forest Tree Seedlings. UNESCO and Parthenon Publishing, New York, pp. 59 -129.

Graham, D., Smydzuc, J., 1965. Use of anthrone in the quantitative determination of hexose phosphates. Anal. Biochem. 11, 246-255. https://doi.org/10.1016/0003-2697(65)90012-6.

Gravatt, D.A., Kirby, C.J., 1998. Patterns of photosynthesis and starch allocation in seedlings of four bottomland hardwood tree species subjected to flooding. Tree Physiol. 18, 411-417. https://doi.org/10.1093/treephys/18.6.411.

Halliwell, B., Gutteridge, J. M., 2015. Free radicals in biology and medicine. Oxford University Press, New York.

Huenneke, L.F., Sharitz, R.R., 1990. Substrate heterogeneity and regeneration of a swamp tree, *Nyssa aquatica*. Am. J. Bot. 77, 413-419. https://doi.org/10.1002/j.1537-2197.1990.tb13570.x.

Ibarra-Manríquez, G., Martínez Ramos, M., Oyama, K., 2001. Seedling functional types in a lowland rain forest in Mexico. Am. J. Bot. 88, 1801-1812. https://doi.org/10.2307/3558356.

Infante-Mata, D., Moreno-Casasola, P., Valverde, T., Maza-Villalobos, S., 2019. Effects of soil flooding, sunlight and herbivory on seedlings of *Annona glabra* and *Pachira aquatica* in a tropical swamp. Wetl Ecol Manag. 27, 539-551. https://doi.org/10.1007/s11273-019-09676-1.

Irfan, M., Hayat, S., Hayat, Q., Afroz, S., Ahmad, A., 2010. Physiological and biochemical changes in plants under waterlogging. Protoplasma 241, 3-17. https://doi.org/10.1007/s00709-009-0098-8.

Jackson, M., Davies, D., Lambers, H., 1991. Plant Life under Oxygen Deprivation. Ecology, Physiology and Biochemistry. SPB Academic, The Hague.

Keller, H.A., Velazco, S.J., Krauczuk, E.R., 2016. Regeneración de plantas leñosas bajo arbustos aislados en un sector de los esteros del Iberá, Corrientes, Argentina, implicâncias etnoecológicas. Bonplandia, 25, 103-114. www.jstor.org/stable/26413081.

Kozlowski, T.T., 1997. Responses of woody plants to flooding and salinity. Tree Physiol. Monogr. 1, 1-29. https://doi.org/10.1093/treephys/17.7.490.

Larré, C.F., Fernando, J.A., Marini, P., Bacarin, M.A., Peters, J.A., 2013. Growth and chlorophyll a fluorescence in *Erythrina crista-galli* L. plants under flooding conditions. Acta Physiol Plant. 35,1463-1471. https://doi.org/10.1007/s11738-012-1187-4.

Leck, M., Parker, V., Simpson, R., 2008. Seedling Ecology and Evolution, Cambridge University Press, Cambridge.

Liu, B., Rennenberg, H., Kreuzwieser, J., 2015. Hypoxia affects nitrogen uptake and distribution in young poplar (*Populus* × *canescens*) trees. PloS one. 10, e0136579. https://doi.org/10.1371/journal.pone.0136579.

Lorenzi, H., 2009. Árvores brasileiras: manual de identificaçâo e cultivo de plantas arbóreas nativas do Brasil, primeira ed. vol. 3, Instituto Plantarum, São Paulo.

Lucas, C.M., Mekdeçe, F., Nascimento, C.M., Holanda, A.S.S., Braga, J., Dias, S., Souza, S., Rosa, P., Suemitsu, C., 2012. Effects of short-term and prolonged saturation on seed germination of Amazonian floodplain forest species. Aquat Bot. 99,4 9-55. https://doi.org/10.1016/j.aquabot.2012.02.004.

Malik, A.I., Colmer, T.D., Lambers, H., Schortemeyer, M., 2001. Changes in physiological and morphological traits of roots and shoots of wheat in response to different depths of waterlogging. Aust. J. Plant Physiol. 28, 1121-1131. https://doi.org/10.1071/PP01089.

Marcílio, T., Barbeiro, C., Firmino, T.P., Romagnolo, M.B., de Souza, L.A., Pastorini, L.H., 2019. Flooding and submersion-induced morphological and physiological adaptive strategies in *Lonchocarpus cultratus*. Aquat Bot. 159, 103146. https://doi.org/10.1016/j.aquabot.2019.103146.

McCready, R.M., Guggols, J., Silveira, V., Owens, H.S., 1950. Determination the starch and amylose in vegetables: applications to pea. Anal. Chem. 22, 1156-1158. https://doi.org/10.1021/ac60045a016.

Oliveira, V.C, Joly, C.A., 2010. Flooding tolerance of *Calophyllum brasiliense* Camb. (Clusiaceae): morphological, physiological and growth responses. Trees–Struct Funct, 24,185-193. https://doi.org/10.1007/s00468-009-0392-2.

Oliveira, A.C.S, Martins, G.N., Silva, R.F., Vieira, H.D., 2015. Testes de vigor em sementes baseados no desempenho de plântulas. InterSciPlace1, 1-21.

Parolin, P.D., De Simone, O., Haase, K., Waldhoff, D., Rottenberger, S., Kuhn, U., Junk, W.J., 2004. Central Amazonian floodplain forests: tree adaptations in a pulsing system. Bot. Rev. 70, 357-380. https://doi.org/10.1663/00068101(2004)070[0357:CAFFTA]2.0.CO;2.

Parolin, P., Ferreira, L., Junk, W., 2003. Germination characteristics and establishment of trees from central Amazonian flood plains. Trop. Ecol. 44, 157–169.

Patel, P.K., Singh, A.K., Tripathi, N., Yadav, D., Hemantaranjan, A., 2014. Flooding: abiotic constraint limiting vegetable productivity. Adv. Plants Agric. Res. 1, 00016. doi:10.15406/apar.2014.01.00016.

Paz, A.A., Ribeiro, C., Azevedo, A.A., de Lima, E.R., da Silva Carmo, F.M., 2017. Induced flooding as environmental filter for riparian tree species. Envir. Exp. Bot. 139, 31-38. https://doi.org/10.1016/j.envexpbot.2017.03.017.

Peng, Y.J., Dong, Y.M., Tu, B.K., Zhou, Z.X., Zheng, B., Luo, L.W., Shi, C.M., Du, K.B., 2013. Roots play a vital role in flood-tolerance of poplar demonstrated by reciprocal grafting. Flora 208, 479–487. https://doi.org/10.1016/j.flora.2013.08.001.

Peng, Y., Zhou, Z., Tong, R., Hu, X., Du, K., 2017. Anatomy and ultrastructure adaptations to soil flooding of two full-sib poplar clones differing in flood-tolerance. Flora 233, 90-98. https://doi.org/10.1016/j.flora.2017.05.014.

Perata, P., Armstrong, W., Voesenek, L.A.C.J., 2011. Plants and flooding stress. New Phytol. 190:269–273. https://doi.org/10.1093/aob/mci205.

Perez-Harguindeguy, N., Diaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., ... & Urcelay, C., 2016. Corrigendum to: new handbook for standardised measurement of plant functional traits worldwide. Aust. J. Bot. 64, 715-716. https://doi.org/10.1071/BT12225_CO.

Puiatti M., Sodek L., 1999. Waterlogging affects nitrogen transport in the xylem of soybean. Plant Physiol. Biochem. 37, 767-773. https://doi.org/10.1016/S0981-9428(00)86690-5.

Rasband, W.S., 2016. ImageJ. U. S. National Institutes of Health. Bethesda, Maryland, USA, 1997–2016. https ://image j.nih.gov/ij/. (Accessed August 2019).

Reboita, M.S., Kruche, N., 2018. Provisional climatological normals from 1991 to 2010 for Rio Grande RS. Rev. Bras. Meteorol. 33, 165-179. http://dx.doi.org/10.1590/0102-7786331010.

Santos Junior, U.M., Gonçalves, J.F.C., Strasser, R.J., Fearnside, P.M., 2015. Flooding of tropical forests in central Amazonia: what do the effects on the photosynthetic apparatus of trees tell us about species suitability for reforestation in extreme environments created by hydroelectric dams? Acta Physiol. Plant. https://doi.org/10.1007/s11738-015-1915-7.

Shimamura, T., Momose, K., 2005. Organic matter dynamics control plant species coexistence in a tropical peat swamp forest. Proc. Roy. Soc. B–Biol. Sci. 272, 1503-1510. https://doi.org/10.1098/rspb.2005.3095.

Shimamura, T., Momose, K., Kobayashi, S., 2006. A comparison of sites suitable for the seedling establishment of two co-occurring species, *Swintonia glauca* and *Stemonurus scorpioides*, in a tropical peat swamp forest. Ecol. Res. 21, 759-767. https://doi.org/10.1007/s11284-006-0185-7.

Silva, A.D., Van Den Berg, E., Higuchi, P., Oliveira-Filho, A.D., 2007. Comparação florística de florestas inundáveis das regiões Sudeste e Sul do Brasil. Braz. J. Bot. 30, 257-269. http://dx.doi.org/10.1590/S0100-84042007000200010.

Smirnoff, N., Stewart, G.R., 1985. Nitrate assimilation and translocation by higher plants: comparative physiology and ecological consequences. Physiol. Plant. 64, 133-140. https://doi.org/10.1111/j.1399-3054.1985.tb02326.x.

Teixeira, A.D.P., Assis, M.A., Siqueira, F.R., Casagrande, J.C., 2008. Tree species composition and environmental relationships in a Neotropical swamp forest in Southeastern Brazil. Wetl. Ecol. Manag. 16, 451-461. https://doi.org/10.1007/s11273-008-9082-x.

Teixeira, A.P., Assis, M. A., Luize, B.G., 2011. Vegetation and environmental heterogeneity relationships in a Neotropical swamp forest in southeastern Brazil (Itirapina, SP). Aquat. Bot. 94, 17-23. https://doi.org/10.1016/j.aquabot.2010.10.001.

Tewari, S., Mishra, A., 2018. Flooding stress in plants and approaches to overcome. In: Ahmad, P., Ahanger, M.A., Singh, V.P., Tripathi, D.K., Alam, P., Alyemeni, M.N. (Eds.), Plant Metabolites and Regulation Under Environmental Stress. Academic Press, London, pp. 355 - 366.

Van Handel, E., 1968. Direct microdetermination of sucrose. Anal. Biochem. 22, 280-283. https://doi.org/10.1016/0003-2697(68)90317-5.

Velikova, V., Iordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci. 151, 59-66. https://doi.org/10.1016/S0168-9452(99)00197-1.

Viani, R.A.G., Vieira, A.O.S., 2007. Flora arbórea da bacia do rio Tibagi (Paraná, Brasil): Celastrales sensu Cronquist. Acta Bot. Bras. http://dx.doi.org/10.1590/S0102-33062007000200019.

Voesenek, L.A.C.J., Bailey-Serres, J., 2013. Flooding tolerance: O_2 sensing and survival strategies. Curr. Opin. Plant Biol. 16, 647-653. https://doi.org/10.1016/j.pbi.2013.06.008.

Voesenek, L.A., Bailey - Serres, J., 2015. Flood adaptive traits and processes: an overview. New Phytol. 206, 57-73. https://doi.org/10.1111/nph.13209.

Waechter, J.L., Jarenkow J.A., 1998. Composição e estrutura do componente arbóreo nas matas turfosas do Taim, Rio Grande do Sul. Biotemas 11, 45-69.

Wellburn, A.R., 1994. The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol.144, 307-313. https://doi.org/10.1016/S0176-1617(11)81192-2.

Wittmann, F., Junk, W. J., & Piedade, M. T. (2004). The várzea forests in Amazonia: flooding and the highly dynamic geomorphology interact with natural forest succession. Forest ecology and Management, 196, 199-212. https://doi.org/10.1016/j.foreco.2004.02.060.

Wittmann, F., Parolin, P., 2005. Aboveground Roots in Amazonian Floodplain Trees 1. Biotropica: The Journal of Biology and Conservation, 37, 609-619. DOI 10.1111/j.1744-7429.2005.00078.x.

Wittmann, F., Schongart, J., Montero, J.C., Motzer, T., Junk, W.J., Piedade, M.T.F., Queiroz, H.L., Worbes, M., 2006. Tree species composition and diversity gradients in white-water forestsacross the Amazon basin. J. Biogeogr. 33:1334–1347.https://doi.org/10.1111/j.1365-2699.2006.01495.x.

Yu, B., Zhao, C.Y., Li, J., Li, J.Y., Peng, G., 2015. Morphological, physiological, and biochemical responses of *Populus euphratica* to soil flooding. Photosynthetica 53, 110-117. https://doi.org/10.1007/s11099-015-0088-3.

Zanandrea, I., Alves, J.D., Deuner, S., de FP Goulart, P., Henrique, P.D.C., Silveira, N.M., 2010. Tolerance of *Sesbania virgata* plants to flooding. Aust. J. Bot. 57, 661-669. https://doi.org/10.1071/BT09144.

Zamith, L.R., Scarano, F.R., 2010. Restoration of a coastal swamp forest in southeast Brazil. Wetl. Ecol. Manag.18, 435-448. https://doi.org/10.1007/s11273-010-9177-z.

Zhang, L., Shao, H., 2013. Direct plant–plant facilitation in coastal wetlands: a review. Estuar. Coast. Shelf Sci.119, 1-6. https://doi.org/10.1016/j.ecss.2013.01.002.

Zhang, Q., Huber, H., Beljaars, S.J., Birnbaum, D., Best, S., Kroon, H., Visser, E.J., 2017. Benefits of flooding-induced aquatic adventitious roots depend on the duration of submergence: linking plant performance to root functioning. Ann. Bot. 120,171-180. https://doi.org/10.1093/aob/mcx049.

CAPÍTULO II

Germinação de sementes, crescimento e fluorescência da clorofila *a* em plantas jovens de *Allophylus edulis* em diferentes períodos de inundação

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Seed germination, growth and chlorophyll *a* fluorescence in young plants of *Allophylus edulis* in different periods of flooding

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Abstract

The distribution of woody species in flooded environments depends on the duration of stress as well as seed germination strategies and plant morphophysiological adaptations. *Allophylus edulis* is a tree that occurs in temporarily or permanently flooded areas in several countries of South America. In this paper, we evaluate seed germination, growth parameters, photosynthetic pigment contents and chlorophyll *a* fluorescence in young plants to understand the tolerance of the specie to flood events. The evaluations were performed in non-flooded (NFL) and flooded (FL) plants in a temporal scale that included short (up to 30 days) and long (up to 90 days) flood periods. A short flooding (15 days) may favor germination but maintaining stress for 60 days make the seeds unviable. Although 71.4% of the FL plants survived up to 90 days of flooding, injuries such as chlorosis and foliar abscission appeared. An increase in stem height and diameter was only observed in NFL plants, whereas FL plants showed a growth inhibition. At 90 days NFL and FL plants presented total dry mass of 18.35 ± 1.57g and 1.93 ± 0.62g, respectively. The photosynthetic performance indexes indicated acclimatization of the plants on the third day of flooding, but the stress induced a progressive decline in the parameters, signaling damages to the photosystem II. Both seeds and young plants of *A. edulis* tolerate short periods of flooding, but prolonged floods make the seeds unfeasible and damages the photosynthetic apparatus, leading to death of the plants.

Keywords: Stress, Flood acclimatization, Hypoxia, Sapindaceae, Recalcitrant seed

Introduction

Periodic or permanent floods exert strong selective pressure on the species, influencing aspects like the composition and structure of the tree flora in forest formations (Silva et al. 2007). Flooding causes a number of changes in the soil such as decreased or disappeared O_2, accumulation of CO_2, and formation of toxic compounds affecting various the processes of plant development (Kozlowski 1997).

Flood events are challenging in all life stages of the plant, especially during the seed germination and seedlings initial development (Zúñiga-feest et al. 2017). The seeds and seedlings of some species are not tolerant to flooding although adult plants of the same species tolerate stress (da Paz et al. 2017). The oxygen is less available to the embryos in flooded soils, preventing or delaying the germination of seeds of various species (Kozlowski 1997). To withstand flooding events, seeds may have morphological adaptations that improve their fluctuation (Parolin et al. 2004); physical dormancy (Lucas et al. 2012; da Paz et al. 2017); physiological dormancy (Scarano et al. 2003) and energy reserve compounds (Ferreira et al. 2009).

Flooding of the root system can cause a number of consequences to the plant such as increased shoot biomass to the detriment of the root (Liu et al. 2015; Yu et al. 2015), reduced or inhibited growth (Bailey-Serres and Voesenek 2008; Colmer and Voesenek 2009), decreased water and nutrient uptake (Colmer and Voesenek 2009; Liu et al. 2015), leaf fall (Parolin et al. 2004), minor chlorophyll content and decreased photosynthetic performance (Junior et al. 2015). The decrease in photosynthetic activity during flooding has been related to several factors such as decreased stomatal conductance (Medina et al. 2009), lower photosynthetic enzyme activities (Pezeshki 2001), lower chlorophyll content (Parolin et al. 2004), photosystem II damage and photosynthetic electron transport (Reginfo et al. 2005). The stress duration influences the damage to the structure and activity of the photosystem II and the development of photosynthetic adjustments in the plant (Larré et al. 2013).

The occurrence of some species in a predominantly flooded area is a strong but inconclusive indication of their tolerance, since some abiotic and/or biotic factors facilitate the occurrence of less tolerant species in these environments. In swamp forests, microtopographic changes (Teixeira et al. 2011; Gattringer et al. 2018) and positive interactions between plants (Scarano 2002) can attenuate the stress caused by flooding, favoring the germination process and the survival of species less tolerant or living in flood-prone areas.

Flooding events can last for up to seven months in a year in some swamp forests in the extreme south of Brazil. These forests are located on relief depressions where the ground water is close to the surface or emerging most of the year, with periods of high precipitation rates, configuring long flooding events (Waechter and Jarenkow 1998; Duarte 2012). These forests are characterized by great spatial variations in soil water conditions, containing flooded microenvironments (water line formation) and non-flooded microenvironments (humid soil) (Duarte 2012). The presence of arboreal individuals is noticeably greater in non-flooded sites, being *Allophylus edulis* (A.St.-Hil et al.) Hieron. ex Niederl one of the species possibly favored by these microenvironments.

Allophylus edulis is a pioneer tree distributed in several countries of South America, including diverse phytogeographic domains throughout Brazil (Abreu et al. 2005). The plantation of this species is recommended to accelerate forest restoration due to its rapid growth and high amount of fruits that attract dispersers of seeds from other species to the site (Turchetto et al. 2017). This species colonizes from open areas to forest formations subject to flooding, and it is more abundant in temporarily flooded forests than in those permanently flooded (Silva et al. 2007).

The species distribution in environments with different degrees of stress can be inferred through the knowledge about their level of tolerance to factors such as soil water conditions. Although important, this information is still scarce for subtropical and tropical tree species in southern South America (Zúñiga-feest et al. 2017), becoming an obstacle to restoration projects. This information is even more relevant if we consider the climate change scenario, which will increase the frequency of extreme weather events such as rainfall and drought, thereby influencing the hydrological regime in areas subject to flooding (Junk 2013; Parolin and Wittmann 2010). In the extreme south of Brazil, the climate change is associated with an increase in precipitations (Copertino et al. 2016), intensifying forest flood events on a spatial and temporal scale. Such changes may impact the structure and distribution of the tree species in flooded forests.

In this sense, we evaluated the tolerance of seeds and young plants of *Allophylus edulis* to short and long-term flooding. Our hypothesis was that seeds and young plants of the species would not tolerate a long period of flooding. Thus, short floods would reduce seed germination, growth, content of photosynthetic pigments and photosynthetic indexes (PIs) of young plants. While long periods of flooding cause plant death and seed unviability.

Material and methods

Fruits of various trees of *Allophylus edulis* were collected in a swamp forest located in the municipality of Rio Grande, RS, Brazil (32º07'S; 52º09'W). This forest remains flooded for approximately seven months per year, with a depth of about 30 cm of water, and the flood periods can be greater or lesser depending on the rainfall regime (Duarte 2012). The seeds for the experiments I and II were collected in November 2016, and in December 2015 for the experiment III. The fruits were pulped and the seeds were used shortly after the collection in the tests in order to maintain their viability, since the seeds of *A. edulis* have a recalcitrant behavior.

Experiment I: Seed germination in flooded and non-flooded conditions

This experiment consisted of two treatments using eight Gerbox® transparent plastic boxes with 25 seeds each, totaling 200 seeds per treatment: non-flooded (NFL) and flooded (FL), with seed sowing on a sheet of blotting paper moistened with 20 mL and 150 mL of distilled water, respectively, totaling approximately 2 cm of water depth in FL treatment.

The germination tests were carried out for 30 days in a BOD germination chamber under the temperature of 25 ºC and constant light, as recommended by Abreu et al. (2005). The criterion considered for germination was the presence of radicles larger than 2 mm in the seeds. The percentage of germination (%G) and germination speed index (GSI) were evaluated according to the equations described by Oliveira et al. (2009). At the end of the experiment the seedlings that were morphologically perfect had the measured length (cm) and were classified as normal seedlings (NS) and seedlings that had any of their essential structures missing, deformed or severely damaged were classified as abnormal (AS) (Brasil 2009; Oliveira et al. 2009).

Experiment II: Germination with relief from flood-related stress

The experiment II consisted of four tests with eight samples of 25 seeds, totaling 200 seeds per test. All tests were performed in Gerbox® boxes, using a layer of 2 cm of medium-textured, washed and sterilized sand (substrate). The germination tests followed the same criteria of germination, temperature and photoperiod established in experiment I. The seeds were submitted to four temporal levels of flood-related stress. In the control (time 0), the seeds were planted directly on the non-flooded sand, moistened with 20 mL of distilled water. In the other tests, to ensure submersion, the seeds were initially semi-buried in sand substrate and submitted to different flooding periods (15, 30, 60 days), characterizing different stress levels induced by flood duration. To this end, the Gerbox® boxes were filled with 150 mL of distilled water, maintaining a water depth about of 2 cm. At the end of each flooding period, the seeds were removed from the flood conditions (stress relief) and transferred to Gerbox®

boxes with the same conditions of the control treatment, in order to evaluate the germination parameters.

Experiment III: Development of young plants under flood conditions

The seedlings for this experiment were obtained from the germination of *A. edulis* seeds in 290 cm³ tubes. After 60 days, the seedlings were transplanted to 5-L perforated plastic pots containing organic soil obtained from the Federal University of Rio Grande. After seven months, the plants were submitted to two treatments: (I) non-flooded plants (NFL), irrigated daily to keep the substrate moist; (II) flooded (FL) plants, where the pots were placed into a second, to 10-L non-perforated plastic vessel to maintain a water slide of about 3 cm above the substrate. The experiment lasted for 90 days was and conducted in experimental area covered with shade screen that partially diminished the solar radiation incident on the plants (50% shading). Global solar radiation daily (mean ± SD), during 30, 60 and 90 days of flooding were, respectively, 953.30 ± 510.29 KJ/m², 1451.2 ± 569.35 KJ/m², and 1626 ± 681.39 KJ/m². The air temperature for the same periods were 14.9 ± 2,1ºC, 16.1 ± 2,45ºC and 19± 2,66ºC, respectively. Daily values of global solar radiation and air temperature were obtained by Automatic Meteorological Station of Rio Grande, RS, Brazil (32 ° 01'S 52 ° 06'W) distant about 2 km from area of flooding experiment and provided by National Institute of Meteorology (INMET). Climate of the region is classified as Cfa, being characterized as humid subtropical (Alvares et al. 2013).

To evaluate survival and growth, 35 plants of each treatment (NFL and FL) were selected and evaluated after seven different periods: 1, 10, 20, 30, 45, 60 and 90 days after the flooding started. The growth parameters analyzed were: plant height (cm), from the base of the plant until the meristematic apex insertion; number of leaves; and stem diameter (cm), measured at 2 cm above the ground with a pachymeter. Possible visual morphological alterations induced by the treatments, such as hypertrophied lenticels and adventitious roots, were weekly monitored.

At the end of the experiment, ten plants of each treatment were randomly selected to estimate leaf area and dry mass. The leaf area was obtained through the foliar contour method, scanning ten mature leaves of each plant (Benincasa 1988). To estimate the dry mass, the plants were separated into leaves, stem and root, individually stored in paper bags, oven-dried at 70 ° C for 48 h and weighed.

The concentrations of chlorophyll *a (Chl a)*, chlorophyll *b (Chl b),* total chlorophyll (Chl_{a+b}) and carotenoids (C_{x+c}) were determined from mature leaves of four plants of each treatment (NFL and FL) at 30, 60 and 90 days after the onset of flooding. The leaves were wrapped in aluminum foil and transported on ice to the laboratory, where a sample of each leaf was withdrawn and weighed. Subsequently, the samples were macerated with 80% (v/v) acetone (Arnon 1949) and filtered using Whatmann n1 filter paper, obtaining the ketone extract from chloroplast pigments. The absorbance readings were obtained from the ketone extracts at the wavelengths of 470, 646 and 663 nm. From the absorbance and dilution factors, the pigment contents were quantified and the results expressed in microgram per gram of fresh mass of leaf tissue (μg pigment g^{-1} MF), according to the equations described by Wellburn (1994).

The fluorescence of chlorophyll *a* was measured 1, 3, 10, 20, 30, 45 and 60 days after flooding, using 18 plants of each treatment. In each collection, the central leaflets of two mature leaves of each plant were measured, totaling 36 measurements in each water regime (NFL and FL). The emissions of chlorophyll *a* fluorescence emission were measured using a portable fluorometer Handy PEA (Hansatech Intruments). The leaves were adapted to the dark for 90 min and exposed to a saturation pulse of 3,000 μmol $m^{-2}s^{-1}$of photons for 1s after that period. The fluorescence intensity of chlorophyll *a* was measured for 50 μs (initial fluorescence, F_0), 2 ms (F_J), 30 ms (F_I) and maximum fluorescence (F_M). The parameters for the JIP test (Strasser and Strasser 1995; Strasser et al. 2004) were then calculated and are described in Table 1. The values of the JIP test parameters were normalized in relation to the control plants (NFL) levels in each period of data collection.

Statistical analyses

The effects of the water conditions (NFL and FL) on the germination parameters (%G, GSI, AS) of experiment I were evaluated with the *t* test. The effect of the different flood periods on the germination parameters (%G, GSI, AS) of experiment II was evaluated with an analysis of variance (ANOVA) followed by the Tukey‟s test. The effects of water conditions (NFL and FL) in different evaluation periods on plant growth (stem diameter, number of leaves and height) were tested with two-way ANOVA with repetitive measures. The differences in the pigment content of the plants were obtained with a two-way ANOVA. Leaf area, dry mass, PI_{abs} and PI_{total} in different flood regimes (NFL and FL) were evaluated using a *t* test. The percentage data (%G and AS) and dry mass were transformed using, respectively, arcsine and square root of x, in order to achieve normality and homoscedasticity. All other data presented normal and homoscedastic distribution and all the statistical tests followed Zar (1999) with a level of significance of 0.05.

Results

Seed germination in flood conditions and relief of flood-related stress

The flood reduced the percentage of germinated seeds and the GSI (Table 2). In addition, seeds germinated in the FL treatment produced smaller NS and a high number of abnormal seedlings (Fig 1a, Table 2), with short hypocotyls and atrophied radicles (Fig. 1b). The germination began on the sixth day after sowing and the germination rate was high in the control test (NFL). The stress relief after 15 days of flooding resulted in germination on the third day, with high germination rate, high GSI and larger normal seedlings, indicating greater germinative vigor. The potential to germinate continued after 30 days of flooding, with seeds germinating on the fifth day after removed from the flood and transferred to a humid substrate. In spite of this, the germination rate and GSI decreased and the number of abnormal seedlings increased. After 60 days of flooding, the seeds became non-viable (Table 2).

Survival and growth of young plants

Plants of *A. edulis* from the NFL treatment had an increase of approximately 67% in stem diameter at 90 days (Fig. 2a). In contrast, the stem growth of plants in the FL treatment was inhibited, lacking morphoanatomical adaptations such as adventitious roots, hypertrophied lenticels, cortical cracks and thickening of the stem due to the formation of aerenchymal tissue, which are normally observed in species tolerant to flooded environments. NFL plants had a progressive increase in height,48.16% larger at the end of the experiment, differing from the FL plants (Fig. 2b). The flooding induced a stoppage in plant growth, and the plant height measurements on the first day did not differ from the measure after 90 days of flooding. In the NFL plants, after 90 days of flooding, the number of leaves doubled in relation to the first day (Fig. 2c). There was a leaf fall in the FL treatment after 20 days of flooding, followed by a small increase in the number of leaves at 30 days and another fall after 90 days of flood, when we verified a leaf number 26.3% lower than the at the first day of flood.

After 90 days of experiment, all NFL plants were still alive, unlike plants undergoing FL treatment, which showed a mortality of 28.57%. The surviving plants of the FL treatment had severe stress symptoms such as leaf chlorosis and withered leaves (Fig. 1c) as well as lower dry mass for both shoot and root (Fig. 1d). The flood caused a reduction in leaf area, so that FL plants had leaves about six times smaller than the NFL plants (Table 3*)*. The values of

Chl *a*, Chl *b* and Chl_{a+b} were lower in FL plants at 60 days of flooding and remained stable (Fig. 3).

Chlorophyll a fluorescence transient

After 30 and 60 days, there was an increase in the flux of light energy absorption (ABS/RC) and captured energy flow (TR_0/RC) per reaction center in the plants submitted to FL treatment. Additionally, there was an increase in the dissipated energy flow (DI_0/RC) reaching values of 76% (30 days) and 61% (60 days) in relation to the plants submitted to the NFL treatment (control) (Fig. 4). For the ET_0/RC and RE_0/RC parameters, there was no discrepancy between FL and NFL (control) treatments. We verified a tendency of reduced ET_0/TR_0 (Fig. 4) levels in conditions of flood, indicating a lower probability that the captured exciton moves electrons for the CTE besides the Q_A^-. This can be confirmed by the decline in the values of the parameters associated with the quantum electron transport yield of Q_A^-. (i) for the electron acceptors of the intersystem (ET_0/ABS); and (ii) for the final electron acceptor of photosystem I (FSI) (RE_0/ABS). The maximum photochemical quantum yield (TR_0/ABS or F_V/F_M) did not suffer influence of the stress duration, with values close to control levels throughout all periods evaluated.

After the first day of flooding, there was a reduction in the performance indexes PI_{abs} (33%) and PI_{total} (35%) in relation to the control treatment. After three days, the NFL (control) and FL plants resembled in relation to PI_{abs} and PI_{total}. However, on the tenth day of evaluation, FL treatment plants showed a significant drop in the value of the PI_{abs} that remained in decline until 60 days of flooding. In the same way, there was a progressive decrease in the PI_{total} as the flooding time increased. After 30 and 60 days of flooding, the plants had the values of PI_{abs} and PI_{total} reduced in more than 50% in relation to the control (Figs. 4 and 5).

Discussion

Allophylus edulis plants tolerate flooding for a short period, maintaining the seed germination capacity and inhibiting the growth of young plants, though both stages are sensitive to prolonged flooding. These characteristics, associated to the efficient dispersion mechanism, justify the wide distribution of the species in temporarily flooded areas (Silva et al. 2007; Silva et al. 2012).

The success in establishing a species in flooded areas depends initially on the strategy of fruit dispersal associated with factors that allow seed germination and seedling survival during flooding (Marques and Joly 2000). The dispersal of *A. edulis* fruits can occur by zoochory, hydrochory and autochory. Zoochoric dispersal is promoted especially by birds (Abreu et al. 2005; Gagetti et al. 2016) and hydrochoric dispersion occurs due to the frequent location of the specie near watercourses (Silva et al. 2007), contributing to that seeds are dispersed in places that experience a wide variety of hydrological conditions.

Some seeds show great success in germination after flooding (Ferreira et al. 2006; Wittmann et al. 2007) and others maintain viability after a long period of submersion (Scarano and Crawford 1992; Parolin and Wittmann 2010). However, for most species stress has a negative effect causing loss of viability (Kozlowski 1997; Okamoto and Joly 2000). In our study, we verified that some seeds started germination under flooding conditions, forming abnormal seedlings or small size normal seedlings which decreases the chances of establishment in the soil. The fluctuation of seed may have exercised a positive effect on germination under flood conditions. There is variation in seed size of *A. edulis* (Abreu et al. 2005) what can have an effect on both seed fluctuating capacity and on the content of reserves accumulated. In swamp forests submerged seeds are exposed to an environment with less availability oxygen and light due to turbid waters. In experiment II, when the seeds were half-buried and therefore prevented from floating the start of germination occurred only when the seeds were transferred to the non-flooded treatment. Submersion possibly induced the physiological dormancy in seed, postponing germination until conditions were favorable. The seed dormancy is an ecologically advantageous response in flood prone habitats (Scarano et al. 2003).

The stress relief test showed that the germination of *A. edulis* seeds can be favored after a short flood period, since seeds flooded for 15 days and subsequently transferred to the non-flooded treatment had high germination rate, higher germination speed producing also more vigorous seedlings. Other studies have shown that short-term soaking in water increases the germination of forest species seeds (Parolin et al. 2003; Lucas et al. 2012; Conserva et al. 2018). Soaking seeds in water probably breaks the physical barriers imposed on germination by softening the coating and favoring the imbibition process (Lucas et al. 2012). The soaking treatment for 15 days may have promoted the imbibition of *A. edulis* seeds. Thus when the seeds were transferred to the non-flooded substrate they germinated faster.

The seeds of *A. edulis* have a thin integument (Abreu et al. 2005) and imbibition is generally rapid in seeds with permeable coating (Baskin and Baskin 2014). After the germination process has started, the as maintenance of seeds in hypoxic conditions possibly caused gradual damage to embryonic tissue and consequent loss of germination capacity. In addition, other characteristics of *A. edulis* seed such as relatively small size, absence of endosperm and foliar cotyledons (Abreu et al. 2005) indicate that the seeds store low energy reserves. Thus the decline in germination rate may also be linked to the depletion of energy resources to maintain fermentative metabolism. Under low humidity conditions *A. edulis* seeds also lose viability as they are recalcitrant (José et al. 2007). This type of seed has a strong tendency to accumulate soluble sugars, especially sucrose (Berjak and Pammenter 2007) which is an immediate use reserve for energy production (Buckeridge et. al. 2004). Thus, the rapid germination and formation of seedlings when conditions become favorable may be associated with the presence of these sugars. In addition, soluble sugars may have exerted an osmoprotective effect while the seeds were flooded, maintaining cell turgidity, membrane stability and preventing protein degradation (Tewari and Mishra 2018).

In southern Brazil, the ripening of *A. edulis* fruits occurs at the end of the flooded phase, when the water level is falling. Thereby, many seeds must go through a brief period of flooding and begin germination while floating or as soon as conditions of normoxia are reestablished. The germination in the hydrological transition period is beneficial as it prevents the seed from being exposed to extreme flood or dry conditions. The dry phase is characterized by high temperatures and high evaporation (Reboita and Kruche 2018) rates that can be as detrimental to seed germination as a long period of flooding.

Given that plants with recalcitrant seeds do not form seed banks in the soil (Barbedo and Marcos Filho 1998), it is important to invest in seeds with rapid germination to form a seedling bank (Berjak and Pammenter 2007). This investment becomes even more important in floodable forests that offer a narrow window for seedling regeneration (Parolin et al .2003; Wittmann et al. 2007). Furthermore, there is an association between functional type of cotyledon and seed germination speed, being that seedlings with foliar cotyledon develop faster because they have limited energy reserves (Parolin et al. 2003). The rapid germination and emergence of foliar cotyledons when flooding ceases probably maximizes photosynthetic activity and growth seedling in the non-flooded period (Parolin et al. 2003; Conserva et al. 2018).

According to Joly and Crawford (1982), a plant can be considered flood tolerant when it is able to grow and increase its biomass under flood conditions. In our study, we verified that the young plants of *A. edullis* respond to flood conditions with changes in the energy flow of the photosynthetic electron transport chain (ETC), reduction in plant size and biomass, and leaf fall, which indicate reduced tolerance to prolonged flood. The hypoxic and/or anoxic conditions imposed by the flood affect the growth of sensitive plants, as the water and nutrient uptake is compromised and the stomatal conductance of the leaves and photosynthetic capacity are reduced (Batista et al. 2008; Yu et al. 2015; Bidalia et al. 2018). A deviation from aerobic to anaerobic root system metabolism also occurs under these conditions, resulting in a reduced energy efficiency (Drew 1997). To compensate low energy yields, root cells increase their demand for carbohydrates, reducing their availability to other plant functions (Bailey-Serres and Voesenek 2008). Thus, it is common for plants to respond to flooding by inhibiting their growth or reducing their biomass (Andrade et al. 1999; Batista et al. 2008; Peng et al. 2017; Bidalia et al. 2018).

Some plants are able to grow vigorously during long periods of flooding (Kolb and Joly 2009). In general, species that present high flood tolerance tend to inhibit growth when stress begins and return to growth after a period of acclimatization (Larré et al. 2013; Zúñiga-Feest et al. 2017). During this period, the plant performs metabolic (Joly and Crawford 1982; Alves et al. 2013; Larré et al. 2016) and photosynthetic (Medina et al. 2009; Larré et al. 2013) adjustments, developing morphoanatomical structures that allow the plant to survive and thrive in conditions of water saturation (Medina et al. 2009; Oliveira and Joly 2010; Larré et al. 2013). In this context, the formation of hypertrophied lenticels, cortical cracks and adventitious roots are common morphological responses that confer advantage to the species (Zhang et al. 2017). Although individuals withstand a long period of flooding, the plants of *A. edulis* did not develop any morphological changes that could help the plant survive the anoxia conditions. Stress conditions were signaled by injury symptoms, such as chlorosis, loss of leaf mass and reduced growth.

The flood may compromise photosynthesis through stomatal and non-stomatal limitations, decreasing the photosynthetic capacity due to a reduction in chlorophyll concentrations, carboxylation efficiency or electron transport rates (Gravatt and Kirby 1998; Herrera et al. 2008; Larré et al. 2013; Polacik and Maricle 2013; Junior et al. 2015). The distinction between stomatal and non-stomatal limitations is important, since the stomatal closure is a transient regulatory response that can be reverted when stressful conditions cease, whereas the other responses reflect permanent changes (Herrera et al. 2008).

The plants of the FL treatment showed a reduction in chlorophyll content (Fig. 3) and changes in JIP-test parameters (Fig. 4), which reflect the energy flux in the photosynthetic ETC, signaling that long-term flooding promotes a decrease in photosynthetic capacity. The reduction in pigment contents is a typical symptom of stress due to oxidative processes in the chloroplast, resulting in slow synthesis or rapid breakdown of pigments (Smirnoff 1993). Some species with a greater tolerance to flooding do not present a reduction in their content of photosynthetic pigments (Junior et al. 2015), even increasing their pigment content in response to flooding (Parolin 2001). However, many plants, classified as tolerant or sensitive to flooding, show a reduced chlorophyll content in water saturation conditions (Junior et al. 2015; Kozlowski and Pallardy 2002). During flooding, metabolic adjustments can occur, reducing the chlorophyll content in the leaves, resulting in a lower absorption of light energy by leaf area to protect PSII from photo-oxidation (Du et al. 2012).

In our study, the flood increased the absorption flux (ABS/RC) by the antenna pigments, as well as the captured energy flow (TR_0/RC), mainly after the first day, 30 and 60 days of flooding. The decrease in the ET_0/TR_0, ET_0/ABS and RE_0/ABS parameters show a lower electron transfer to the ETC (Fig. 4). This can lead to the inactivation of a fraction of the PSII reaction centers, converting them into non-reducing centers of Q_A^- resulting in an increase in the mean antenna size by active RC (Junior et al. 2015). Consequently, an increase in the energy flux dissipated by PSII reaction center (DI_0/RC) was triggered. The mechanism of dissipating energy in the form of heat or fluorescence may have been employed by *A. edulis* plants as a measure to avoid photoinhibition and consequent damage to the photosystems.

Substantial changes in the electron transport per active reaction center were noted with flooding. In general, the performance indexes (PI_{abs} and PI_{total}), energy flow parameters per reaction center (ABS/RC, TR_0/RC and DI_0/RC) and parameters related to quantum yields (ET_0/TR_0, ET_0/ABS and RE_0/ABS) show increased stress on the first day of flood. However, the stress was followed by an acclimatization mechanism after the third day, when FL treatment plants showed values similar to the control (Fig. 4 and 5). After this short period, the discrepancies between the NFL and flooded treatments increased as the flood time elapsed, culminating in low PI values at 30 and 60 days, which indicate damage to PSII. The maximum quantum yield of primary PSII photochemistry (TR_0/ABS or F_V/F_M), widely used to monitor environmental stress in plants (Oxborough and Baker 1997), was not an effective indicator in our study, which was also observed for other tree species under flood conditions (Larré et al. 2013; Martinazzo et al. 2013).

The distribution of species in flooded forests is regulated by the duration of flooding, their inherent mechanisms to tolerate flood-related stress (Bailey-Serres and Voesenek 2008), and the existence of microenvironments where stress is attenuated and the conditions become favorable for the plant development. In this way, species with different levels of flood tolerance may coexist in a flooded environment seasonally or permanently.

We verified positive germinative response in *A. edulis* seeds after a short period of flooding (15 days) and low mortality of young plants after a long period of flooding (90 days). However, the period of 30 days of flooding was sufficient to cause a sharp drop in seed germination and plant photosynthetic performance indexes (PIs). After 60 days of flooding, the seeds lost viability and the PIs remained low, signaling damage to the photosynthetic apparatus. At 90 days of flooding, the surviving plants presented serious evidence of injury caused by the flood. Thus, we conclude that the initial development of *A. edulis* is associated with environments with short periods of flooding, which explains the greater abundance of individuals of the species in temporarily flooded forests. The occurrence of the species in flooded forests for long periods is conditioned by the occurrence of microenvironments protected from long floods. This species is recommended for the restoration of flooded areas if planted where they are exposed to short periods of flooding.

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Tables

Table 1 The JIP-test parameters, formulas and definitions. Note that PSI, PSII, RC, and Q_A^- are for photosystem I, photosystem II, total number of active PSII reaction centers, and the first plastoquinone electron acceptor of PSII, respectively.

Parameters of fluorescence	Description
Extracted fluorescence parameters	
FO = F50μs (O)	Fluorescence intensity at 50 μs
FJ = F2ms (J)	Fluorescence intensity at 2 ms
FI = F30ms (I)	Fluorescence intensity at 30 ms
FP = FM (P)	Maximum fluorescence
F100μs	Fluorescence intensity at 100 μs
F300μs	Fluorescence intensity at 300 μs
Calculated parameters	
Fv = (FM - F50μs)	Variable fluorescence
VJ = (F2ms - F50μs)/(Fm - F50μs)	Relative variable fluorescence at 2 ms
M0 = [4.(F300μs - F50μs)/(Fm - F50μs)]	Net rate of PSII closure
Specific energy fluxes	
ABS/RC = (M0 /VJ) /φP0	Apparent antenna size of an active PSII
TR0/RC = M0 /VJ	Maximum trapped exciton flux per active PSII
ET0/RC = M0 /VJ × ψE0	The flux of electrons transferred from QA- to PQ per active PSII
DI0/RC = ABS/RC - TR0/RC	The flux of energy dissipated in processes other than trapping per active PSII
RE0/RC = RE0/RC = (M0/VJ) × ψR0	The flux of electrons transferred from QA- to final PSI acceptors per active PSII
Efficiencies and quantum yields	
TR0/ABS = φP0 = Fv/FM	Maximum quantum yield of primary PSII photochemistry
ET0/ABS = φE0 = φP0 × ψE0	Quantum yield of electron transport from QA- to PQ
ET0/TR0 = ψE0 = 1 – VJ	Efficiency with which a PSII trapped electron is transferred from QA- to PQ
RE0/ET0 = δR0 = ψR0/ψE0	Efficiency with which an electron from PQH2 is transferred to final PSI acceptors
RE0/ABS = φR0 = φP0 × ψR0	Quantum yield of electron transport from QA- to final PSI acceptors
Performance indexes	
PIABS = (RC/ABS) × [φP0/(1 – φP0)] × [ψE0/(1 – ψE0)]	Performance index on absorption basis
PIABS,total = PIABS × [δR0/(1 – δR0)]	Total performance index on absorption basis measured for FSI and electron acceptors

For review see Strasser et al. (2004)

Table 2 Average germination percentage, germination speed index (GSI), percentage of abnormal seedlings **(AS),** and total length of *Allophylus edulis* seedlings. Experiment I: seeds submitted to the non-flooded (NFL) and flooded (FL) regimes; Experiment II: germination after relief from stress by flooding periods of 15, 30 and 60 days (n = 8*,* mean ± SE).

Treatment	Germination (%)	GSI	**AS** (%)	Length of seedlings (cm)
Experiment I				
NFL	93.00 ± 1.96 a	2.33 ± 0.04 a	1.66 ± 1.15 a	5.46 ± 0.13 a
FL	32.00 ± 3.93 b	1.03 ± 0.29 b	35.27 ± 12.63 b	1.65 ± 0.39 b
Experiment II				
0 days (NFL)	84.50 ± 3.81 a	2.15 ± 0.10 a	3.33 ± 1.11 a	7.56 ± 0.34 a
15 days	92.50 ± 2.06 a	3.77 ± 0.14 b	8.06 ± 1.34 a	9.51 ± 0.21 b
30 days	62.00 ± 3.38 b	1.88 ± 0.14 a	22.64 ± 3.40 b	7.18 ± 0.34 a
60 days	0.00	-	-	-

Means followed by equal letters in the columns did not differ (P> 0.05) after *t* test (Experiment I) or Tukey's test (Experiment II).

Table 3 Mean (±SE; n=10) values of dry mass of the stem, leaves and root, total dry mass and leaf area of flooded and non-flooded young plants of *Allophylus edulis* after 90 days of flooding, and statistical values [t statistics and probability associated with a type I error (p)] obtained from the t test

	Flooded	Non-flooded	*t*	*p*
Dry leaf mass (g)	0.37 ± 0.06	6.55 ± 0.57	- 10.82	0.000001
Dry stem mass (g)	0.84 ± 0.11	5.36 ± 0.63	7.07	0.00001
Dry root mass (g)	0.73 ± 0.06	6.44 ± 0.87	6.59	0.00003
Total dry mass (g)	1.93 ± 0.62	18.35 ± 1.57	11.28	0.000001
Leaf area (mm^2)	172.0 ± 27.8	997.0 ± 53.69	-14.33	0.000001

Figures

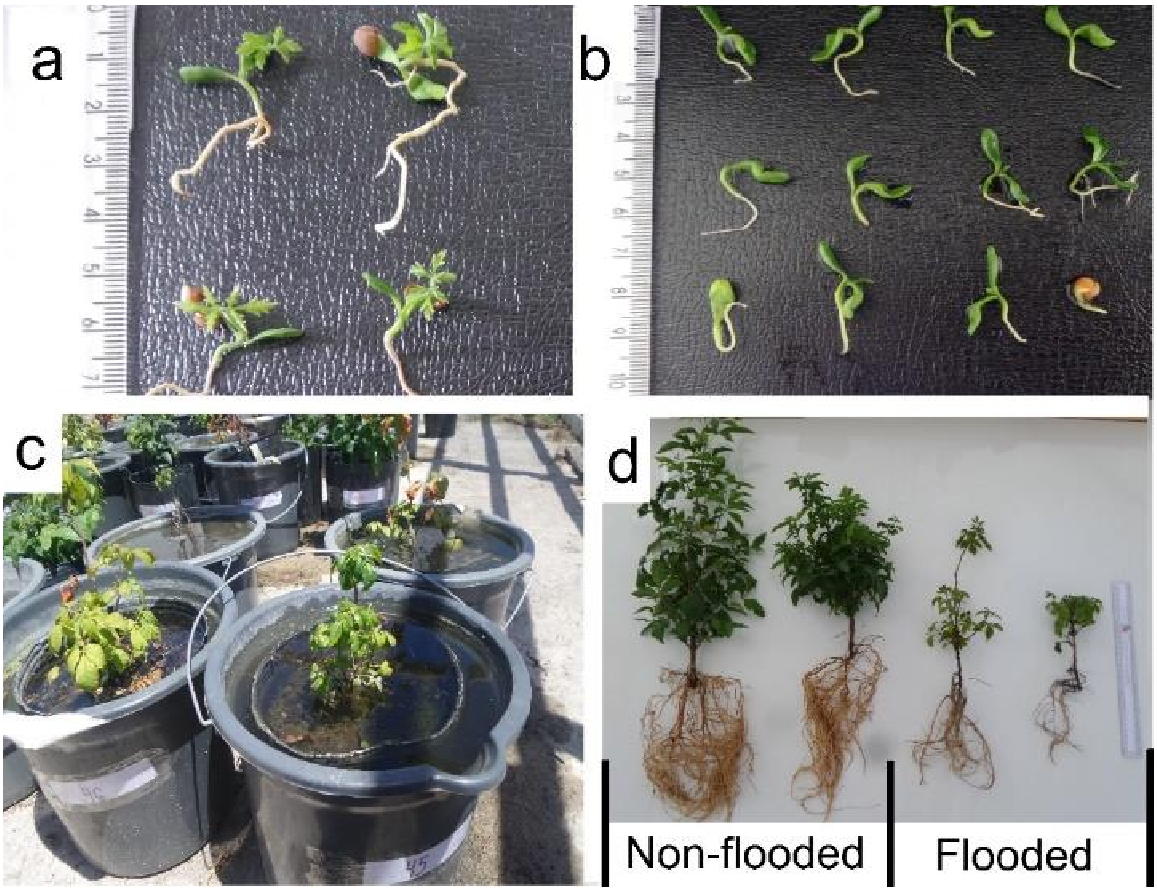

Fig. 1 Morphological aspects of seedlings and plants of *Allophylus edulis*. Normal seedlings originated from non-flooded treatment (a); abnormal seedlings originated from flooded treatment (b); young plants after 90 days of flooding presenting symptoms such as wilted leaves and chlorotic spots (c); non-flooded and flooded plants at 90 days (d)

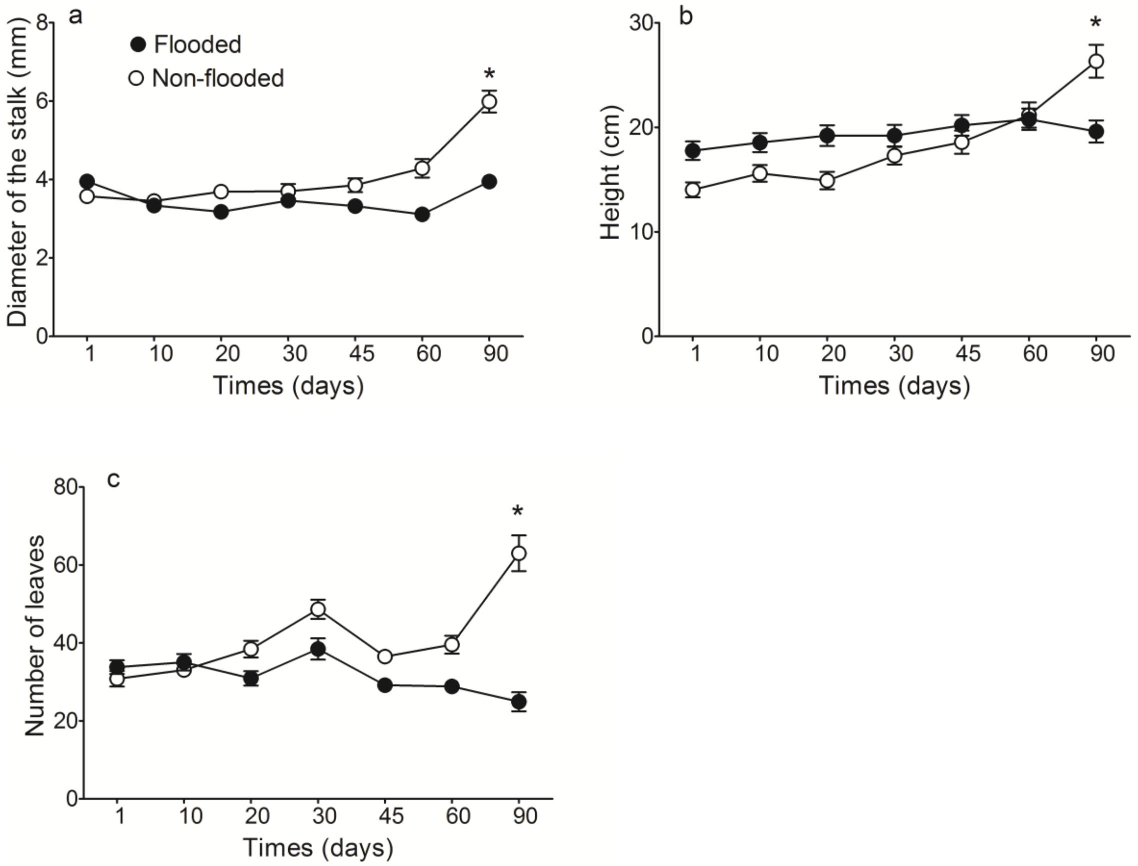

Fig. 2 Average stem diameter (a), stem height (b) and number of leaves (c) in young plants of *Allophylus edulis* submitted to seven flooding periods. ns: not significant, * $p<0.05$ by the Tukey‟s test (n = 35, mean ± SE)

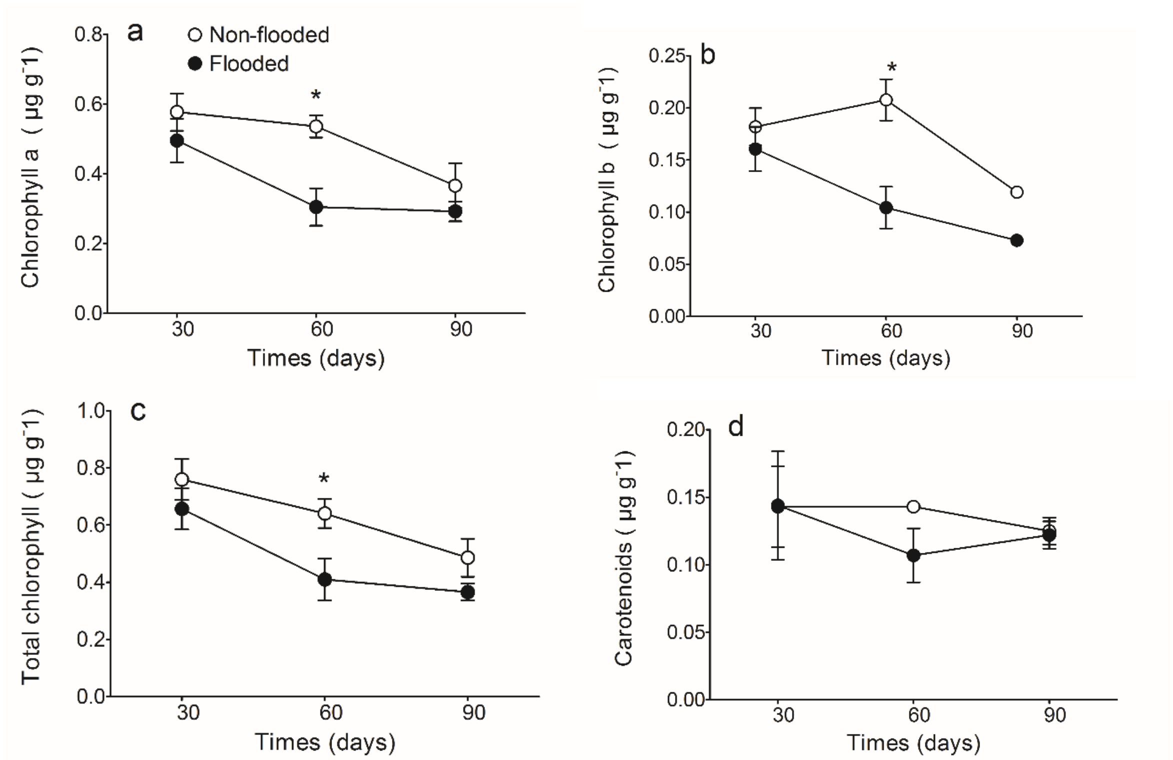

Fig.3 Average photosynthetic pigments of leaves of young *Allophylus edulis* plants in non-flooded (NFL) and flooded (FL) treatments during three periods of evaluation. * $p < 0.05$ by the Tukey‟s test (n=4, mean ± SE).

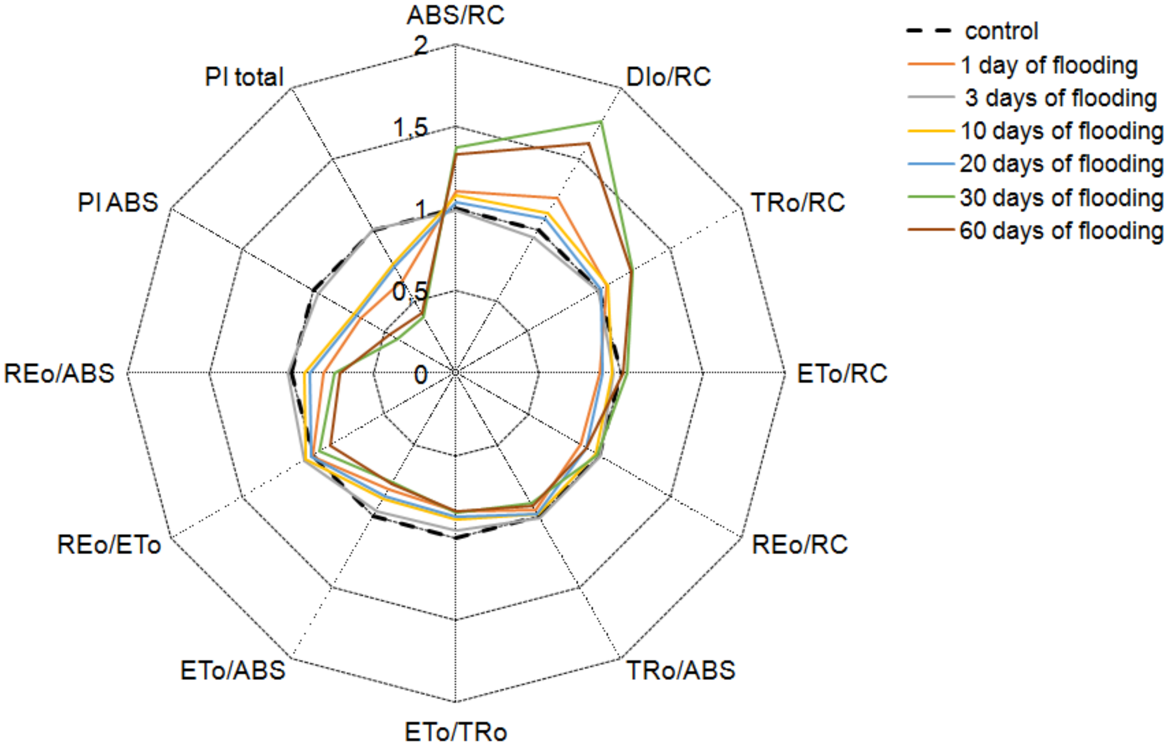

Fig. 4 Photosynthetic parameters obtained by JIP test and transient analysis of the chlorophyll a fluorescence in young plants of *Allophylus edulis* subjected to six flooding periods (1, 3, 10, 20, 30 and 60 days) (n = 18).

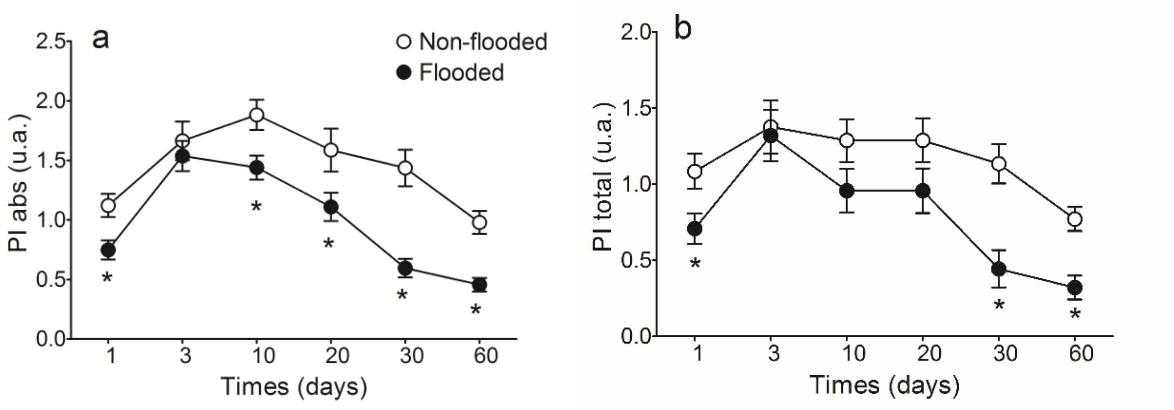

Fig. 5 Average performance index on the basis of absorption PI_{abs} (a) and total performance index PI_{Total} (b) of young plants of *Allophylus edulis* in non-flooded (NFL) and flooded (FL) treatments for different periods of evaluation. * $P < 0.05$ by the t test (n=18, mean ± SE)

References

Abreu DCA, Kuniyoshi YS, Nogueira AC, Medeiros A (2005) Caracterização morfológica de frutos, sementes e germinação de *Allophylus edulis* (St.-Hil.) Radlk. (Sapindaceae). Rev. bras. Sementes 27:59-66.

Alvares CA, Stape JL, Sentelhas PC, Moraes G, Leonardo J, Sparovek G (2013). Köppen's climate classification map for Brazil. Meteorol. Z. 22: 711-728.

Alves JD, Zanandrea I, Deuner, S, Goulart PFP, Souza KRD, Santos MO (2013) Antioxidative responses and morpho-anatomical adaptations to waterlogging in *Sesbania virgata*. Trees 27:717-728.

Andrade ACSD, Ramos FN, Souza AFD, Loureiro MB, Bastos R (1999) Flooding effects in seedlings of *Cytharexyllum myrianthum* Cham. and *Genipa americana* L.: responses of two neotropical lowland tree species. Rev. bras. Bot. 22:281-285.

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphrenol oxidase in *Beta vulgaris*. Plant Physiol 24:1-15.

Bailey-Serres J, Voesenek LACJ (2008) Flooding stress: acclimations and genetic diversity. Annu Rev Plant Biol 59:313-339.

Barbedo CJ, Marcos Filho J (1998) Tolerância à dessecação em sementes. Acta Bot. Bras.12:145-164.

Baskin CC, Baskin JM (2014) Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination. Academic Press, San Diego.

Batista CUN, Medri ME, Bianchini E, Medri C, Pimenta JA (2008) Flood tolerance in *Cecropia pachystachya* Trec. (Cecropiaceae): ecophysiological and morpho-anatomical aspects. Acta Bot Bras 22:91-98.

Benincasa MMP (2003) Análise de crescimento de plantas: noções básicas. Funep, Jaboticabal.

Berjak P, Pammenter NW (2007) From *Avicennia* to *Zizania*: seed recalcitrance in perspective. Ann. Bot. 101: 213-228.

Bidalia A, Okram Z, Hanief M, Rao KS (2018) Assessment of tolerances in *Mitragyna parvifolia* (Roxb.) Korth and *Syzygium cumini* Keels. seedlings to waterlogging. Photosynthetica 56: 707-717.

Brasil, Ministério da Agricultura, Pecuária e Abastecimento (2009). Regras para análise de sementes. MAPA, Brasília.

Buckeridge MS, Aidar MPM, Santos HP, Tiné MAS (2004) Acúmulo de reservas. In: Ferreira AG, Borghetti F (eds) Germinação: do básico ao aplicado, Artmed, Porto Alegre, pp 31-50.

Colmer T, Voesenek L (2009) Flooding tolerance: suites of plant traits in variable environments. Funct Plant Biol 36:665-681.

Conserva A, Camargo JLC, Santana DG, Piedade MTF (2018) Germinative behaviour of ten tree species in white-water floodplain forests in central Amazonia. Folia Geobot 53: 89-101.

Copertino MS, Creed JC, Lanari MO, Magalhães K, Barros K, Lana PC, Sordo L, Horta PA (2016) Seagrass and submerged aquatic vegetation (VAS) habitats off the coast of Brazil: state of knowledge, conservation and main threats. Braz. J. Oceanogr. 64: 53-80.

da Paz AA, Ribeiro C, Azevedo AA, Lima ERD, Carmo FMDS (2017) Induced flooding as environmental filter for riparian tree species. Environ Exp Bot 139: 31-38.

Drew MC (1997) Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. Annu Rev Plant Physiol Plant Mol Biol 48:223-250.

Du K, Xu L, Wu H, Tu B, Zheng B (2012) Ecophysiological and morphological adaption to soil flooding of two poplar clones differing in flood-tolerance. Flora 207:96-106.

Duarte CI (2012) Influência da variação hidrológica e da luminosidade na composição e estrutura do componente herbáceo em uma floresta paludosa no extremo sul do Brasil. Dissertation, Universidade Federal do Rio Grande.

Ferreira CS, Piedade MTF, Bonates LC (2006) Germinação de sementes e sobrevivência de plântulas de *Himatanthus sucuuba* (Spruce) Wood em resposta ao alagamento nas várzeas da Amazônia Central. Acta Amaz 36:413-418.

Ferreira CS, Piedade MTF, Tiné MAS, Rossatto DR, Parolin P, Buckeridge MS (2009) The role of carbohydrates in seed germination and seedling establishment of *Himatanthus sucuuba*, an Amazonian tree with populations adapted to flooded and non-flooded conditions. Ann. Bot. 104:1111-1119.

Gagetti BL, Piratelli AJ, Piña-Rodrigues FCM (2016) Fruit color preference by birds and applications to ecological restoration. Braz J Biol 76:955-966.

Gattringer JP, Ludewig K, Harvolk-Schöning S, Donath TW, Otte A (2018) Interaction between depth and duration matters: flooding tolerance of 12 floodplain meadow species. Plant Ecol 219:973-984.

Gravatt DA, Kirby CJ (1998) Patterns of photosynthesis and starch allocation in seedlings of four bottomland hardwood tree species subjected to flooding. Tree Physiol 18:411-417.

Herrera A, Tezara W, Marín O, Rengifo E (2008) Stomatal and non-stomatal limitations of photosynthesis in trees of a tropical seasonally flooded forest. Physiol Plant 134:41-48.

Joly CA, Crawford RMM (1982) Variation in tolerance and metabolic responses to flooding in some tropical trees. J Exp Bot 33:799–809.

José AC, Silva EA, Davide AC (2007) Classificação fisiológica de sementes de cinco espécies arbóreas de mata ciliar quanto a tolerância à dessecação e ao armazenamento. Rev. bras. sementes 29: 171-178. http://dx.doi.org/10.1590/S0101-31222007000200023.

Junior UMS, Gonçalves JFC, Strasser RJ, Fearnside PM (2015) Flooding of tropical forests in central Amazonia: what do the effects on the photosynthetic apparatus of trees tell us about species suitability for reforestation in extreme environments created by hydroelectric dams? Acta Physiol Plant 37:166.

Junk WJ (2013) Current state of knowledge regarding South America wetlands and their future under global climate change. AquatSci 75:113-131.

Kolb RM, Joly CA (2009) Flooding tolerance of *Tabebuia cassinoides*: metabolic, morphological and growth responses. Flora 204:528-535.

Kozlowski TT (1997) Responses of woody plants to flooding and salinity. Tree Physiol https://doi.org/10.1093/treephys/17.7.490.

Kozlowski TT, Pallardy SG (2002) Acclimation and adaptive responses of woody plants to environmental stresses. Bot Rev 68:270-334.

Larré C, Leivas-Moraes C, Borella J, Amarante L, Deune S, Peters JA (2016) Antioxidant activity and fermentative metabolism in the plant *Erythrina crista-galli* L. under flood conditions. Semina: Ciênc Agrár 37:567-580.

Larré CF, Fernando JA, Marini P, Bacarin MA, Peters JA (2013) Growth and chlorophyll a fluorescence in *Erythrina crista-galli* L. plants under flooding conditions. Acta Physiol Plant 35:1463-1471.

Liu B, Rennenberg H, Kreuzwieser J (2015) Hypoxia Affects Nitrogen Uptake and Distribution in Young Poplar (*Populus* × *canescens*) Trees. PLoS ONE 10(8):e0136579.

Lucas CM, Mekdeçe F, Nascimento CM. Holanda ASS, Braga J, Dias S, Souza S, Rosa P, Suemitsu C (2012) Effects of short-term and prolonged saturation on seed germination of Amazonian floodplain forest species. Aquat Bot 99:49-55.

Marques M, Joly CA (2000) Germinação e crescimento de *Calophyllum brasiliense* (Clusiaceae), uma espécie típica de florestas inundadas. Acta Bot. Bras. 14:113-120

Martinazzo EG, Perboni AT, Oliveira PV, Bianchi VJ, Bacarin MA (2013) Atividade fotossintética em plantas de ameixeira submetidas ao déficit hídrico e ao alagamento. Cienc Rural 43:35-41.

Medina CL, Sanches MC, Tucci MLS, Sousa CA, Cuzzuol GRF, Joly CA (2009) *Erythrina speciosa* (Leguminosae-Papilionoideae) under soil water saturation: morphophysiological and growth responses. Ann Bot 104:671-680.

Okamoto J, Joly CA (2000) Ecophysiology and respiratory metabolism during the germination of *Inga sessilis*(Vell.) Mart. (Mimosaceae) seeds subjected to hypoxia and anoxia. Braz J Bot 23:51-57.

Oliveira ACS, Martins GN, Silva RF, Vieira HD (2009) Testes de vigor em sementes baseados no desempenho de plântulas. InterSciencePlace 1:1-21.

Oliveira VC, Joly CA (2010) Flooding tolerance of *Calophyllum brasiliense* Camb. (Clusiaceae): morphological, physiological and growth responses. Trees 24:185-193.

Oxborough K, Baker NR (1997) Resolving chlorophyll *a* fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components-calculation of qP and Fv-/Fm-; without measuring Fo. Photosynth Res 54:135-142.

Parolin P (2001) *Senna reticulata*, a pioneer tree from Amazonian várzea floodplains. Bot Rev 67:239-254.

Parolin P, Ferreira LV, Junk WJ (2003) Germination characteristics and establishment of trees from central Amazonian flood plains. Trop. Ecol. 44: 157-169.

Parolin P, Wittman F (2010) Struggle in the flood: tree response to flooding stress in four tropical floodplain systems. AoB Plants https://doi.org/10.1093/aobpla/plq003.

Parolin P, De Simone O, Haase K, Waldhoff D, Rottenberger S, Kuhn U, Kesselmeier J, Kleiss B, Schmidt W, Piedade M et al (2004) Central Amazonian floodplain forests: tree adaptations in a pulsing system. Bot Rev 70: 357-380.

Peng Y, Zhou Z, Tong R, Hu X, Du K (2017) Anatomy and ultrastructure adaptations to soil flooding of two full-sib poplar clones differing in flood-tolerance. Flora 233: 90-98.

Pezeshki SR (2001) Wetland plant responses to soil flooding. Environ Exp Bot 46: 299-312.

Polacik KA, Maricle BR (2013) Effects of flooding on photosynthesis and root respiration in salt cedar (*Tamarix ramosissima*), an invasive riparian shrub. Environ Exp Bot 89:19-27.

Reboita MS, Kruche N (2018). Normais Climatológicas Provisórias de 1991 a 2010 para Rio Grande, RS. Rev. bras. meteorol. 33: 165-179.

Rengifo E, Tezara W, Herrera A (2005) Water relations, chlorophyll a fluorescence, and contents of saccharides in tree species of a tropical forest in response to flood. Photosynthetica 43: 203-210.

Scarano FR (2002) Structure, function and floristic relationships of plant communities in stressful habitats marginal to the Brazilian Atlantic rainforest. Ann Bot 90:517-524.

Scarano FR, Crawford RM (1992) Ontogeny and the concept of anoxia-tolerance: The case of the Amazonian leguminous tree Parkia pendula. J. Trop. Ecol. 8:349-352.

Scarano FR, Pereira TS, Rôças G (2003) Seed germination in Seed germination during floatation and seedling growth of Carapa guianensis, a tree from flood-prone forests of the Amazon. Plant Ecol 168: 291-296.

Silva AC, Higuchi P, Van den Berg E, Nunes MH, Carvalho DA (2012) Florestas Inundáveis: Ecologia, florística e adaptações das espécies, Editora UFLA, Lavras.

Silva AC, Van den Berg E, Higuchi P, Oliveira Filho AT (2007) Comparação florística de florestas inundáveis das regiões sudeste e sul do Brasil. Rev Bras Bot 30:257-269.

Smirnoff N (1993) The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol 125:27-58.

Strasser BJ, Strasser RJ (1995) Measuring fast fluorescence transients to address environmental questions: the JIP-test. In: Mathis P (ed) Photosynthesis: from light to biosphere. Kluwer Academic Publishers, Dordrecht, pp 977-980.

Strasser RJ, Tsimilli-Michael M, Srivastava A (2004) Analysis of the chlorophyll a fluorescence transient. In: Papageorgiou GC, Govindjee (eds). Chlorophyll a Fluorescence: A Signature of Photosynthesis. Advances in photosynthesis and respiration series. Springer, Dordrecht, pp 321-362.

Teixeira AP, Assis MA, Luize BG (2011) Vegetation and environment relationships in a Neotropical swamp forest in southeastern Brazil (Itirapina, SP). Aquat Bot 94:17-23.

Tewari S, Mishra A (2018) Flooding stress in plants and approaches to overcome. In: Ahmad P, Ahanger MA, Singh VP, Tripathi DK, Pravej Alam P, Alyemeni MN (Eds) Plant Metabolites and Regulation Under Environmental Stress, Academic Press, London, pp 356-366.

Turchetto F, Araujo MM, Callegaro RM, Griebeler AM, Mezzomo JC, Berghetti ÁL, Rorato DG (2017) Phytosociology as a tool for forest restoration: a study case in the extreme South of Atlantic Forest Biome. Biodivers Conserv 26:1463-1480.

Waechter JL, Jarenkow JA (1998) Composição e estrutura do componente arbóreo nas matas turfosas do Taim, Rio Grande do Sul. Biotemas 11:45-69.

Wellburn AR (1994) The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J Plant Physiol 144:307-313.

Wittmann AO, Piedade MTF, Parolin P, Wittmann F (2007) Germination of four low-varzea tree species in central Amazonia. Aquat Bot 86:197-203.

Yu B, Zhao CY, Li J, Li JY, Peng G (2015) Morphological, physiological, and biochemical responses of *Populus euphratica* to soil flooding. Photosynthetica 53:110-117.

Zar JH (1999) Biostatistical analysis. Prentice-Hall, Upper Saddle River.

Zhang Q, Huber H, Beljaars SJ, Birnbaum D, Best S, Kroon H, Visser EJ (2017) Benefits of flooding-induced aquatic adventitious roots depend on the duration of submergence: linking plant performance to root functioning. Ann Bot 120:171-180.

Zúñiga-Feest A, Bustos-Salazar A, Alves F, Martinez V, Smith-Ramírez C (2017) Physiological and morphological responses to permanent and intermittent waterlogging in seedlings of four evergreen trees of temperate swamp forests.Tree Physiol 37:779-789.

CONSIDERAÇÕES FINAIS E PERSPECTIVAS

As respostas da germinação de sementes e o crescimento de plantas de *Citronella gongonha* e *Allophylus edulis* refletem a sua distribuição em ambientes sujeitos a inundação. A coexistência dessas espécies em ambientes palustres é possivelmente determinada por suas estratégias de desenvolvimento afinadas as variações nas condições hidrológicas do solo.

As sementes de *C. gongonha* não germinam sob inundação, perdendo a capacidade germinativa conforme aumenta o tempo de inundação das sementes. Embora a inundação tenha exercido efeito negativo na germinação desta espécie, quando as sementes foram removidas das condições de estresse, o tempo necessário para germinar as sementes viáveis foi reduzido. Já *A. edulis* apresenta sementes com baixa taxa de germinação em condições de inundação. No entanto, quando as sementes passam por um curto período de imersão em água (até 15 dias) e posteriormente são postas em condições não inundadas verifica-se um ótimo desempenho germinativo. A germinação rápida é uma característica ecologicamente vantajosa para espécies que colonizam florestas inundadas por longos períodos, uma vez que, as condições apropriadas para o estabelecimento das plântulas nesses ecossistemas podem durar pouco tempo (WITTMANN et al., 2006; LUCAS et al., 2012; CONSERVA et al., 2018).

As duas espécies estudadas apresentam plântulas do tipo fanero epigeal com cotilédones foliáceos. Esse tipo morfofuncional de plântula apresenta cotilédones fotossintetizantes que favorecem o rápido crescimento e acúmulo de carboidratos de reserva. Os resultados obtidos no Capítulo 1, da presente tese, indicam que o uso de carboidratos de reserva é importante para que as plantas de *C. gongonha* possam enfrentar com sucesso um longo período de inundação durante o estágio inicial de seu desenvolvimento.

As plantas jovens de *C. gongonha* e *A. edulis* sobrevivem às condições de inundação. No entanto, ambas as espécies tiveram menor investimento em altura, número de folhas e área foliar sob as condições de inundação. Essa resposta, provavelmente, visa a economia de energia mediante as condições reduzidas de O_2 no solo. Além disso, a redução da superfície transpiracional através da redução da área foliar pode ser uma estratégia para evitar a perda de água por meio da transpiração.

Outras respostas ecofisiológicas indicam que as plantas jovens das duas espécies estudadas diferem quanto ao nível de tolerância ao estresse por inundação. As plantas de *C. gongonha* tiveram crescimento hipertrófico na base do caule e aumento de biomassa da raiz devido à formação de raízes adventícias. Enquanto, as plantas inundadas de *A. edulis* tiveram inibição total do crescimento e alterações na cadeia transportadora de elétrons fotossintéticos. A análise da fluorescência da clorofila *a* através do teste de JIP, mostrou aumento na dissipação de energia por processos não fotoquímicos nos tecidos foliares das plantas inundadas o que, possivelmente, reduziu os danos no aparato fotossintético e possibilitou a sobrevivência das plantas sob as condições de estresse. Apesar disso, houve redução nos índices de desempenho fotossintéticos (PI_{ABS} e PI_{TOTAL}) em plantas de *A. edulis* conforme aumentou o tempo de inundação. O PI_{ABS} e PI_{TOTAL} são variáveis que quantificam as principais etapas fotoquímicas dos fotossistemas II e I (FSII e FSI, respectivamente) incluindo a absorção de energia luminosa, interceptação da energia de excitação e conversão da energia de excitação em fluxo elétrico. A queda acentuada dos PIs a partir dos 30 dias de inundação indicam danos no FSII.

O conjunto de dados apresentados ajuda a explicar a distribuição das espécies estudadas ao longo do gradiente de inundação, sendo *C. gongonha* predominante em locais inundados em caráter quase que permanente e *A. edulis* mais abundante em locais temporariamente inundados (SILVA et al. 2007). Além disso, os resultados permitem entender a distribuição dos indivíduos das espécies estudadas predominantemente sobre microrrelevos em florestas paludosas.

As variações espaço-temporais nas condições hidrológicas do solo exercem forte influência na composição florística e estrutura da vegetação de florestas paludosas (SHIMAMURA; MOMOSE, 2005; TEIXEIRA; ASSIS, 2009; TEIXEIRA; ASSIS; LUIZE, 2011; DUARTE, 2011; FREUND et al., 2018). Essas características tornam as florestas paludosas ecossistemas de alta complexidade ambiental e, portanto, difíceis de restaurar (ZAMITH; SCARANO, 2010). A restauração de florestas paludosas degradadas, geralmente, requer forte intervenção humana (SCARANO; RIOS; ESTEVES, 1998) devido às condições de inundação prolongadas que tornam o ambiente inóspito para o estabelecimento da maioria das plantas (ZAMITH; SCARANO, 2010). Estudos ecofisiológicos fornecem informações que podem aperfeiçoar as práticas de restauração nessas florestas. As duas espécies estudadas neste trabalho são adequadas para programas de restauração de áreas inundáveis. Os dados obtidos podem ser empregados durante a semeadura e o plantio das mudas a fim de alcançar maior sucesso na germinação das sementes e sobrevivência das plantas.

As sementes de *C. gongonha* devem ser dispostas em locais não inundados para germinar, porém depois de desenvolvidas as mudas podem ser plantadas em locais sujeitos a longos períodos de inundação. É possível que depois de estabelecidas, as próprias árvores criem condições que favorecem o estabelecimento de plantas com maiores restrições a saturação hídrica do solo através da elevação do solo. Sendo assim, estudos que investiguem o possível papel de *C. gongonha* como uma potencial planta facilitadora (nurse plant) em florestas paludosas é importante, pois contribui para o conhecimento sobre os processos que determinam estruturação e a diversidade desses ecossistemas e, portanto, podem fornecer base para sua conservação e restauração.

Para *A. edulis,* recomenda-se a imersão das sementes em água, logo após a coleta dos frutos, por até 15 dias antes da semeadura para aumentar a taxa e a velocidade de germinação, bem como vigor das plântulas formadas. A semeadura ou plantio das mudas deve ser feito em locais expostos a curtos períodos de inundação (até 30 dias) tendo em vista que a inundação prolongada prejudica o desempenho fotossintético das plantas jovens dessa espécie, o que pode causar a sua mortalidade. O comportamento germinativo de *A. edulis* indica uma resposta adaptativa à hidrologia de florestas paludosas. Vários fatores podem ter influenciado essa resposta, entre eles os compostos armazenados nas sementes. Portanto, mais estudos são necessários para elucidar essa questão.

Ambas as espécies estudadas, tiveram maior crescimento e melhor desempenho fisiológico sob as condições não inundadas. Portanto, os indivíduos que se estabelecerem sobre os microrrelevos em florestas paludosas, provavelmente terão seu desenvolvimento favorecido. Assim, os projetos que visam a revegetação de áreas palustres podem avaliar a possibilidade de plantio das mudas dessas espécies sobre substrato elevado, tais como os microrrelevos naturais, objetivando favorecer o desempenho fisiológico das plantas e aumentar as suas chances de sobrevivência durante o longo período inundado.

Tendo em vista, que alterações climáticas podem alterar o regime hidrológico de florestas paludosas (MARENGO; DIAS, 2006), mais estudos com enfoque na ecofisiologia das espécies palustres são necessários para verificar os efeitos dos eventos climáticos extremos, como as secas e inundações prolongadas que são previstos para o sul do Brasil. Tais efeitos podem ser especialmente prejudiciais às espécies que tem sementes recalcitrantes. Assim como *A. edulis*, muitas espécies arbóreas de áreas úmidas apresentam sementes recalcitrantes (LUCAS et al., 2012). As sementes recalcitrantes representam um desafio para o armazenamento em longo prazo, pois perdem a viabilidade em condições de umidade reduzida (BARBEDO; MARCOS FILHO, 1998; BERJAK; PAMMENTER, 2007). As plantas com sementes recalcitrantes formam banco de plântulas ao invés de banco de sementes (BERJAK; PAMMENTER, 2007), portanto alterações nas condições favoráveis à germinação de sementes e estabelecimento de plântulas podem comprometer a regeneração dessas espécies.

Referências Bibliográficas

BARBEDO, C.J.; MARCOS FILHO, J. Tolerância à dessecação em sementes. **Acta Botanica Brasilica**, v. 12, n. 2, p. 145-164, 1998.

BERJAK, P.; PAMMENTER, N.W. From Avicennia to Zizania: seed recalcitrance in perspective. **Annals of botany**, v. 101, n. 2, p. 213-228, 2007.

CONSERVA, A. et al. Germinative behaviour of ten tree species in white-water floodplain forests in central Amazonia. **Folia geobotanica**, v. 53, n. 1, p. 89-101, 2018.

DUARTE, C. I. **Influência da variação hidrológica e da luminosidade na composição e estrutura do componente herbáceo em uma floresta paludosa no extremo sul do Brasil**. 2012. Dissertação de mestrado. Universidade Federal do Rio Grande, Rio Grande, RS, 36p.

FREUND, C.A. et al. Microtopographic specialization and flexibility in tropical peat swamp forest tree species. **Biotropica**, v. 50, n. 2, p. 208-214, 2018.

LUCAS, C.M. et al. Effects of short-term and prolonged saturation on seed germination of Amazonian floodplain forest species. **Aquatic botany**, v. 99, p. 49-55, 2012.

MARENGO, J. A.; DIAS, P. S. Mudanças climáticas globais e seus impactos nos recursos hídricos. **Águas doces no Brasil: capital ecológico, uso e conservação**, v. 3, p. 63-109, 2006.

SCARANO, F.R.; RIOS, R.I.; ESTEVES, F.A. Tree species richness, diversity and flooding regime: case studies of recuperation after anthropic impact in Brazilian flood-prone forests. **International Journal of Ecology and Environmental Sciences**, v. 24, p. 223-235, 1998.

SILVA, A. C.et al. Comparação florística de florestas inundáveis das regiões Sudeste e Sul do Brasil. **Brazilian Journal of Botany**, v. 30, n. 2, p. 257-269, 2007.

SHIMAMURA, T.; MOMOSE, K. Organic matter dynamics control plant species coexistence in a tropical peat swamp forest. **Proceedings of the Royal Society B: Biological Sciences**, v. 272, n. 1571, p. 1503-1510, 2005.

TEIXEIRA, A.; ASSIS, M. A. Relação entre heterogeneidade ambiental e distribuição de espécies em uma floresta paludosa no Município de Cristais Paulista, SP, Brasil. **Acta Botanica Brasilica**, p. 843-853, 2009.

TEIXEIRA, A.P; ASSIS, M. A.; LUIZE, B. G. Vegetation and environmental heterogeneity relationships in a Neotropical swamp forest in southeastern Brazil (Itirapina, SP). **Aquatic botany**, v. 94, n. 1, p. 17-23, 2011.

WITTMANN, F. et al. Tree species composition and diversity gradients in white- water forests across the Amazon Basin. **Journal of biogeography**, v. 33, n. 8, p. 1334-1347, 2006.
ZAMITH, L.R.; SCARANO, F.R. Restoration of a coastal swamp forest in southeast Brazil. **Wetlands ecology and management**, v. 18, n. 4, p. 435-448, 2010.