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JAILTON DE JESUS SILVA

FENOLOGIA, QUALIDADE FISIOLÓGICA E CONSERVAÇÃO DE SEMENTES DE *Handroanthus spongiosus* (RIZZINI) S. GROSE (BIGNONIACEAE), ESPÉCIE ENDÊMICA DA FLORESTA TROPICAL SAZONALMENTE SECA DA CAATINGA

Feira de Santana – BA

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Tese apresentada ao Programa de Pós-Graduação em Botânica, da Universidade Estadual de Feira de Santana como requisito parcial para obtenção do título de Doutor em Botânica. Linha de Pesquisa: Ecologia da Vegetação e Conservação

Orientadora: Prof.ª Dr.ª Claudineia Regina Pelacani (UEFS)
Coorientadora: Prof.ª Dr.ª Bárbara França Dantas (Embrapa Semiárido)

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Dra. Ligia Silveira Funch
Universidade Estadual de Feira de Santana (UEFS)

CRPelacani

Dra. Claudinéia Regina Pelacani Cruz
Universidade Estadual de Feira de Santana (UEFS)
Orientadora e Presidente

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SILVA, J. J. Fenologia, qualidade fisiológica e conservação de sementes de *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae), espécie endêmica da floresta tropical sazonalmente seca da Caatinga. UEFS- Universidade Estadual de Feira de Santana. Tese. p. 112, 2023.

Orientadora: Prof.ª Dr.ª Claudineia Regina Pelacani

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RESUMO

Estudos voltados à conservação das espécies vegetais dependem de um monitoramento e compreensão da dinâmica da vegetação, principalmente em áreas onde há escassez de informações a respeito das espécies nativas. O presente estudo teve como objetivo geral determinar o comportamento fenológico e a qualidade fisiológica das sementes de *Handroanthus spongiosus* (Rizzini) S. Grose em diferentes regiões da Caatinga no Estado de Pernambuco. As áreas da observação fenológica e coleta de sementes estão inseridas no Domínio Caatinga nos municípios de Petrolina e Lagoa Grande. O capítulo I (submetido à revista *FLORA*) avaliou a fenologia vegetativa e reprodutiva durante 36 meses em 87 indivíduos distribuídos em quatro populações, onde os resultados mostraram variações nas frequências e nos índices de atividade entre as populações. As fenofases vegetativas e reprodutivas foram sazonais e influenciadas pela precipitação pluviométrica. O capítulo II (submetido à revista *Seeds MDPI*) avaliou as condições ideais para desenvolvimento do teste de tetrazólio utilizando sementes coletadas em diferentes populações e anos com objetivo de determinar a viabilidade e vigor das sementes, onde sementes imersas por 1h e 4h não promoveu uma clara diferença de coloração. Por outro lado, sementes imersas na concentração de 0,01% por 3h foi mais eficiente na avaliação da viabilidade. O capítulo III (publicado na *Journal of Seed Science*) avaliou a resposta temporal a diferentes embalagens e ambientes de armazenamento na conservação da qualidade fisiológica de sementes de *H. spongiosus*, sendo observado que em ambiente de laboratório apresentaram decréscimo na porcentagem de germinação e de formação de plântulas normais, enquanto aqueles com temperaturas baixas e ultrabaixas propiciaram as melhores condições de qualidade e vigor até os 24 meses, sendo recomendado para o armazenamento da espécie. O capítulo IV (submetido à *Journal of Forestry Research*) avaliou as melhores condições de cultivo para produção de mudas de *Handroanthus spongiosus*, onde sementes foram coletadas em cinco populações no estado de Pernambuco e cultivadas em seis substratos: solo, areia, solo + vermiculita, solo + esterco de cabra, solo + vermiculita + esterco de cabra e solo + esterco de cabra + 30% biocarvão. Os substratos com adição de matéria orgânica favoreceram aumento do comprimento da parte aérea, diâmetro do caule e massa seca. A adição de esterco caprino e biocarvão na formulação dos substratos pode melhorar o desenvolvimento de mudas de *H. spongiosus* e aumentar os benefícios econômicos. As informações obtidas poderão auxiliar programas de colheita de sementes com qualidade fisiológica e produção de mudas para restauração ecológica.

Palavras-chave: Caatinga, Fenologia, Sazonalidade, Sementes florestais, qualidade fisiológica

SILVA, J. J. Phenology, physiological quality and conservation of seeds of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae), an endemic species of the seasonally dry tropical forest of the Caatinga. UEFS- Universidade Estadual de Feira de Santana. Tese. p. 112, 2023.

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Coorientadora: Prof.ª Dr.ª Bárbara França Dantas

ABSTRACT

Investigations into the conservation of plant species depend on monitoring and comprehension of the dynamics of vegetation, especially in areas with scarce information regarding native species. The general objective of the present study was to determine the phenological behavior and physiological quality of seeds of *Handroanthus spongiosus* (Rizzini) S. Grose in different regions of the Caatinga biome in the Brazilian state of Pernambuco. The areas of phenological observation and seed collection were located in the municipalities of Petrolina and Lagoa Grande. The first chapter (submitted to *FLORA*) evaluated the vegetative and reproductive phenology during 36 months of 87 individuals distributed in four populations. The results revealed variations in the frequencies and indices of activity among these populations. The vegetative and reproductive phenophases were seasonal and influenced by the amount of rainfall. Chapter II (submitted to *Seeds MDPI*) ascertained the ideal conditions for performance of the tetrazolium test using seeds collected from the different populations and years, with the aim of determining the seeds' viability and vigor. In this case, immersion of seeds for 1 and 4 hours did not cause a clear difference in coloration, while immersion at a concentration of 0.01% for 3 hours was most efficient to assess viability. Chapter III (published in *Journal of Seed Science*) assessed the temporal response to different packaging and storage conditions on the conservation of the physiological quality of the *H. spongiosus* seeds. The results indicated that storage at room temperature decreased the germination percentage and formation of normal plantlets, while storage at low and ultralow temperatures provided better conditions of quality and vigor in the short and medium term, so these conditions are recommended for conservation of the species. Chapter IV (submitted to *Journal of Forestry Research*) evaluated the best conditions for cultivation of *Handroanthus spongiosus* seedlings, where seeds were selected from five populations in the state of Pernambuco and cultivated in five substrates: soil, sand, soil + vermiculite, soil + goat manure, soil + vermiculite + goat manure, and soil + goat manure + 30% biochar. The substrates with addition of organic matter favored the growth of the plants, specifically the shoot length, stem diameter and dry mass. The results showed that adding goat manure and biochar in the formulation of substrates can improve the development of *H. spongiosus* seedlings and increase the economic returns. The information obtained may help seed collection programs with physiological quality and the production of seedlings for ecological restoration.

Keywords: Caatinga, Phenology, Seasonality, Forest seeds, Physiological quality

Sumário

Introdução Geral

Atualmente a perda da diversidade biológica com a redução das florestas naturais tem se tornado uma grande preocupação e atraído a atenção da comunidade científica. A alta vulnerabilidade nessas florestas está relacionada às mudanças previstas nos principais fatores de mudança ecológica que são as mudanças climáticas e ações antrópicas (a exemplo de queimadas, extrativismo predatório, práticas agrícolas de forma não adequada) que levam à perda de habitats das espécies vegetais, da qualidade do solo e à destruição de vários biomas, dentre eles as Florestas Tropicais Sazonalmente Secas (FTSS) (Dirzo et al., 2011).

As FTSS é o bioma mais ameaçado e menos estudado em todo o mundo onde mais de 60% de sua área original já foi perdida e as que ainda restam possuem níveis altos de fragmentação (Pennington et al., 2018). Apesar de os trabalhos nessas áreas terem avançado nos últimos dez anos, ainda existem muitas lacunas de pesquisas nessas regiões, inclusive são menos protegidas quando comparadas com outros biomas (Miles et al., 2006; Meave et al., 2012; Sanchez-Azofeifa et al., 2013; Pennington et al., 2018).

Apesar de as FTSS ocorrerem na maioria dos continentes, mais da metade de sua superfície é encontrada na América do Sul, onde as suas terras férteis para a agricultura e ocupação humana provocaram desmatamento dessas áreas para estabelecimento de agricultura e pastagem extensiva para o gado (Miles et al., 2006; Pezzini et al., 2014). Mas é na região Nordeste do Brasil que se encontra a maior área contínua de FTSS, especificamente na região conhecida como Domínio Caatinga (Andrade-Lima, 1981; AB’Saber, 2003). A Caatinga cobre uma área de 912.529 km^2 restrita ao Nordeste brasileiro, tem como característica peculiar fisionomia xerófila e florística endêmica, revestida por florestas secas adaptadas às características do clima (Silva et al., 2017; Tabarelli et al., 2018). As temperaturas médias nessa região são elevadas entre 25 e 30 °C e baixos índices de precipitação pluviométrica variando entre 400 e 1200 mm anuais (Tabarelli et al., 2018).

As FTSS do Domínio Caatinga apresentam composição florística diversa com grandes áreas de florestas e vegetações arbustivas, que se destacam durante o período chuvoso, com vários níveis de endemismo e deciduidade, dominadas frequentemente pelas famílias Cactaceae e Bromeliaceae (Prado, 2003; Tabarelli et al., 2018).

Na presença dos atuais cenários de pressões antrópicas, ações quantitativas precisas de florestas ameaçadas são essenciais para o entendimento e previsão de como as populações, comunidades e ecossistemas são afetados para a formulação de ações adequadas de conservação (Ribeiro et al., 2009; Lustig et al., 2017). Sendo assim, faz-se necessário o desenvolvimento de estudos direcionados à compreensão do comportamento das espécies nesses ecossistemas.

Para melhor conservação das espécies que vivem em ambientes com uma sazonalidade ambiental marcada, como é o caso do Domínio Caatinga, estudos envolvendo o desenvolvimento vegetativo e reprodutivo das espécies, além do comportamento fisiológico de suas sementes, poderão auxiliar no entendimento da dinâmica nesses ecossistemas. Com esses estudos ainda podemos entender a ocorrência e disponibilidade de recursos florestais (por exemplo, frutos, sementes) em escala espacial e temporal (Morellato, 2013), além do auxílio com informações para o desenvolvimento de estratégias de armazenamento e manejo dessas espécies.

A fenologia em regiões áridas, onde estão as FTSS, é impulsionada principalmente pela distribuição temporal das chuvas, onde as plantas tendem a produzir ou perder folhas em resposta às mudanças na umidade do solo (Guzmán et al., 2019). Devido à sua fragmentação e perturbação, as FTSS tendem a estar associadas a manchas florestais de diferentes idades, onde a composição das espécies e sua estrutura depende dos estágios sucessionais dessas manchas (Kalacska et al., 2004; Hilje et al., 2015; Sánchez-Azofeifa et al., 2017).

A falta de conhecimento da fenologia em FTSS reflete a deficiência no entendimento dos padrões da biologia reprodutiva de árvores nativas nessas florestas, contribuindo para a ausência de estratégias eficientes sobre o momento ideal da disponibilidade de sementes com boas qualidades (Buisson et al., 2017; Luna-Nieves et al., 2017; Neves et al., 2017).

Essa região de clima semiárido no Nordeste do Brasil apresenta uma alta heterogeneidade ambiental devido a vários fatores como a variabilidade de chuvas, tipos de solos, física e química do solo, altas temperaturas e fotoperíodos (Andrade-Lima, 1981). Nesses ambientes, a heterogeneidade da disponibilidade dos recursos pode controlar os padrões fenológicos, a produção e emergência de sementes, além da sobrevivência e estabelecimento de plântulas (Brown, 1994; Miller, 1999).

Neste sentido, estudos que relacionam os processos fenológicos (vegetativos e reprodutivos) e que analisem a qualidade fisiológica de sementes para indivíduos da mesma espécie, mas que pertencem a diferentes populações em um gradiente ambiental em regiões de clima semiárido, são importantes precursores de mecanismos para recuperação e conservação de populações vegetais naturais.

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Objetivo Geral

Compreender o comportamento fenológico, qualidade fisiológica e conservação das sementes em Bignoniaceae submetidas às condições ambientais em uma Floresta Tropical Sazonalmente Seca.

Objetivos Específicos

- Analisar o efeito da precipitação, temperatura e fotoperíodo no comportamento fenológico de *Handroanthus spongiosus* (Rizzini) S. Grose;
- Investigar os padrões fenológicos vegetativos e reprodutivos, a fim de fornecer informações essenciais para o planejamento da época de coleta de sementes e recrutamento de plântulas;
- Desenvolver metodologias para determinar a viabilidade das sementes de *H. spongiosus*;
- Analisar a qualidade fisiológica das sementes colhidas em diferentes populações;
- Avaliar qual a melhor condição para o desenvolvimento de mudas de *H. spongiosus*.
- Avaliar a qualidade fisiológica das sementes armazenadas em diferentes ambientes.

Referencial Teórico

Domínio Caatinga

A Caatinga ocupa uma área correspondente a 54% da Região Nordeste, 10,7% do território nacional e constitui o chamado Polígono das Secas, compreendendo uma área de 912.529 km² que engloba partes dos territórios pertencentes aos estados do Maranhão, Piauí, Ceará, Rio Grande do Norte, Pernambuco, Paraíba, Alagoas, Sergipe, Bahia e parte de Minas Gerais (Vasconcelos et al., 2017; Silva et al., 2017).

A Caatinga é endêmica do Brasil, abriga o maior núcleo de FTSS, além disso, é uma das maiores regiões semiáridas da América do Sul (Silva et al., 2019). Possui uma formação vegetal tipicamente xerófita, localizada inteiramente no semiárido nordestino (Silva et al., 2017; Queiroz et al., 2017). Encontrada em áreas com solos férteis, baixa precipitação anual e alta sazonalidade (Pennington et al., 2000), compõe um dos biomas tropicais mais ameaçados do mundo (Miles et al., 2006). No entanto, os estudos nesse bioma ainda são escassos em relação a outros biomas, com isso sua flora ainda permanece pouco conhecida (Fernandes et al., 2020).

A vegetação é esparsa, espalhando-se pelos maciços e tabuleiros por onde correm rios, em geral intermitentes (Dantas et al., 2014). Possui pelo menos 135 unidades geoambientais e nove ecorregiões distintas, o que sustenta uma alta diversidade de plantas, anfíbios e mamíferos (Silva et al., 2017). Possui alta radiação solar, com temperaturas que podem variar entre 23 e 27 °C, baixa umidade relativa, geralmente inferior a 50%, alta evapotranspiração (> 1500 mm ano^{-1}) resultando em balanços hídricos negativos por 7-11 meses a cada ano, a precipitação é pouco distribuída no espaço e no tempo ocorrendo em pouco mais de quatro meses com média de 400 e 800 mm, os solos são rasos e cristalinos (Silva et al., 2017; Silva et al., 2020).

As chuvas em regiões semiáridas geralmente ocorrem em forma de pequenos eventos concentrados em poucos meses do ano com duração e intensidade altamente variáveis e imprevisíveis, provocando uma disponibilidade de água e nutrientes no solo em um estado descontinuo. Esses pequenos eventos de precipitação funcionam como gatilhos que acionam as atividades fisiológicas que determinam o desenvolvimento das espécies (Andrade et al., 2006). Eles podem afetar o comportamento em nível individual e gerar inúmeras respostas em nível de população (Wen-Zhi et al., 2011).

Espécies com sistema radicular profundo ou que armazenam água no caule ou no sistema radicular podem apresentar padrões fenológicos independentes das chuvas (Borchert; Rivera, 2001). No entanto, algumas espécies como é o caso das pertencentes ao gênero *Handroanthus*, família Bignoniaceae, apresentam alta densidade de madeira (1,08 g/cm³) (Lorenzi, 2008) e não possuem raízes profundas devido aos solos rasos dos locais onde habitam, sendo totalmente dependente dos pequenos eventos de precipitação para alcançar seu período de crescimento vegetativo e reprodução.

Devido a essa heterogeneidade ambiental (níveis diferenciados de precipitação e tipos de solos) que esses ambientes apresentam em escala espacial relativamente pequena, podem provocar diferentes respostas fenológicas e produzir sementes com diferentes qualidades fisiológicas para indivíduos de mesma espécie (Medina, 1995).

A Caatinga apresenta extensas superfícies planas com altitudes que variam entre 300 e 500 m, revestidas por florestas secas e vegetação arbustiva decíduas, onde as folhas podem ser perdidas durante a estação seca (Tabarelli et al., 2018).

Apesar dessa vasta diversidade de ambientes com características que sustentam uma alta diversidade de plantas e animais endêmicos, cerca de 200.000 km² de matas naturais da Caatinga nordestina foram substituídas por culturas agrícolas e/ou pastagens, favorecendo o aumento da fragmentação e perda de habitat de muitas espécies nativas (DryFlor et al., 2016; Tabarelli et al., 2018).

Esse desmatamento já vem se arrastando desde alguns anos por meio da intensificação do uso da terra, transformação das florestas em pastagens (principalmente para criação de caprinos), extração da madeira para lenha, aliado a falta de estudos científicos (Leal et al., 2005; Vasconcelos et al., 2017). No entanto, esse descaso só veio a público no início da década de 90 quando informações relacionadas a biodiversidade foram necessárias para subsidiar a eleição de áreas prioritárias para a conservação da Biodiversidade e políticas públicas para sustentar iniciativas para o desenvolvimento sustentável da região (MMA, 2002). Após sínteses de informações disponíveis na época, foi revelado que 50% do território da Caatinga foi negligenciado em estudos de biodiversidade e que muitos grupos taxonômicos ainda permaneciam ausentes (MMA, 2002; Queiroz, et al., 2005).

Levando-se em conta a riqueza biológica das florestas e bosques sazonais, a presença de espécies altamente ameaçadas de extinção, a quantidade de áreas protegidas, percebe-se que há necessidade de aumento de estudos para compreensão e implementação de medidas visando a conservação da Caatinga (Brasil, 2015; Fernandes et al., 2020).

Caracterização e importância da família Bignoniaceae

A família Bignoniaceae possui distribuição Pantropical e é composta por 80 gêneros com aproximadamente 840 espécies (Menezes Filho et al., 2020). Na região Neotropical se concentra a maior diversidade de Bignoniaceae, com cerca de 80% das espécies, presentes tanto em florestas secas quanto em florestas úmidas (Gentry, 1980, 1982; Lohmann; Ulloa, 2015; Lohmann, 2018).

As Bignoniaceae podem apresentar hábito arbóreo, arbustivos e lianescente, com filotaxia em sua maioria oposta, folhas palmaticompostas, pinadas, flores vistosas frequentemente em inflorescências axilares ou terminais, cimosas ou racemosas, zigomorfas, corola tubular, gamopétalas e gamossépalas, androceu epipétalo com estames didínamos e um estaminódio, possui um disco nectarífero que circunda o ovário e apresenta frutos do tipo cápsulas com deiscência paralela ou perpendicular ao septo (Fisher et al., 2004; Lohmann, 2004). Possuem sementes aladas e anemocórica, geralmente achatadas, com tricomas tectores, glandulares ou peltados (Gentry, 1980; Umana et al., 2011).

Atualmente são reconhecidas seis tribos monofiléticas Bignonieae, Catalpeae, Jacarandeae, Oroxyleae, Tecomeae e Tourrettieae, além de duas denominadas informalmente de "Aliança Tabebuia" e "Clado Paleotropical" (Olmstead et al., 2009; Lohmann, 2018). Dentre essas tribos, a maior é a Bignonieae, endêmica do Novo Mundo, compreende aproximadamente 393 espécies distribuídas em 21 gêneros, representando o principal componente lianescente na região Neotropical (Gentry, 1992; Olmstead et al., 2009; Lohmann; Taylor, 2014).

O Brasil é o centro de diversidade desta família, onde é possível encontrar 420 espécies distribuídas em 34 gêneros (Gentry, 1980; Lohmann et al., 2020). Essas espécies estão distribuídas nas tribos Jacarandeae, Bignonieae e no clado Aliança Tabebuia (Lohmann, 2015).

A tribo Jacarandeae Fenzl é representada por árvores ou arbustos, geralmente com folhas tipicamente bipinadas, flores com um estaminódio alongado, glandular e exserto, frutos amplos, deiscentes, oblongo a circular ou elíptico e cápsulas loculicidas, achatadas perpendicularmente ao septo (Gentry, 1992a; Olmstead et al., 2009; Ragsac et al., 2019). Inclui dois gêneros nativos do Brasil, *Digomphia* Benth. com apenas três espécies, duas das quais ocorrem no Brasil e *Jacaranda* Juss. que inclui 49 espécies, 39 encontradas no Brasil (Gentry, 1992a; Farias-Singer, 2020).

Membros do gênero *Jacaranda* Juss. ocupam uma ampla faixa de biomas e uma variedade de hábitos de crescimento, desde árvores do dossel da floresta tropical úmida até subarbustos xilopodiais adaptados ao fogo (Ragsac et al., 2019).

A Aliança Tabebuia é o segundo maior clado da família composto por árvores, arbustos, com folhas compostas digitadas e cápsulas loculicidas (Olmstead, 2009). Possui 147 espécies distribuídas em 14 gêneros, das quais dois terços estão nos gêneros *Handroanthus* Mattos *e Tabebuia* Gomes ex DC (Olmstead, 2009; Lohmann, 2015).

O caráter que une os membros da Aliança Tabebuia e os distingue de outras Bignoniaceae são as folhas digitalmente compostas (Grose; Almstead, 2007). Esse clado possui um papel importante ecologicamente, pois é um dos elementos mais abundantes distribuídos em vários biomas brasileiros (Brito et al., 2018).

O gênero *Tabebuia* ex A. P. de Candolle está circunscrito com 100 espécies, é o maior gênero em Bignoniaceae e está distribuído do sudoeste dos EUA até o norte da Argentina e Chile (Grose; Olmstead, 2007; Santos, 2017). Devido à grande variação morfológica, esse gênero sofreu várias alterações em sua circunscrição original desde 1938 quando foi criado por De Candolle para agrupar as espécies de Bignoniaceae arbóreas que apresentavam folhas simples.

Esse gênero foi dividido em *Tabebuia* e *Handroanthus* por Mattos (1970) que reconheceu a existência de um grupo dos "ipês" como um grupo brasileiro (*Handroanthus*). No entanto, Gentry (1972, 1992) retornou a incluir todas as espécies em apenas um gênero, insistindo que *Tabebuia* como foi organizado anteriormente era um grupo natural. Posteriormente, com ênfase em estudos filogenéticos, Grose e Olmstead (2007) reorganizaram o gênero *Tabebuia* em três: *Tabebuia*, *Handroanthus* e *Roseodendron*, reconhecendo a existência de linhagens diferentes dentro do grupo (Santos, 2017).

O gênero *Tabebuia* é formado por árvores e arbustos com 7-9-foliolada, folhas palmaticompostas, cálices densamente lepidotos, corolas brancas a vermelhas, as vezes amarela (Grose; Olmstead, 2007).

O gênero *Handroanthus* possui distribuição na América do Sul, Central e nas Antilhas (Grose; Olmstead, 2007). Caracterizado por possuir hábito arbóreo, 3-5-9-foliolada, folhas palmaticompostas, cálices com 5-lóbulos, tricomas simples, dendríticos ou estrelados e corolas geralmente amarelas (Grose; Olmstead, 2007).

Os gêneros que também são encontrados no Brasil são *Crescentia* L., *Godmania* Hemsl., *Paratecoma* Kuhlm. e *Sparattosperma* Mart. ex Meisn. (Lohmann et al., 2020).

A região Nordeste concentra cerca de 201 espécies, sendo 59 dessas espécies encontradas no Estado de Pernambuco (Lohmann et al., 2020). No entanto, ainda são poucas as informações sobre a família no Nordeste do Brasil, especialmente para esse Estado (Costa et al., 2019).

Van Steenis (1978) em sua obra "Bignoniaceae. In: "Flora Malesiana", declarou que não existia qualidades marcantes em Bignoniaceae como plantas úteis. Posteriormente Gentry (1992b) publicou um trabalho evidenciando a importância tanto econômica quanto etnobotânica desta família, mostrando que há uma gama de usos de espécies desta família para horticultura, ornamentação de vias públicas (Menninger, 1970; Gentry, 1982, 1984), alimentação humana (Gentry, 1980; Lovett, 1990), artesanato, construção civil (Lohmann, 2004), corantes, medicina e rituais religiosos (Gentry, 1992b).

As espécies de *Jacaranda* são conhecidas por terem uma variedade de usos hortícolas e etnobotânicos, com destaque para *Jacaranda mimosifolia* (Gentry, 1992a).

Muitas espécies da Família Bignoniaceae são ricas em uma variedade de diferentes classes de metabólitos secundários com diversas atividades farmacológicas para ações antimicrobianas, anti-inflamatórias e anticâncer (Jimenez-Gonzalez et al., 2018; Hamed et al., 2020).

O principal uso é na indústria madeireira, dada a alta qualidade da madeira encontrada nos gêneros *Handroanthus* Mattos e *Paratecoma* Kuhlm. (Gentry, 1992b) o que compromete a recuperação de suas populações em razão de baixas densidades de indivíduos adultos em fase reprodutiva e baixo recrutamento de plântulas (Schulze et al., 2008). Devido a isso, espécies destes grupos foram frequentemente extraídos das matas, o que levou à ameaça de extinção local de várias espécies dos gêneros acima citados (Lohmann et al., 2013).

Introdução à Fenologia

Estudos em fenologia ajudam a entender o processo de regeneração, reprodução das plantas, organização temporal dos recursos e da interação planta-animal (Van Schaik et al., 1993). O período de ocorrência das fases do ciclo de vida das plantas não está relacionado apenas ao seu desenvolvimento, mas também ao de organismos que dela dependem. Assim, mudanças na fenologia das plantas podem impactar negativamente na densidade de organismos ou mesmo provocar mudanças evolutivas (Miller-Struttmann et al., 2015).

A fenologia não envolve apenas o monitoramento do ciclo vegetativo das plantas como emissão de novas folhas (brotamento) e queda de folhas (senescência), mas também o monitoramento do ciclo reprodutivo, por meio das observações da formação dos botões florais, floração e frutificação (presença de frutos imaturos e maduros) atrelados ao clima e às respostas dos organismos às mudanças sazonais do ambiente onde vivem (Mariano et al., 2016). Esse conhecimento pode ser utilizado tanto como fundamento para a coleta de material fértil como para estudos de reprodução de espécies (Biondi et al., 2007).

Compreender os mecanismos responsáveis pelas mudanças fenológicas observadas nas plantas é importante para conhecer como se organiza a estrutura dos ecossistemas, prever futuras mudanças na distribuição das espécies e seus impactos (Piao et al., 2019). Além disso, possuem efeitos na biodiversidade, ciclagem de nutrientes, recrutamento de plântulas, na agricultura e até na economia (Morellato et al., 2016).

Os estudos sobre fenologia no Brasil geralmente são baseados na sazonalidade climática e correlacionados com fatores abióticos. Com o passar dos anos, tem-se aumentado a preocupação com os diversos métodos aplicados nos estudos em fenologia, pois podem dificultar a interpretação dos resultados como também comparações entre os estudos (Bencke; Morellato, 2002; Almeida Neto, 2004).

Os estágios de desenvolvimento das folhas, também chamado de período de crescimento, tem sua importância pois ajuda a prever os impactos das mudanças climáticas, controla os processos cruciais do ecossistema como ciclagem de nutrientes e sequestro de carbono e está relacionada ao estresse hídrico (Reich et al., 1992; Piao et al., 2019; Lima et al., 2021). Compreender essas alterações e o que as impulsionam contribuem para o entendimento dos efeitos na dinâmica da vegetação e pode evitar a perda de funcionalidades dos ecossistemas (Polgar; Primack, 2011; Richardson et al., 2013).

Em ecossistemas sazonalmente secos podem ser encontradas diferentes estratégias ligadas à manutenção e troca de folhas, desde espécies com folhas perenes, decíduas e semidecíduas que frequentemente ocorrem (Eamus, 1999). As espécies perenes mantêm alta cobertura da copa ao longo do ano, são distinguíveis por suas estratégias de tolerância à seca e maior tempo de vida das folhas (Fu et al., 2012; Rossatto, 2013; Vico et al., 2014). As espécies decíduas permanecem sem folhas durante parte do período seco, exibindo estratégias ligadas à redução das atividades fisiológicas, evitam a seca, promovem menor investimento na produção de tecidos não fotossintetizantes nas folhas, associadas à maior eficiência no uso da água (Franco et al., 2005; Fu et al., 2012).

Os tipos funcionais de troca de folhas (perenes, decíduas e semidecíduas) definem a dinâmica dos processos do ecossistema, podendo ser caracterizado pelo tempo, comprimento e intensidade da estação de crescimento foliar (Reich, 1995; Singh; Kushwaha, 2005).

Em florestas tropicais que apresentam climas sazonais podem apresentar maior periodicidade na produção foliar, onde a alternância das estações seca e úmida funcionam como principal fator envolvendo as fenofases desencadeadoras (Van Schaik et al., 1993; Morellato et al., 2013). No entanto, os mecanismos fisiológicos e as interações com fatores externos que controlam as respostas ao desenvolvimento das plantas ainda não foram determinados para a maioria das espécies que habitam os ambientes tropicais.

A fenologia reprodutiva compreende a formação de botões florais, floração, formação de frutos imaturos, maduros e o período de dispersão de sementes. Essas fenofases possuem sua importância no que reflete a época do ano onde há uma alta disponibilidade de sementes com boa qualidade fisiológica para coleta (Luna-Nieves et al., 2017). O tempo de amadurecimento dos frutos, que podem ser dirigidos por fatores abióticos ou mesmo bióticos, afetam os eventos subsequentes do ciclo de vida da planta, pois inclui a dispersão de sementes e o estabelecimento de plântulas (Mendoza et al., 2017; Cortés-Flores, et al., 2019).

Os ciclos fenológicos reprodutivos dos vegetais estão relacionados muitas vezes com as atividades dos animais, uma vez que influenciam os polinizadores que dependem dos recursos florais para sobreviver. Além disso, as atividades limitadas dos dispersores de sementes podem limitar o alcance de uma espécie e influenciar a dinâmica da população (Newstrom; Frankie, 1994).

Nos últimos anos vem crescendo o número de pesquisas em fenologia de florestas neotropicais utilizando conjuntos de dados de médio e longo prazo (Neves et al., 2017; Wright et al., 2019; Souza et al., 2020; Lima et al., 2021; Neves et al., 2022; Ribeiro et al., 2022). No entanto, os estudos com espécies tropicais ganharam destaque a partir da década de 1960, com estudos de comunidades realizados principalmente na Costa Rica e em ecossistemas sazonalmente secos (Vico et al., 2014). Esses trabalhos abordaram comparações entre florestas secas e úmidas (Frankie et al., 1974), comparações entre áreas e influência das chuvas na floração (Opler et al., 1976; 1980) e influência do estresse hídrico sobre a fenologia na América Central (Borchert, 1983). Esses trabalhos forneceram resultados importantes para o entendimento das respostas fenológicas em relação aos fatores climáticos determinantes nesses processos. Esses estudos influenciaram o desenvolvimento de novas linhas de pesquisa em fenologia e nortearam o desenvolvimento de pesquisa em outras partes do mundo.

Em regiões temperadas, o crescimento e o desenvolvimento das plantas são determinados principalmente pela variação na temperatura, na qual o inverno sincroniza a maioria das espécies em ciclos anuais (Borchert et al., 2005). No entanto, quando se trabalha com fenologia em regiões tropicais os padrões podem divergir em diferentes níveis de análise, tanto em larga quanto em pequena escala geográfica (Newstrom; Frankie, 1994; Bencke; Morellato, 2002), principalmente em ecossistemas secos onde as chuvas apresentam forte sazonalidade e a fenologia das plantas é impulsionada pela disponibilidade de água (Borchert, 2005; Wright et al., 2019; Lima et al., 2021). Os ecossistemas tropicais secos, de ocorrência nas Américas, África e Austrália estão sujeitos à forte sazonalidade hídrica, com extensos períodos de baixa pluviosidade ou ausência completa de chuvas em alguns meses do ano (Vico et al., 2014).

O comportamento fenológico das comunidades de plantas das florestas tropicais sazonalmente secas (FTSS) se diferencia das florestas tropicais úmidas e temperadas, pois as FTSS são caracterizadas por apresentar fortes mudanças sazonais na precipitação pluviométrica, onde podem perder até 100% de suas folhas durante o período sem chuvas, que pode durar de 5-6 meses (Reich, 1995; Ribeiro et al., 2022). Com essa marcada sazonalidade e forte limitação de água nesses ambientes, é provável que a fenologia vegetativa e reprodutiva esteja fortemente ligada aos fatores ambientais (Lima et al., 2021).

Os trabalhos de fenologia desenvolvidos em FTSS teve como princípio a descrição de padrões a nível de comunidades (Cortés-Flores et al., 2017), enquanto a níveis de populações ainda são poucos explorados e tem recebido menos atenção. Os padrões a níveis de comunidade podem ocultar uma grande diversidade de respostas fenológicas exibidas pelas espécies, que só surgem quando os padrões são avaliados no nível da população (Luna-Nieves et al., 2017).

Qualidade fisiológica de sementes

O conhecimento da tecnologia e ecofisiologia das sementes de espécies nativas são essenciais para a sua conservação, devido à diversidade de tipos e comportamento que essas sementes apresentam (Fenner et al., 2005; Mulkey et al., 2012). Na maioria das vezes, pela falta de informações a respeito das necessidades ecofisiológicos das espécies, elas são plantadas em locais com condições edafoclimáticas inadequadas, resultando em insucessos, a exemplo de operações de recuperação/restauração florestal (Bezerra et al., 2017). Além disso, ainda há uma escassez de informações a respeito do comportamento dessas sementes para o armazenamento a longo prazo (Solberg et al., 2020). Visto que a biodiversidade está em perigo em todo o mundo, programas de conservação são necessários para conservar as espécies (Peres, 2016; O'Donnell; Sharrock, 2017).

A qualidade das sementes é determinada por fatores ou características que podem ser agrupados em genéticos (espécie), físicos (pureza, danos físicos), sanitários (incidência de pragas e doenças) e fisiológicos (germinação, vigor). Entender esses fatores é fundamental para se ter sementes de alta qualidade para produção de mudas e restauração ecológica (Delouche, 2016). Apesar de esses fatores serem abordados separadamente a nível de explicação, isso não significa que eles atuam isoladamente, pelo contrário, o cuidado que se atribui a um ou mais fatores interferem na qualidade do outro. No nosso trabalho vamos focar principalmente na qualidade fisiológica das sementes.

As sementes possuem atributos intrínsecos que determinam a sua capacidade de emergir ou germinar rapidamente de forma homogênea sob uma variedade de condições ideais ou adversas (Delouche, 2016). Esses atributos estão ligados diretamente aos processos que envolvem desde os estádios de desenvolvimento do embrião, passando por todo o período de completa maturação e dessecação da semente (no caso daquelas classificadas como ortodoxas) até o desprendimento fisiológico da planta-mãe, que é a dispersão.

A partir da maturidade fisiológica e a consequente dispersão/coleta, a semente tende a começar a perder a sua qualidade fisiológica progressivamente ao longo do tempo, rompendo as conexões tróficas com a planta mãe. Nesse momento a única fonte de translocação de reservas de nutrientes para o eixo embrionário são os compostos orgânicos de reserva da própria semente, endosperma, perisperma ou cotilédones. Com isso, faz-se necessários estudos que acompanham as fases reprodutivas (floração e frutificação), especialmente do desenvolvimento da semente, reconhecendo a partir de índices físicos e fisiológicos (tamanho, teor de água, massa da matéria seca, germinação e vigor), índices morfológicos (a exemplo da coloração e secagem dos frutos, coloração do tegumento e secagem das sementes) e o período de maturidade fisiológica. Admite-se que sementes na maturidade fisiológica apresentam máxima qualidade fisiológica que coincide com o momento adequado de coleta (Marcos-Filho, 2015).

O teste de germinação é utilizado para conhecer as condições ideais para que o processo de rompimento dos tecidos que envolvem a radícula ocorra normalmente (Carvalho; Nakagawa, 2012). Assim, as condições do teste devem corresponder às exigências das sementes em termos das condições ambientais como, temperatura, substrato, umidade e luz (Ferraz; Calvi, 2011).

O teste de germinação é considerado como padronizado, com ampla repetição dos resultados, no entanto possui limitações, pois na maioria das vezes não corresponde a porcentagem de emergência em campo, onde com frequência são considerados inferiores aos de experimentos desenvolvidos em laboratórios (Ferraz; Calvi, 2011; Delouche et al., 2016).

O desenvolvimento de testes de vigor surge para proporcionar informações adicionais aos testes de germinação, como detectar diferenças significativas na qualidade fisiológica de populações com teste de germinação semelhantes. O potencial fisiológico das sementes, ou vigor, compreende todas as propriedades das sementes que possibilitam uma germinação e desenvolvimento de plântulas adequadas sob condições de campo (Marcos-Filho, 2015).

Alguns órgãos foram criados ao longo do tempo com o objetivo de desenvolver e publicar procedimentos padrão no campo de sementes no mundo, como o ISTA (*International Seed Testing Association*) e o AOSA/SCST (*Association of Official Seed Analysts* / *Society of Commercial Seed Technologists*). O órgão responsável no Brasil para análise de sementes é o Ministério da Agricultura, Pecuária e Abastecimento (MAPA) que fiscaliza as pessoas físicas e jurídicas que produz, beneficia, analisa, certifica, armazena, transporta, importa, exporta, utiliza ou comercializa sementes ou mudas. É o órgão responsável pela Rede Nacional de Laboratórios Agropecuários do Sistema Unificado de Atenção à Sanidade Agropecuária e possui dentre suas atribuições estabelecer, uniformizar e oficializar métodos para a realização de análises. Essa padronização no Brasil é feita por meio das Regras de Análises de Sementes (RAS) que disponibiliza métodos, sendo estes de uso obrigatório nos Laboratórios de Análise de Sementes credenciados no MAPA (Brasil, 2009).

Os testes de vigor disponibilizados pela RAS são: testes de tetrazólio, testes de raio x, envelhecimento acelerado, testes de frio e condutividade elétrica. Os testes de vigor mais utilizado e que os comitês de vigor da ISTA e da AOSA/SCST consideram os mais convenientes são teste de tetrazólio, condutividade elétrica, taxa de crescimento de plântulas, envelhecimento acelerado, dentre outros (Marcos-Filho, 1999). Mais recentemente têm sido considerados os testes de protrusão da raiz primária e avaliação de imagens de plântulas computadorizadas, possibilitando a agilidade das análises e a obtenção de resultados com alto nível de confiabilidade (Medeiros et al., 2019). Vários trabalhos já foram desenvolvidos utilizando o processamento digital de imagens na avaliação do vigor (Noronha et al., 2019; Sarigu et al., 2019).

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ARTIGO I - Phenological dynamics of populations of *Handroanthus spongiosus* (Rizzini) S. Grose in a seasonally dry tropical forest in Brazil

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Jailton de Jesus Silva[a], Edson Ferreira Duarte[b], Lúcia Helena Piedade Kiill[c], Raquel Araujo Gomes[a], Bárbara França Dantas[c,*], Claudineia Regina Pelacani[a], Ligia Silveira Funch[a]

[a] Universidade Estadual de Feira de Santana, Departamento de Ciências Biológicas, Avenida Transnordestina, s/n - Novo Horizonte, 44036-900 Feira de Santana, BA, Brazil.

[b] Universidade Federal de Goiás, Instituto de Ciências Biológicas (ICB), Departamento de Botânica (DBOT), Goiânia, GO, Brasil.

[c] Embrapa Semiárido, Rodovia BR-428, Km 152, Zona Rural, 56302-970 Petrolina, PE, Brazil.

*Corresponding author.

Phenological dynamics of populations of *Handroanthus spongiosus* (Rizzini) S. Grose in a seasonally dry tropical forest in Brazil

Abstract: The scarcity of observational studies on the phenological behaviors of different populations of tropical forest trees limits seed management and collection for reforestation efforts. Precipitation is the primary factor driving tropical plant phenology in seasonal environments, although other environmental variables and plant traits can affect plant phenology under similar rainfall regimes. We examined the seasonality, synchrony, and intensities of the vegetative and reproductive phenophases of four populations of *Handroanthus spongiosus* (Rizzini) S. Grose under similar climate regimes in a seasonally dry tropical forest ecosystem during a three-year period. We expected to observe some divergence of the phenological behaviors of those four populations related to distinct functional traits selected for by differences in the environmental variables of rainfall volume and soil properties. The vegetative and reproductive phenophases of 87 trees were monitored. Seasonality was examined using circular statistics, and the influences of environmental variables on phenophases were investigated using generalized additive models. Variations in frequencies and activity indices were identified among the different populations. Vegetative phenophases were seasonal, with leaf longevity of up to 7 months; budding peaked in February-March, while leaf fall peaked in April and October. The reproductive phenophases were found to be seasonal and of short duration during the transition between the dry and rainy seasons. Our hypothesis that precipitation played a key role was confirmed, although differences in soil properties and plant traits (such as tree height and stem diameter) were also found to be important drivers of vegetative and reproductive phenophases.

Keywords: Caatinga; Environmental variables; Leaf phenology; Plant traits; Reproductive phenology; Seasonality

1. Introduction

Analyses of different populations of the same species can contribute to better understanding their phenological patterns and their relationships with local climatic factors (Duboscq-Carra et al., 2020; Santos et al., 2020; Silva et al., 2022a). Flowering and leaf production have been the most studied phenomenon among tropical trees (e.g., Pires et al., 2013; Souza et al., 2019; Wright and Calderón, 2018), followed by fruiting and seed dispersal – which also play very fundamental roles in population ecology (e.g., Chapman et al., 2018; Dunham et al., 2018). Phenological patterns in tropical trees may be affected by a variety of local factors, such as rainfall, temperature, day length, irradiance, relative humidity (Chapman et al., 2018; Dunham et al., 2018; Mendoza et al., 2017), and species traits (Babweteera et al., 2018; Silva et al., 2022a).

The Seasonally Dry Tropical Forests (SDTF) biome, which includes Caatinga vegetation in northeastern Brazil, is among the least studied but most threatened phytophysiognomies in the world, having already lost more than 60% of its original area (Miles et al., 2006). SDTF are found on most continents, although more than half of their total area is found in South America (Pezzini et al., 2014; Pennington et al., 2018). Caatinga vegetation covers approximately 900,000 km^2, and consists of a mosaic of xerophytic and deciduous vegetation whose physiognomy and floristic composition varies considerably on small spatial scales, from open formations to forest patches (Prado, 2003; Queiroz et al., 2017; Barbosa et al., 2019). The distributions of those vegetation mosaics are mainly linked to reduced rainfall, geological formations, and soil attributes (Prado, 2003; Andrade et al., 2017). Rainfall in the Caatinga evidences wide spatial-temporal variations, with annual means between 400-800 mm generally distributed into just four months (Reddy, 1983), marking distinct dry and rainy periods (Andrade et al., 2017). Temperatures and solar radiation, on the other hand, are relatively constant (Pennington et al., 2018; Paloschi et al., 2020).

In general, woody plants growing in arid ecosystems, such as the Caatinga, exhibit highly seasonal vegetative and reproductive events, with the onset of leaf and flower production at the beginning of the rainy period, with fruit set during the late rainy season at times of greatest water availability, followed by leaf fall during the dry season (Lima et al., 2012; 2021; Quirino and Machado, 2014; Neves et al., 2017; 2022). Studies comparing phenological events across populations can reveal much about the survival strategies of species and how abiotic factors influence their phenological variability (Pennington et al., 2006; Apgaua et al., 2014). Some Caatinga species, such as *Ziziphus joazeiro* Mart. and *Croton heliotropiifolius* Kunth, for example, exhibit fine adjustments and adaptations to local environmental factors (Nadia et al., 2007; Santos et al., 2015; Costa et al., 2021). In the last ten years, there have been advances in terms of the number of phenological studies undertaken in SDTF areas, although there are still significant gaps in our understanding of the diversity found there and much to be done in terms of their conservation as vital resources in tropical countries (Meave et al., 2012; Oliveira et al., 2017; Pennington et al., 2018; Lima et al. 2021; Wright et al. 2021; Neves et al. 2022).

Handroanthus spongiosus (Rizzini) S. Grose (Bignoniaceae) is an arboreal species endemic to the semiarid Caatinga Domain that preferentially develops in sandy soils in seasonally dry forests. It is considered “Endangered” (EN) in terms of the possibility of its extinction, and has been negatively impacted by deforestation for agriculture, livestock raising and logging (CNCFlora, 2012). The plant is locally known as “cascudo”, “ipê-amarelo”, “ipê-cascudo”, and “sete-cascas”. Its main commercial use is by the timber industry, given the high quality of its wood. It occurs preferentially in sandy soils of the Caatinga, in forests with intermediate stages of succession, generally with low density of individuals and low recruitment of seedlings (Lohmann, 2020).

We sought then to understand how abiotic factors and plant traits are linked to the phenological patterns of four populations of *H. spongiosus* growing in northeastern Brazil. The scarcity of studies covering more than two years focusing on the phenological behavior of this species limits our ability to manage its populations and to collect seeds for Caatinga reforestation efforts. There is a great need for native seeds to execute Brazilian revegetation policies (Brasil, 2017) and to fulfill international proposals (such as the decade of restoration proposed by the UN [United Nations, 2019]) that could help mitigate the effects of global climate change. The main goal of this study was therefore to better understand the phenological behavior of *H. spongiosus*, examine how environmental aspects affect the phenology of this species over space and time in SDTF sites, and to provide support for seed harvesting. Those objectives were approached by studying the vegetative and reproductive phenologies of *H. spongiosus* in different SDTF sites and then examining their relationships with temporal changes in abiotic factors and plant traits. To that end, we addressed the following questions: (i) are there spatial-temporal variations and/or variations in the intensities of phenophases among populations? (ii) Which abiotic variables trigger the vegetative and reproductive phenophases in those populations? (iii) When should *H. spongiosus* seeds be harvested? We expected to find the seasonal patterns among *H. spongiosus* populations in the SDTF to be concentrated in times of greatest water availability, but with divergences among the phenological behaviors of their populations related to distinct functional traits selected for by differences in certain environmental variables (rainfall volumes and soil properties).

2. Materials and methods

2.1. Species and study sites

The phenological monitoring of *H. spongiosus* trees was focused on individuals approximately 10 meters tall growing in four fragmented SDTF remnants in Pernambuco State, Brazil (Fig. 1). The study sites were located near the villages of Pau ferro (8°55’57.4” S, 40°43’19.8” W, 431 m) (HUEFS 243297), Caiçara (9° 07'24.5” S, 40° 23'16.1” W, 393 m) (HUEFS 252490), Cristália (8°5'56.3” S, 40°19'27.1” W, 403 m) (HUEFS 259093), and Jutaí (8°33'35.7” S, 40°12'1.9” W, 418 m) (HUEFS 259094). The above listed vouchers for botanical materials collected at each site were deposited in the Universidade Estadual de Feira de Santana (HUEFS) herbarium. All of the sites were dominated by anthropized vegetation composed of secondary forests and pasture; the sites were each separated by more than 10 km. The study region has a low mean annual precipitation rate (between 300 mm and 1000 mm) and a high annual potential evapotranspiration rate (between 1.500 mm and 2.000 mm) (Prado, 2003). The climate is semiarid (classified as BSh), with mean annual temperatures between 25 and 30° C; both the rainy season (usually from November to April – the austral summer) and the dry season (usually from May to September) are extremely variable in their lengths and intensities in different years (Sampaio, 2010; Alvares et al., 2013).

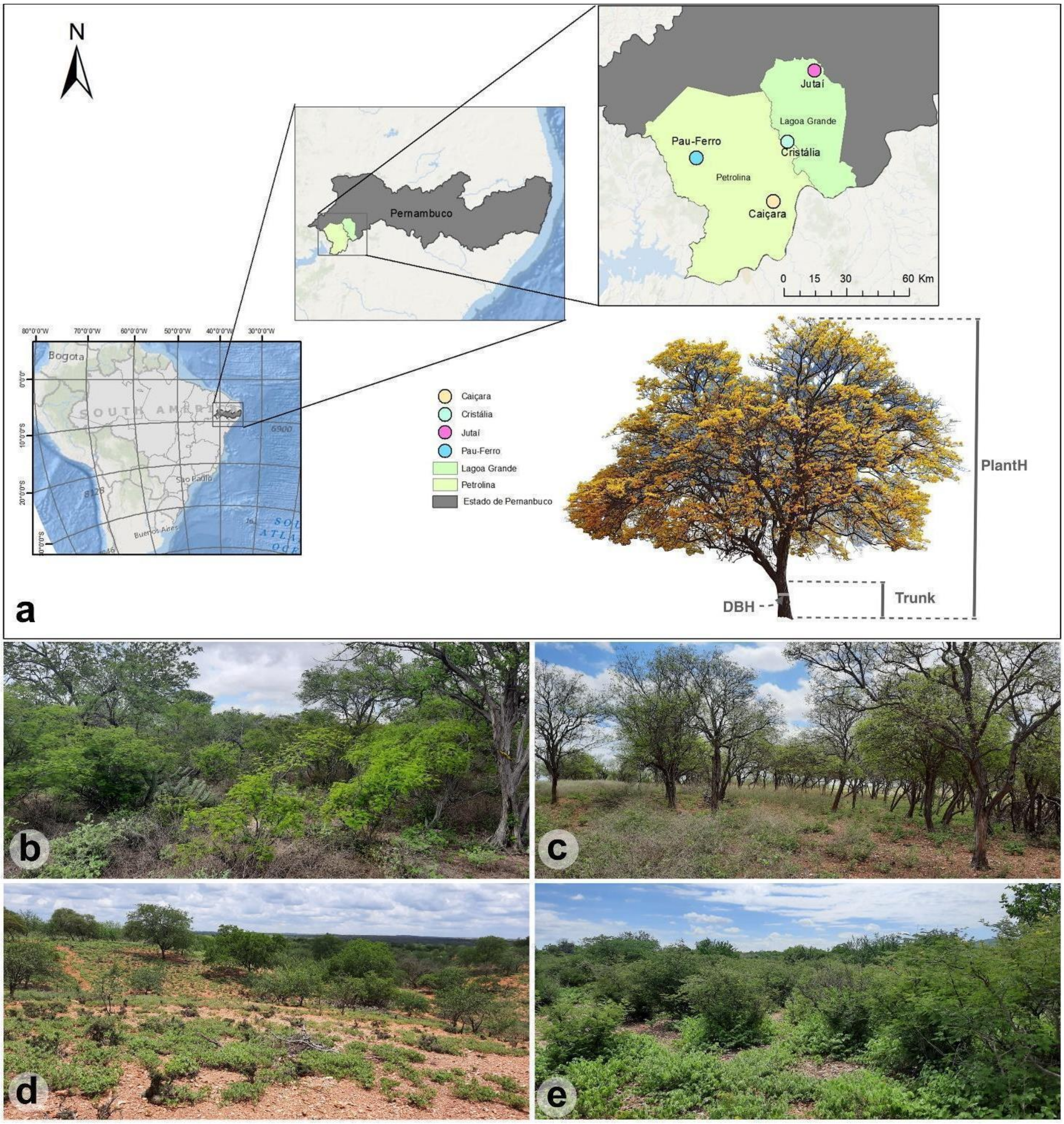

Fig. 1. Study sites and physiological traits of *Handroanthus spongiosus* (Rizzini) S. Grose in four sites in northeastern Brazil. (a). Location map of the study area. (b). Pau ferro. (c). Jutaí. (d). Cristália. (e). Caiçara. DBH = Diameter at Breast Height. PlantH = Plant Height. TrunkH = Trunk Height.

The predominant soil types in the Pau ferro and Caiçara sites are eutrophic yellow latosols developed from sedimentary materials, with sandy and clayey textures in their A horizon (Embrapa, 2018); the soils in the Cristália and Jutaí sites are orthic chromic luvisols typical of semiarid regions, with high concentrations of gravel and stones in the surface horizon, which are generally susceptible to erosion (Santos et al., 2018; Embrapa, 2018).

2.2. Study area

2.2.1 Environmental variables

Meteorological data (Fig. 2) were obtained from Embrapa Semiárido meteorological stations, located in Cristália (8° 48' 39.5” S, 40° 22', 2.6” W) (Cristália site), Bebedouro (9° 08' 12.3” S, 40° 18' 31.6” W) (Caiçara site), Cruz de Salinas (8° 50' 26.1” S, 40° 36' 5.2” W) (Pau ferro site), and Vini Brasil (08° 48' 55.6” S, 40° 41' 37.2” W) (Jutaí site). The photoperiods of the study sites were obtained using ModelE AR5 Simulations (NASA, 2020) during the 36-month duration of the study (June/2019 - May/2022). The meteorological data are separated by periods: Period 1 = June/2019 - May/2020, Period 2 = June/2020 - May/2021, and Period 3 = June/2021 - May/2022. Four composite soil samples (0-20 cm) were collected in each study site. Each composite sample was taken by mixing soils from four random points, totaling 16 composite samples. The physical and chemical properties of these samples were analyzed in the according Donagema et al. (2011). Total Porosity and Soil Density were determined by the tension table (tension of 6 kPa) and the volumetric ring methods respectively; soil granulometry was determined by the pipette and Textural Triangle method (Embrapa, 2009). The chemical analyses consisted of determinations of E.C. in saturation paste, pH in water (1:2.5 soil:water ratio); Al, Ca, Mg (exchangeable extracted with 1 mol L^{-1} KCl, analyzed by titration); P, K and Na (extracted by Mehlich extractor 1); Cu, Fe, Mn and Zn (Mehlich 1 Extraction and determination by Flame Atomic Absorption Spectrophotometry), SB and CTC (Calcium Acetate Extraction), according to Embrapa (2009).

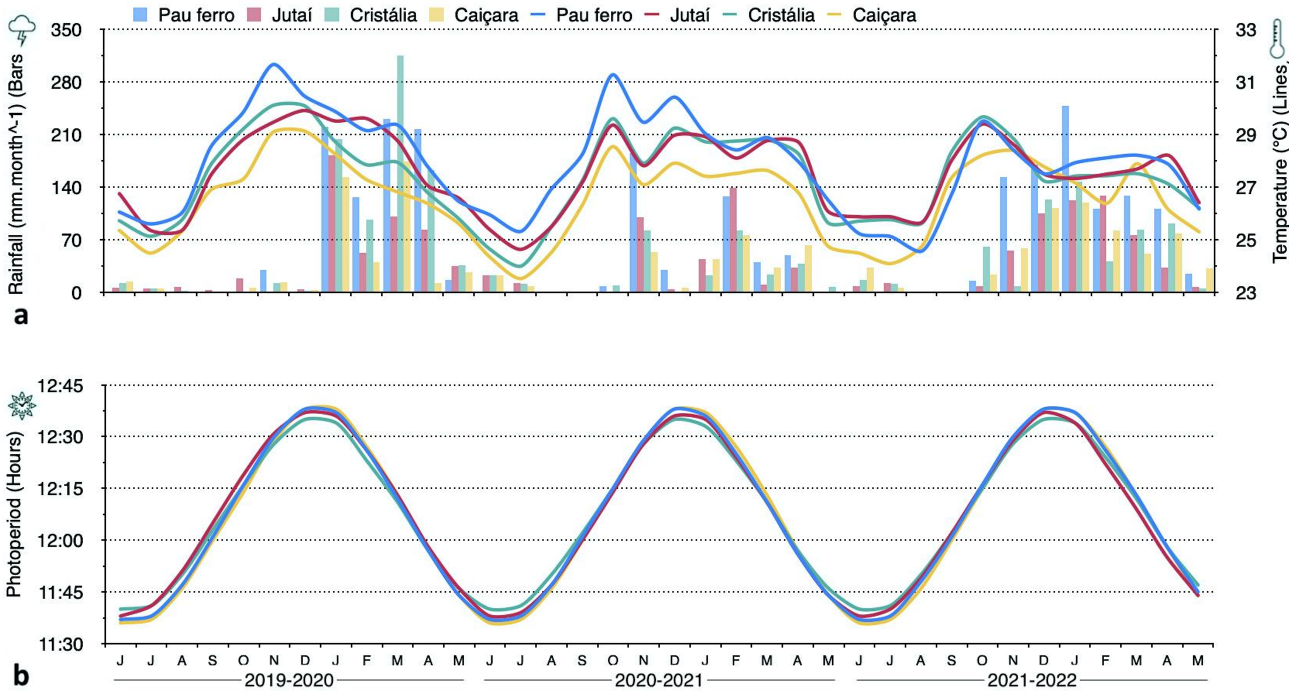

Fig. 2. Meteorological data for the *Handroanthus spongiosus* (Rizzini) S. Grose study sites were evaluated for 36 months (June/2019 to May/2022) in northeastern Brazil. (a). Monthly average air temperature (lines), and accumulated monthly rainfall (bars). (b). Photoperiod.

2.2.2. Phenological data and plant traits

A total of 87 healthy adult individuals of *H. spongiosus* (growing at least 20 m apart) were geo-referenced and marked with aluminum tags. The numbers of individuals accompanied in each of the four sites varied according to their population sizes: Cristália (n = 20 individuals), Caiçara (n = 17 individuals), Pau ferro (n = 30 individuals), and Jutaí (n = 20 individuals). The plant traits examined were: total plant height (PlantH), trunk height (TrunkH), and diameter at breast height (DBH) (Silva et al., 2022a) (Fig. 1). Phenological observations were undertaken for 36 months (June/2019 - May/2022) during three different periods: Period 1 = June/2019 - May/2020, Period 2 = June/2020 - May/2021, and Period 3 = June/2021 - May/2022. The vegetative phenophases evaluated were: (i) leaf flushing (shoots in formation, having light green tones); (ii) young leaves (developed leaves, but light green in color); (iii) mature leaves (developed and expanded leaves, with dark green tones); (iv) leaf fall (absence of leaves on the branches). The reproductive phenophases were: (v) flower buds; (vi) flowering (anthesis); (vii) immature fruits (newly formed fruits, greenish); (viii) Mature fruits (brown colored fruits); (ix) seed dispersal (fruits starting to open and dispersing seeds). Phenophase intensities were estimated during field observations using a semi-quantitative scale consisting of five categories (0-4) at 25% intervals (Fournier, 1974). The intensities of the phenophases were measured as the ratio of the sum of each category multiplied by 100, and the maximum Fournier number (4) multiplied by the number of individuals (Martin-Gajardo and Morellato, 2003). We determined the activity index based on the percentage of individuals in each population manifesting a certain phenological event (Bencke and Morellato, 2002). Leaf longevity was estimated through evaluations of the peaks of leaf flushing and leaf fall of individuals (scores 3 or 4 on the Fournier scale). We calculated average leaf longevity in months between the respective phenophases in the observed phenological cycles (Oliveira et al., 2014; Santos et al., 2020). We evaluated flowering patterns (flower phenophase) for the species based on data from the four populations, considering the frequency and duration of the episodes (Newstrom et al. 1994); flowering strategies were classified according to Gentry (1974).

2.3. Data analysis

The seasonality of the phenological events of *H. spongiosus* at each site in each year were evaluated using circular statistical analyses (Morellato et al., 2010), employing R environment software (R Core Team, 2022) with the addition of the “circular” package (Agostinelli and Lund, 2017). The frequency of each phenophase was calculated based on the total number of individuals accompanied every month. Months were converted into angles at intervals of 30° (0° representing June, 30° representing July, and so forth, until 330° representing May). The mean angles and (r) vector lengths were calculated. Angle significance was tested using the Rayleigh test (z) for circular distributions (Zar, 2010). The phenological events with significant mean angles ($p < 0.05$) were transformed into mean dates. Phenophases whose vector lengths (r) were > 0.5 (and whose Rayleigh tests indicated as significant) were considered seasonal (Morellato et al., 2010). The nonparametric Mardia-Watson-Wheeler test (W) (Batschelet, 1981; Mardia and Jup, 2000) was used to assess whether there were differences in the phenophases among the populations, and whether there were differences in the phenophases of the populations among the evaluation periods. This test consists of evaluating whether two or more circular samples (angles) differed among the mean dates or mean months ($p < 0.05$). The multiple factor analysis (MFA) used the R package determine the relationships between environmental variables, plant traits, and phenophases, considering the directions and lengths of vectors (Novaes et al., 2020).

The influences of temporal meteorological conditions on plant phenophases were evaluated using generalized additive models (GAMs) of the "mgcv" package (Wood, 2011), using a Gaussian error structure to model the relationships between dependent variables (phenophases) and predictors (mean air temperature, monthly accumulated rainfall, and photoperiod) as a smoothing function. This relationship was tested and evaluated through the significance (*p-value*) for each smoothing term (s), Deviance (explained) and R^2 (adjusted). From these analyses, partial dependence (PD) graphs were constructed using the "ggplot2" package (Villanueva and Chen, 2019) to show how each environmental variable interacts with the phenophases. A complete model was used for each phenophase, inserting all meteorological variables. The formula for GAM is:

$$g(\mu) = \sum f(x) \quad (1)$$

where g is a specific binding function, (μ) the average expected response and $\sum f(x)$ are smooth functions of the covariates (Ma et al., 2018). The Gaussian distribution and the identity link function were used to model the data. GAMs are valuable tools as they maximize predictive qualities by estimating nonparametric functions of predictor variables through connections with the dependent variable that are established by a linkage function (Wood, 2011; Murphy et al., 2019). All analyzes were performed using R Software (R Core Team, 2022).

3. Results

3.1. Environmental variables and plant traits

The accumulated rainfall varied between periods and study sites (Table 1). The highest and lowest monthly mean temperatures were observed in Pau ferro in the first period (28.40±0.58° C) and Caiçara in the second (26.36±0.48° C), (Table 1). The tallest plants (PlantH) and greatest diameters at breast height (DBH) were found in Cristália, while the tallest trunks (TrunkH) were found in Jutaí (Table 1). Regarding the physical-chemical parameters of the soil, there were higher densities and greater amounts of sand in the Pau ferro site, while the Cristália and Jutaí sites had the highest levels of clay and the highest sums of bases (SB), CEC, Fe and Zn (Table 2).

Table 1. Study sites of *Handroanthus spongiosus* (Rizzini) S. Grose in the SDTF in northeastern Brazil. Mean environmental variables, including elevation, soil type, temperature, rainfall, photoperiod, and plant traits are indicated.

Variable/Site	**Pau ferro**	**Jutaí**	**Cristália**	**Caiçara**
Period (12 months)	Accumulated monthly rainfall (mm y^{-1})			
Period 1	841.47±28.49	497.29±16.05	849.16±29.93	452.12±17.38
Period 2	440.32±17.28	366.26±12.93	301.96±8.38	308.42±7.92
Period 3	962.64±24.44	556.97±14.28	590.19±14.80	596.65±11.91
	Mean air temperature (°C)			
Period 1	28.40±0.58	27.89±0.48	27.58±0.50	26.83±0.43
Period 2	28.19±0.52	27.45±0.48	27.36±0.56	26.36±0.48
Period 3	27.11±0.44	27.34±0.34	27.24±0.37	26.49±0.44
	Photoperiod (hours)			
Period 1	11.90±0.12	11.92±0.12	11.90±0.11	11.90±0.12
Period 2	11.90±0.12	11.89±0.12	11.90±0.11	11.90±0.12
Period 3	11.91±0.12	11.90±0.12	11.91±0.11	11.90±0.12
Altitude (m)	431±2.51	418±1.13	403±0.65	393±0.22
Soil	EYL	OCL	OCL	EYL
	Plants			
PlantH (m)	5.72±0.15	6.68±0.18	7.76±0.17	5.16±0.23
TrunkH (m)	1.87±0.08	2.25±0.10	1.98±0.08	2.14±0.10
DBH (cm)	14±0.1	18±0.1	26±0.1	16±0.1

OCL = Orthic Chromic Luvisols; EYL = Eutrophic Yellow Latosols; PlantH = Total plant height; TrunkH = Trunk height; DBH = Diameter at breast height; Period 1 = June/2019 to May/2020; Period 2 = June/2020 to May/2021; Period 3: June/2021 to May/2022. Means are followed by the standard error.

Table 2. Physical and chemical soil characteristics of *Handroanthus spongiosus* (Rizzini) S. Grose sites in the SDTF in northeastern Brazil.

Parameters	**Pau ferro**	**Jutaí**	**Cristália**	**Caiçara**
Soil Density (mg m^{-3})	1.52±0.02 a	1.34±0.02 b	1.20±0.03 c	1.35±0.05 b

Particle Density (mg m^{-3})	2.58±0.02 a	2.56±0.02 a	2.46±0.02 b	2.56±0.02 a
Total Porosity (%)	41.80±0.54 c	46.60±0.72 b	52.00±1.35 a	46.00±1.83 b
Total Sand (g kg)	699.09±26.67 a	539.00±36.74 b	427.77±46.64 c	464.00±22.35 c
Silt (g kg)	235.00±33.68 c	355.00±33.08 b	391.00±9.12 b	427.00±23.31 a
Clay (g kg)	67.00±22.81 c	104.08±5.96 b	180.70±8.97 a	66.80±22.60 c
EC (mS cm)	1.01±0.39 c	1.06±0.24 b	1.96±0.96 a	1.96±0.41 a
pH	4.92±1.23 b	5.93±0.15 a	5.00±0.21 b	5.38±0.15 a
P (mg dm^{-3})	6.72±0.67 b	4.66±0.40 c	4.90±1.44 c	12.45±1.20 a
K (mg dm^{-3})	0.22±0.03 c	0.67±0.10 a	0.55±0.06 a	0.36±0.10 b
Na (mg dm^{-3})	0.08±0.00 b	0.21±0.03 a	0.25±0.04 a	0.18±0.03 a
Ca (mg dm^{-3})	1.50±1.20 c	3.90±0.26 a	2.24±0.26 b	2.23±1.00 b
Mg (mg dm^{-3})	0.71±0.53 c	1.68±0.09 a	1.26±0.03 b	0.93±0.32 c
AL (cmolc dm^{-3})	0.28±0.01 a	0.00±0.00 c	0.14±0.03 b	0.19±0.09 b
SB (cmolc dm^{-3})	2.48±1.19 c	6.64±1.27 a	4.20±0.22 b	3.83±1.01 b
CEC (cmolc dm^{-3})	2.48±1.23 c	6.68±0.26 a	4.24±0.22 b	3.83±1.29 c
Cu (mg dm^{-3})	0.74±0.06 c	0.82±0.16 c	1.09±0.06 b	1.65±0.16 a
I (mg dm^{-3})	18.60±1.12 a	22.80±3.15 a	21.60±4.00 a	15.70±0.58 b
Mn (mg dm^{-3})	25.20±13.50 b	63.20±2.33 a	23.20±1.58 b	23.50±3.56 b
Zn (mg dm^{-3})	1.50±0.26 c	4.40±0.47 a	2.87±0.83 b	1.84±0.24 c

EC = electrical conductivity; pH = potential of hydrogen determined in water; P = phosphorus, Mg2+ = magnesium; Ca2+ = calcium; Na = sodium; K+ = exchangeable potassium; H + Al = potential acidity; SB = sum of bases (Ca+2 + Mg+2 + Na+1 + K+1); CEC = cation exchange capacity (H + Al + SB); V% = base saturation (SB/CTC)*100; Cu = Copper; I = Iron; Mn = manganese; Zn = zinc. Means followed by different lowercase letters on the same line differ statistically by the Tukey test at a 0.05 degree of probability. Means (± standard error).

3.2. Vegetative phenology and leaf longevity

All of the vegetative phenophases of *H. spongiosus* were seasonal in the four monitored populations during the three observation periods, with small variations of their intensity and activity indices (Fig. 3; Table 3). The peaks of activity of leaf flushing (late October - early November), young leaves (October - November), and mature leaves (February - March) occurred during the rainy season (Fig. 3a-b). Leaf flushing and young leaf events were concentrated in just a few months (Table 3), with activity values above 80% and intensities generally below 60% (Fig. 3a-b). The mature leaves exhibited activity values above 80% and intensities above 60%, distributed from December to June (Fig. 3c; Table 3). The markedly seasonal leaf fall occurred between April and October, during the dry season, with activity and intensity peaks in July and August (100 %) (Fig. 3d; Table 3). The entire crown was renewed during each study period, with clear and separate episodes of leaf flushing and total leaf fall (up to 100%), with leaf longevity of 7.2-7.7 months (deciduous habit), with no variations among sites and periods (Supplementary Table 1). The populations differed during at least one of the evaluation periods for each vegetative phenophase (Supplementary Table 2).

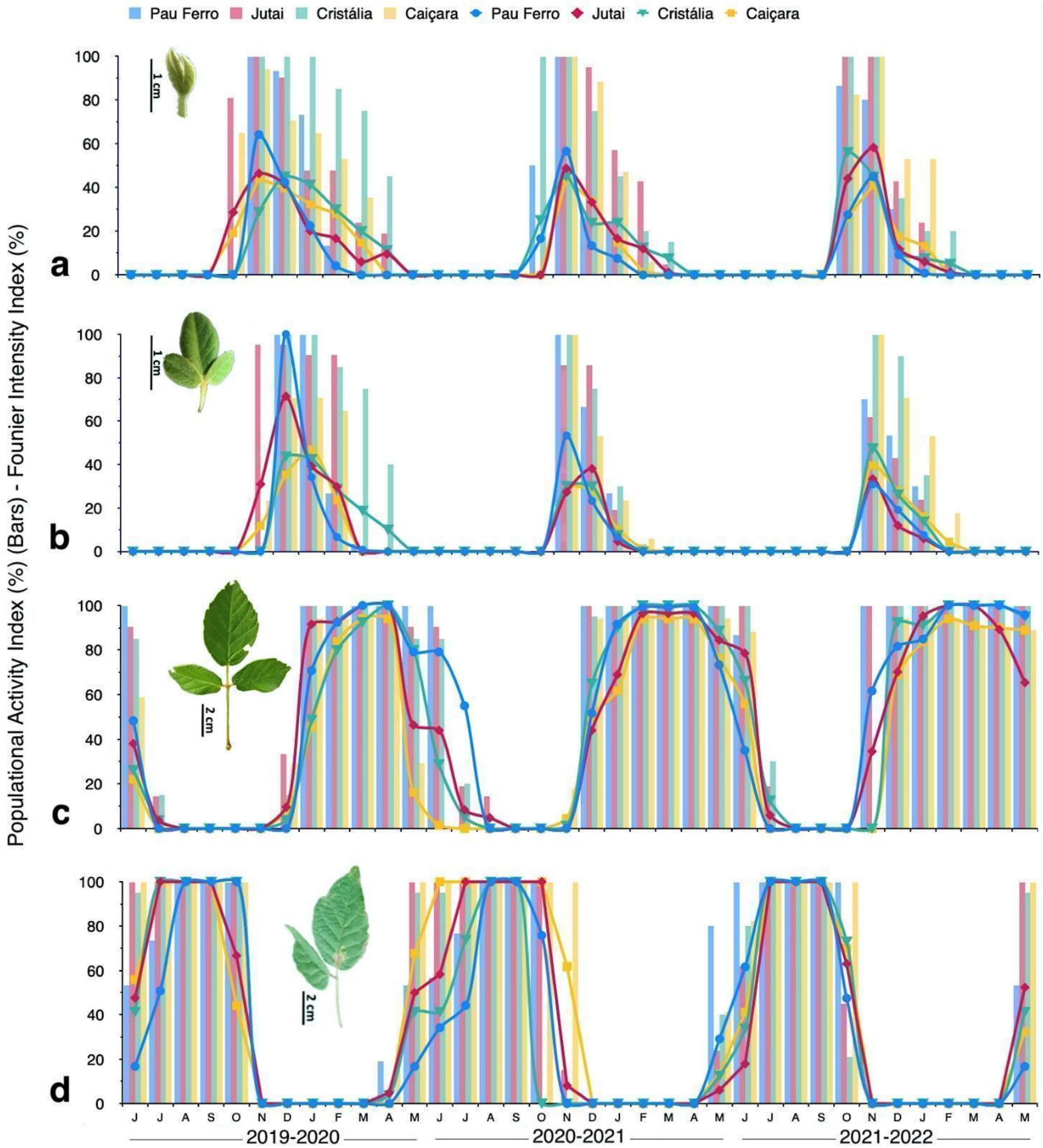

Fig. 3. Vegetative phenophases of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae) during 36 months (June/2019 to May/2022) in populations located in SDTF, in northeastern Brazil. (a). Leaf flushing. (b). Young leaf. (c). Mature leaf. (d). Leaf fall. Activity Index (Bars) and Fournier Intensity Index (%) (lines).

Table 3. Circular statistical analyses of the occurrences of vegetative phenophases in populations of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae), monitored from June/2019 - May/2022 in SDTF in northeastern Brazil.

Variable	Pau ferro			Jutaí			Cristália			Caiçara		
	Period1	**Period2**	**Period3**	**Period1**	**Period2**	**Period3**	**Period1**	**Period2**	**Period3**	**Period1**	**Period2**	**Period3**
						Leaf flushing						
Mean angle	179.41°	152.56°	142.05°	176.70°	184.28°	148.76°	212.61°	162.57°	151.22°	182.08°	174.19°	157.07°
Mean date	Nov	Nov	Oct	Nov	Dec	Oct	Jan	Nov	Nov	Dec	Nov	Nov
MLV (r)	0.89	0.89	0.92	0.67	0.85	0.87	0.69	0.77	0.83	0.70	0.91	0.85
RT (z)	0.894	0.892	0.923	0.669	0.848	0.872	0.689	0.769	0.825	0.699	0.912	0.853
RT (p)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
						Young leaves						
Mean angle	200°	168.07°	171.85°	194.11°	169.33°	170.60°	228.13°	169.43°	171.07°	203.38°	168.02°	177.47°
Mean date	dec	nov	nov	dec	nov	nov	jan	nov	nov	dec	nov	nov
MLV (r)	0.93	0.93	0.92	0.84	0.94	0.92	0.78	0.93	0.93	0.88	0.91	0.88
RT (z)	0.933	0.932	0.922	0.837	0.943	0.923	0.783	0.931	0.931	0.876	0.915	0.88
RT (p)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
						Mature leaves						
Mean angle	285°	270°	252.71°	279.50°	272.19°	257.40°	281.06°	270.47°	274.15°	265.09°	252.38°	267.93°
Mean date	Mar	Mar	Feb	Mar	Mar	Feb	Mar	Mar	Mar	Feb	Feb	Feb
MVL (r)	0.64	0.53	0.43	0.58	0.49	0.39	0.61	0.51	0.49	0.65	0.62	0.53
RT (z)	0.644	0.533	0.431	0.584	0.487	0.393	0.608	0.511	0.492	0.649	0.622	0.535
RT (p)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
						Leaf fall						
Mean angle	57.24°	54.83°	51.92°	45°	58.77°	40.76°	46.26°	39.13°	35.71°	42.83°	68.98°	46.95°
Mean date	Jul	Jul	Jul	Jul	Jul	Jul	Jul	Jul	Jul	Jul	Aug	Jul
MVL (r)	0.64	0.65	0.68	0.64	0.69	0.68	0.65	0.79	0.72	0.62	0.59	0.64
RT (z)	0.637	0.648	0.682	0.644	0.694	0.683	0.647	0.789	0.716	0.622	0.589	0.642
RT (p)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

MVL (r) = Mean vector length; RT (z) = Rayleigh Test (z); RT (p) = Rayleigh Test (p); Period 1 = June/2019 to May/2020; Period 2 = June/2020 to May/2021; Period 3; June/2021 to May/2022.

3.3. Reproductive phenophases

All of the reproductive phenophases of *H. spongiosus* were strongly seasonal, occurring between October and December, during the rainy season, in all of the four monitored populations and during the three observation periods, with only small variations in their intensity and activity indices (Fig. 4; Table 4). Flower bud production initiated at the end of October and beginning of November (Fig. 4a), 48 h after rains with >10 mm of accumulated volume, temperatures >26º C, and photoperiods >12 hours of sunlight (Fig. 2). The populations showed low variations of peak activity and flower bud intensity among periods and among study sites (Fig. 4a). The flowering phenophase is annual, of intermediate duration, concentrated in the months of October and November, and with peak intensity in the last month (Fig. 4b; Table 4). Flowering is of the big-bang type, massive, with the flowers remaining in anthesis for up to two days, followed by senescence events, wilting, and corolla abscission. Regarding fruit set, there was a peak of production of immature fruits and mature fruits with greater intensity in the month of November for all populations and for all periods evaluated (Fig. 5a-b; Table 4). The first immature fruits began to appear between the sixth and eighth day after flower opening. The phenophases mature fruit and seed dispersing occurred between the eighth and twelfth day after the appearance of the first immature fruits, usually in November (occasionally in early December). Seed dispersal occurred concomitantly with mature fruit between November and December, with peak intensity and activity in November (Fig. 5c; Table 4). The populations differed in at least one of the periods evaluated for each reproductive phenophase (Supplementary Table 2).

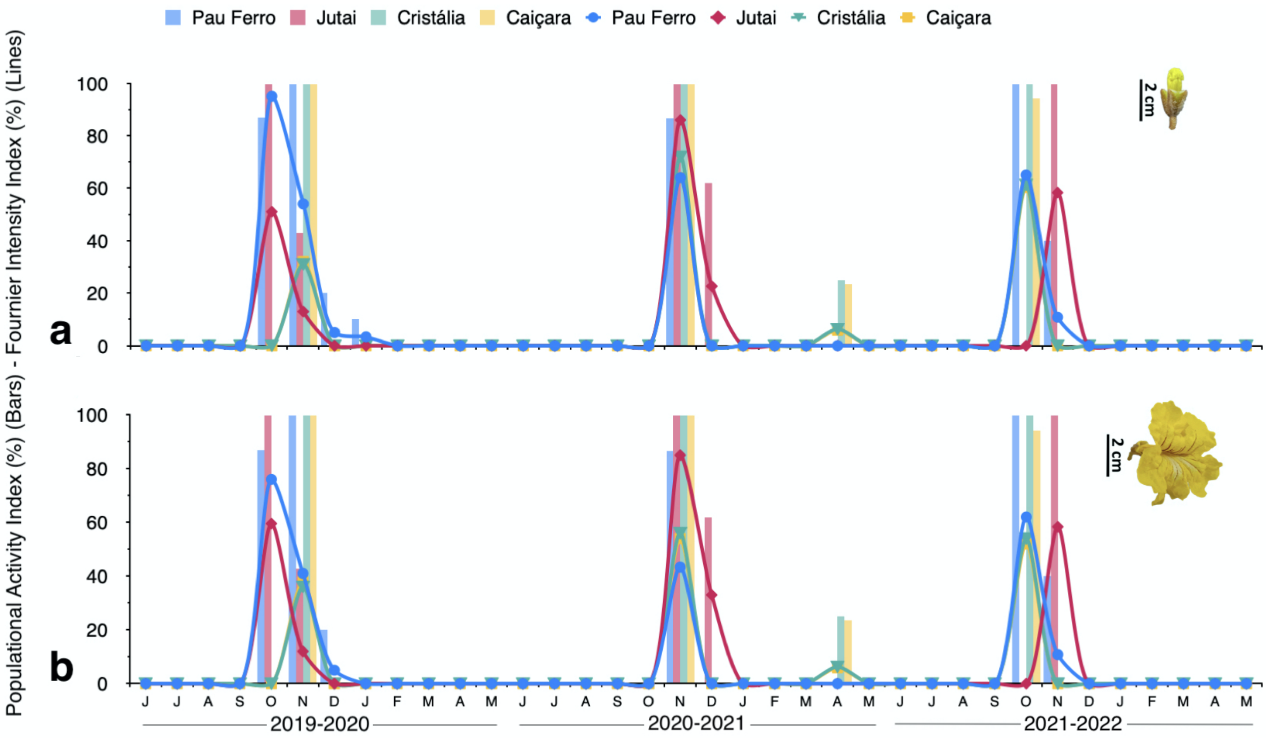

Fig. 4. Reproductive phenophases of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae) along 36 months (June/2019 to May/2022) in populations located in SDTF in northeastern Brazil. (a). Bud flower. (b). Flower. Activity Index (Bars) and Fournier Intensity Index (%) (lines).

Table 4. Circular statistical analyses of the occurrence of reproductive phenophases in populations of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae), monitored from June/2019 – May/2022 in SDTF populations in northeastern Brazil.

Variable	Pau ferro			Jutaí			Cristália			Caiçara		
	Period1	Period2	Period3	Period1	Period2	Period3	Period1	Period2	Period3	Period1	Period2	Period3
						Flower buds						
Mean angle	142.84°	150°	128.45°	128.75°	161.29°	150°	150°	159.06°	120°	150°	158.17°	120°
Mean date	Oct	Nov	Oct	Oct	Nov	Nov	Nov	Nov	Oct	Nov	Nov	Oct
MVL (r)	0.92	1	0.97	0.97	0.97	1	1	0.63	1	1	0.66	1
RT (z)	0.918	0.998	0.972	0.972	0.968	0.997	0.997	0.635	0.992	0.998	0.658	0.997
RT (p)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
						Flowers						
Mean angle	140.14°	150°	128.45°	128.75°	161.29°	150°	150°	159.06°	120°	150°	158.17°	120°
Mean date	Oct	Nov	Oct	Oct	Nov	Nov	Nov	Nov	Oct	Nov	Nov	Oct
MVL (r)	0.94	1	0.97	0.972	0.97	1	1	0.63	1	1	0.66	1

RT (z)	0.945	0.998	0.972	0.971	0.968	0.997	0.997	0.635	0.997	0.998	0.658	0.997
RT (p)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Immature fruits												
Mean angle	154.61°	150°	156.79°	150°	161.29°	150°	150°	154.93°	150°	150°	158.13°	150°
Mean date	Nov	Nov	Nov	Nov	Nov	Nov	Nov	Nov	Nov	Nov	Nov	Nov
MVL (r)	0.98	1	0.98	1	0.97	1	1	0.76	1	1	0.54	1
RT (z)	0.975	0.998	0.976	0.989	0.968	0.997	0.997	0.759	0.997	0.998	0.536	0.997
RT (p)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Mature fruits												
Mean angle	156.62°	154.87°	159.34°	155.48°	161.29°	158.75°	155.87°	153.13°	155.87°	150°	164.74°	158.99°
Mean date	Nov	Nov	Nov	Nov	Nov	Nov	Nov	Nov	Nov	Nov	Nov	Nov
MVL (r)	0.97	0.98	0.97	0.98	0.97	0.97	0.98	0.83	0.98	1	0.53	0.97
RT (z)	0.970	0.981	0.971	0.979	0.968	0.972	0.978	0.832	0.978	0.998	0.535	0.971
RT (p)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Seed dispersal												
Mean angle	162.06°	151.63°	153.34°	155.48°	154.66°	153.57°	155.87°	153.13°	153.80°	150°	169.30°	159.35°
Mean date	Nov	Nov	Nov	Nov	Nov	Nov	Nov	Nov	Nov	Nov	Nov	Nov
MVL (r)	0.88	0.99	0.99	0.98	0.98	0.99	0.98	0.83	0.98	1	0.55	0.9
RT (z)	0.878	0.993	0.986	0.979	0.982	0.986	0.978	0.832	0.985	0.998	0.547	0.901
RT (p)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

MVL (r) = Mean vector length; RT (z) = Rayleigh Test (z); RT (p) = Rayleigh Test (p); Period 1 = June/2019 to May/2020; Period 2 = June/2020 to May/2021; Period 3; June/2021 to May/2022.

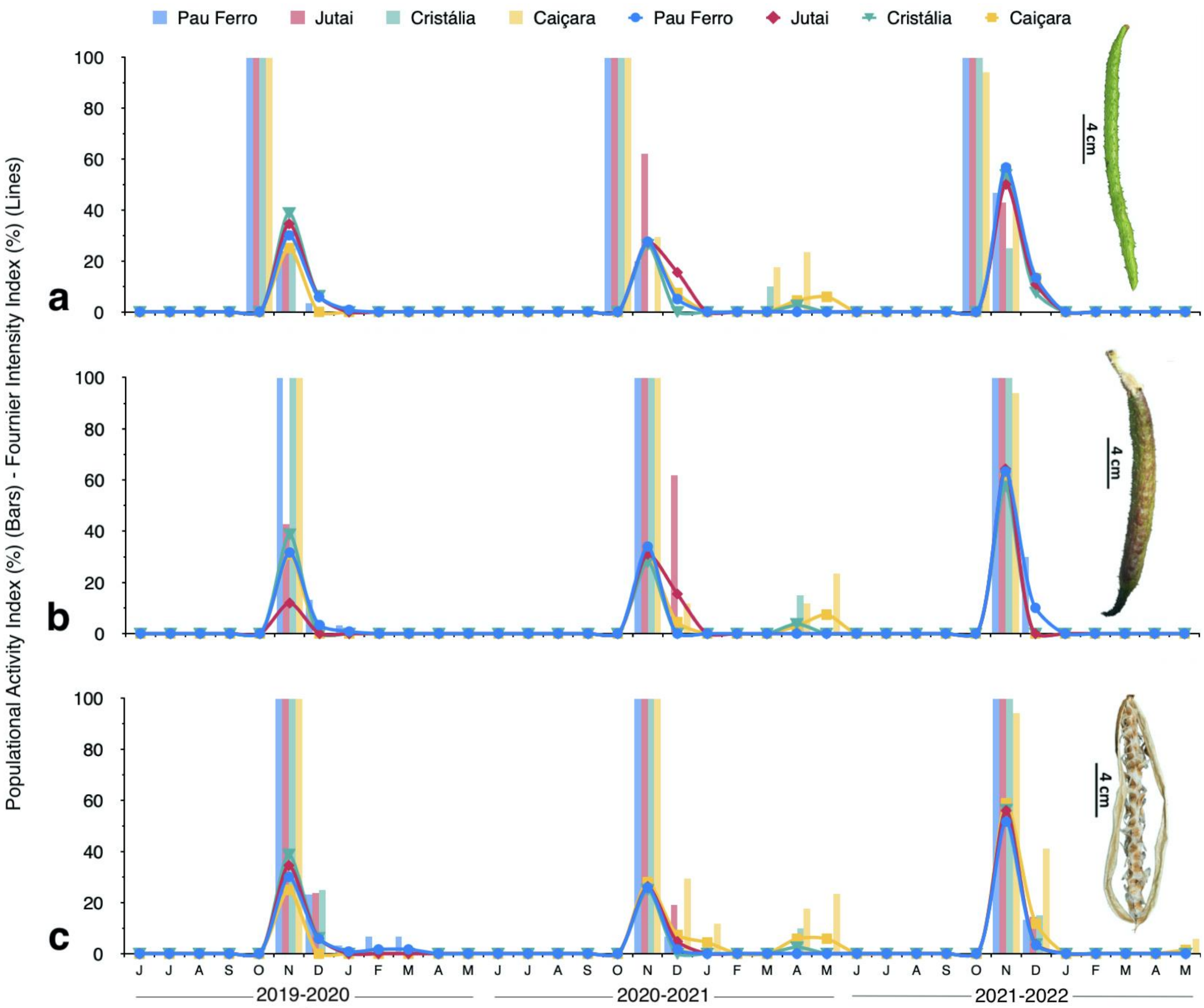

Fig. 5. Reproductive phenophases of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae) during 36 months (June/2019 to May/2022) in populations located in SDTF, in northeastern Brazil. (a). Immature fruit. (b). Mature fruit. (c). Seeds dispersing. Activity Index (Bars) and Fournier Intensity Index (%) (lines).

3.4. Effects of environmental variables and plant traits on phenology

Vectors close to each other have positive relationships, while vectors positioned opposite each other (at 180°) show negative relationships (Fig. 6). Multiple Factorial Analysis (MFA) evidenced that the vegetative phenophases (LeafFlush and MatureLeaf) have positive relationships with each other, and that they increase when the presence of YoungLeaf is reduced. The reproductive phenophases have stronger relationships (vector directions and lengths) among floral buds (Buds) and flowers and the formation of immature and mature fruits (ImmatureF and MatureF). The effects of environmental variables and plant traits on the phenology of *H. spongiosus* evidenced that leaf formation (LeafFlush and MatureLeaf) is related to plant height (PlantH and TrunkH), especially among trees that grow in more pedogenically developed soils (Orthic Chromic Luvisols in Jutaí and Cristália) that contain more clay and higher cation exchange capacities (CEC) (Table 1-2). It is important to note that the populations with smaller diameters at breast height (DBH) (Pau ferro and Caiçara) had greater numbers of plants producing buds and flowers (Table 1; Fig. 4).

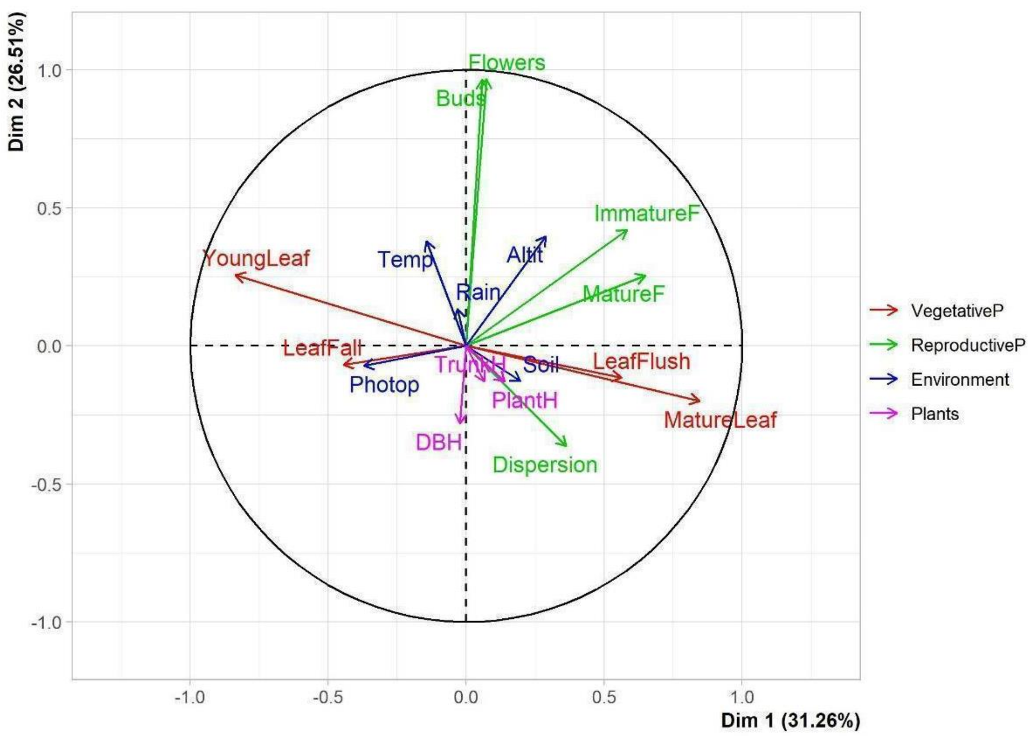

Fig. 6. Multiple Factorial Analysis (MFA) of the phenology (VegetativeP and ReproductiveP), plant traits of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae), and the abiotic variables of the

study sites in SDTF, in northeastern Brazil. Rain = Rainfall; Photop = Photoperiod; Temp = Mean temperature; Soil = Soil type; Altit = Altitude; PlantH = Total plant height; TrunkH = Trunk height; DBH = Diameter at breast height; LeafFlush = Leaf flushing; YoungLeaf = Young leaf; LeafFall = Leaf fall; MatureF = Mature fruits; ImmatureF = Immature fruits; Dispersion = Dispersed seeds; VegetativeP = Vegetative phenophases; ReproductiveP = Reproductive phenophases.

The generalized additive models (GAMs) evidenced meteorological variables as smoothing aspects and produced models with explanatory powers varying between 0.47 and 0.93 for R2, and Deviance values oscillating between 0.52 and 0.95 (Supplementary Table 3). The GAMs indicate how the dependent variables (phenophases) were influenced along the distribution interval by each predictor (meteorological variables) (Fig. 7), which had not been detected in the MFA (Fig. 6). The solid lines indicate the mean distribution of each phenophase, while the shaded areas indicate their respective 95% confidence intervals. Precipitation and photoperiod were the meteorological variables that most explained the vegetative phenophases (Supplementary Table 3; Fig. 7b-c). It can be noted that the vegetative phenophases are sensitive to low precipitation volumes (slightly more than 30 mm) and day lengths (with an average of 12 h), as they promote significant increases in leaf emission. Temperature reduced uncertainty in shoot emission between 26 and 28° C (Fig. 7a). There were low positive linear relationships between temperature, young leaves, and leaf fall, although those relationships were not significant (Supplementary Table 3). High temperatures and high rainfall volumes caused high uncertainty in the vegetative phenophase data (increased confidence intervals), suggesting little or no effect. Temperature and photoperiod were the environmental variables that most influenced the reproductive phenophases over time (Fig. 7d-e). Low rainfall has been shown to be the sensitive threshold for initiating flower bud formation and flowering, even if that effect is not significant (Supplementary Table 3; Fig. 7e).

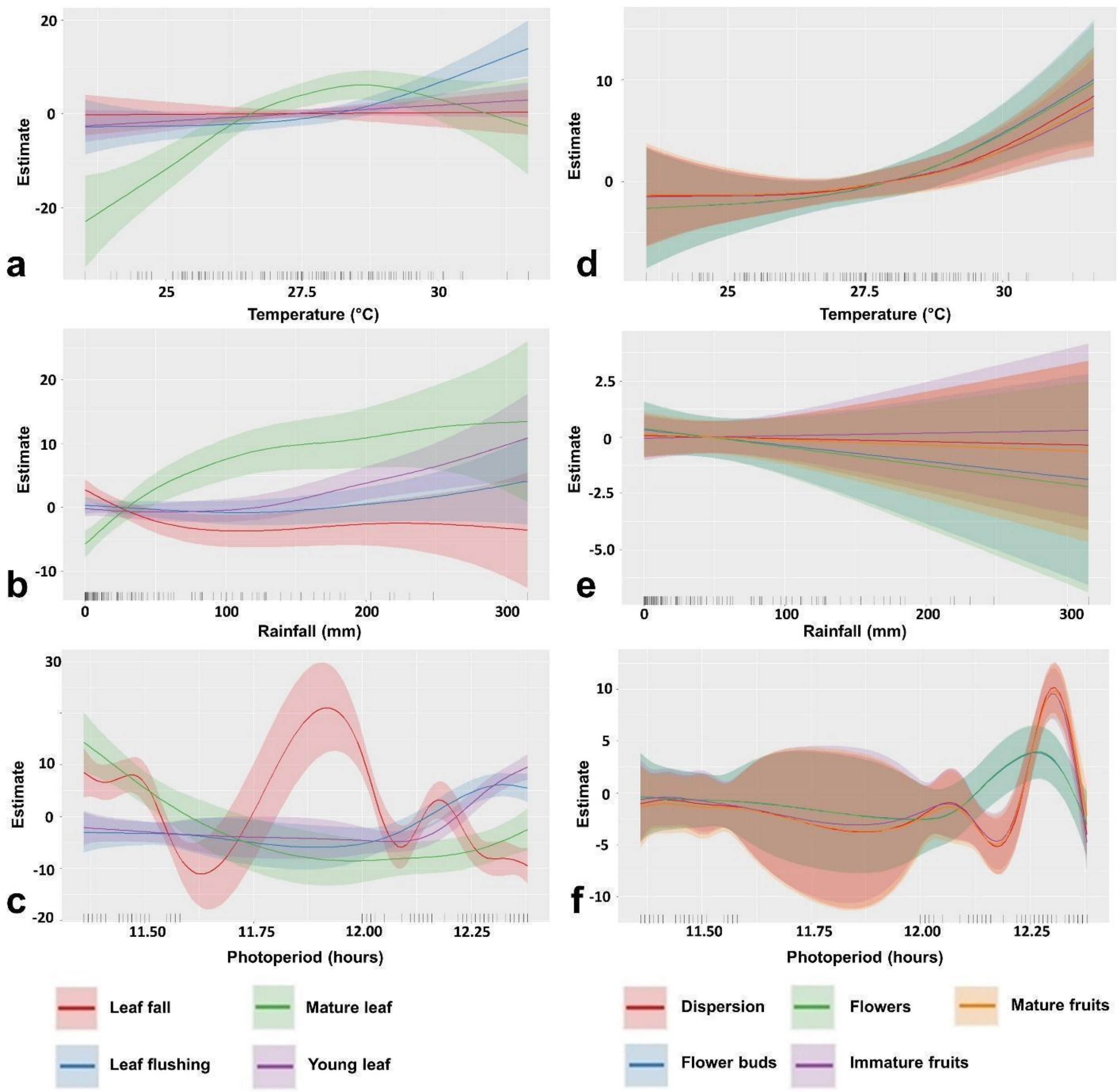

Fig. 7. Partial dependence of the generalized additive model (GAM) for the vegetative and reproductive phenophases of *Handroanthus spongiosus* (Rizzini) S. Grose and the abiotic variables of study sites in SDTF, in northeastern Brazil. (a) Partial dependence of the vegetative phenophases on the average monthly temperature; (b) Partial dependence of the vegetative phenophases on accumulated monthly rainfall; (c) Partial dependence of the vegetative phenophases on Photoperiod; (d) Partial dependence of the reproductive phenophases on the mean monthly Temperature; (e) Partial dependence of the reproductive phenophases on accumulated monthly Rainfall (f) Partial dependence of the reproductive phenophases on photoperiod. The shaded areas represent the 95% confidence interval. The Estimate Value indicates the intensity with which a meteorological variable influences a given phenophase.

4. Discussion

Based on a temporal set (36 months) of vegetative and reproductive data from four populations of *H. spongiosus*, the seasonal phenological patterns in the SDTF were found to be concentrated during times of greatest water availability and evidenced only slight spatial-temporal variations. The regional austral summer climate in the study area, with high rainfall and long day lengths, favored flushing and flower bud production in all of the populations. Our hypotheses that precipitation plays a key role, and that differences in soil properties and plant traits are important drivers of the vegetative and reproductive phenophases, were confirmed.

4.1. Vegetative phenology

The vegetative phenophases showed immediate responses to water availability at the onset of the rainy season, with all individuals responding simultaneously (Pezzini et al., 2014; Alberton et al., 2019; Borchert, 1994a, 1994b; Neves et al., 2017; Souza et al., 2020). Those responses corresponded well to the assumed paradigm for tropical vegetation, in which rainfall seasonality is considered the principal driver of plant phenology (Morellato et al., 2013). The initiations, durations, and intensities of the phenophases varied among the populations and were associated with spatial-temporal variations of the quantities and distributions of rainfall (Venter and Witkowski, 2019). Changes in vegetative phenophases are known to be stimulated by changes in rainfall levels (Rivera et al., 2002; Lima et al., 2018), which helps to explain the rapid emission of leaf buds by *H. spongiosus* immediately after the first rains. That species is characterized by having high density wood (Grose and Olmstead, 2007), which limits its water storage capacity (Borchert, 1994b). The phenology of species having high density wood has been found to be directly related to the availability of soil water, especially among species living in seasonally dry tropical ecosystems (Lima and Rodal, 2010; Lima et al. 2021; Neves et al. 2017; Neves et al. 2022).

Handroanthus spongiosus evidenced a decrease in the formation of both young and mature leaves as well as increased leaf fall with increasing temperatures under dry conditions – which is considered a strategy for efficient water use and water loss avoidance due to evapotranspiration in SDTF habitats (Reich, 1995; Goldstein et al., 2008; Toledo et al., 2012; Araújo and Lobo., 2020; Silva et al., 2022a). Leaf fall in plants growing in seasonal ecosystems can be influenced by increases in temperature, photoperiod, or soil water availability, factors then can act independently or together (Borchert et al., 2002; Asgarzadeh et al., 2010; Luna-Nieves et al., 2022). The present work evidenced that clayey soils with high porosity favored greater leaf formation. Luizão et al. (2004) noted that well-drained clayey soils favor the growth of tall trees with large biomasses (high leaf formation, for example). Soils with high percentages of sand and reduced porosity, however, resulted in reduced growth, although with greater formation of buds and flowers (which may reflect abscisic acid production by their roots under water stress conditions) (Tuteja, 2007; Taiz et al., 2017).

Leaf fall was strongly influenced by a photoperiod <12 hours, coinciding with the period of reduced rainfall between April and October (the dry austral winter), with monthly rainfall rates <10 mm, diminished relative humidity of the air and soil water availability, the negative water balance of the plants due to high rates of evapotranspiration, and reduced stomata control by older leaves (Borchert, 2000). Those factors will result in greater water losses by plants and initiation of leaf abscission (Lima et al., 2021).

Leaf fall is a functional characteristic of SDTF tree species that allows them to tolerate long periods of drought (Borchert, 1994b; Hoffman et al., 2011; Chaturvedi et al., 2021). Those trees can survive under severe semiarid conditions by compensating for low soil water availability and water pressure deficits through strong stomata control that limits evapotranspiration and restricts photosynthesis. During the short rainy season, on the other hand, those same trees maximize their growth rates (as seen in the present study) by concentrating the formation of leaf buds and young leaves during the rainy months (October and November). There are, however, other tree species in the same ecoregion that are not deciduous during the dry season, as they make rigorous physiological adjustments that reduce water losses and allow for the continuation of photosynthetic activities (Santos et al., 2015; Chaturvedi et al., 2021).

4.2. Reproductive phenology

The reproductive phenophases of *H. spongiosus* evidenced annual patterns (Newstrom et al., 1994) limited to the rainy season. It is likely that those reproductive phenological events are triggered by precipitation and a combination of increased temperatures and photoperiods (Rivera et al., 2002; Nunes et al., 2012; Pezzini et al., 2014; Lima et al., 2012; Lima et al. al., 2021), as it is well-known that plants can use more than one environmental trigger to regulate flowering (Silva et al., 2021). The emergence of flower buds and flowers in the Caatinga domain has mainly been attributed to the decreased water stress after the first rains in the dry period, and to increasing temperatures (Andrade et al., 2020), as seen, for example, with the reproductive phenology of *Z. joazeiro* Mart. (Nadia et al., 2007). But changes in onset dates, frequencies, and intensities of reproductive phenophases in seasonally dry tropical areas can nonetheless be observed in response to interannual variations in rainfall (Luna-Nieves et al, 2017).

It is known that plant growth also affects the intensity of the reproductive phenophases of trees, as has been reported for *Dalbergia nigra* (Vell.) Allemão ex Benth. (Silva et al., 2022a). In that study, photoperiod was shown to be the environmental factor that most strongly affected reproductive phenological variables (flowering and seed dispersal) when receiving >12 hours of sunlight, indicating that it can be used by individuals of *H. spongiosus* to synchronize flowering among plants in different localities; that same effect was observed in *Croton heliotropiifolius* Kunt. (Costa et al., 2021). The reproductive initiation of *H. spongiosus* occurring after days with more than 12 hours of sunlight suggest that this is a long-day plant (LDP) (Bennie et al., 2016).

The flowering of *H. spongiosus* is characterized as explosive (Gentry, 1982), where all individuals flower at the same time. This synchrony in flowering among individuals facilitates the attraction of a generalized spectrum of potential pollinators, mainly bees (Janzen, 1967), which are attracted by the abundance, even if only momentary, of flowers and nectar, and they may abandon their regular foraging patterns (Gentry, 1974). This attraction is further intensified because the explosive flowering occurs during a period of general moisture shortages, and pollinators will then benefit from the abundant supply of fresh nectar (Janzen, 1967; Gentry, 1974). Another important point is that flowering occurs when there are no leaves in the canopy, making the flowers even more visible at greater distances (Janzen, 1967; Gentry, 1974). Rainfall was found to be a limiting factor for flowering when indices were <10 mm; large variations in flowering were also observed among populations when rainfall exceeded 100 mm, probably because of damage to the petals, mechanical abscission of the buds and flowers, as well as reduced visitation by pollinators – all leading to a lower intensity of fruit formation.

The flowering and fruiting of *H. spongiosus* was concentrated at the beginning of the rainy season, with heavy flowering after the first 10 mm of rainfall (in October), followed by the dispersal of anemochoric seeds 12 days later (in November). This can be considered a strategy that takes advantage of the increased visibility of flowers as well as the efficient dispersal of anemochoric seeds among naked branches – thus facilitating both pollinator attraction and seed dispersal (Elzinga et al. 2007; Mendoza et al. 2017), as well as subsequent seedling establishment during the rainy season (which extends until April).

5. Conclusion

Populations of *Handroanthus spongiosus* showed distinct initiations, peaks, and durations of their vegetative phenophases, mainly in terms of budding and mature leaves; their reproductive phenophases, on the other hand, showed little spatial-temporal variation. The vegetative and reproductive phenophases of the populations studied, with the overlapping formation of new leaves, floral buds, and fruits occurred at the beginning of the rainy season. Photoperiods >12 hours and rainfall >10 mm were found to be drivers for breaking bud dormancy. The effects of secondary plant traits (such as tree height on leaf flushing and mature leaves) were most pronounced among plants growing on more pedogenically developed soils with higher clay contents. Tree populations growing on sandy soils and having smaller diameter at breast height, on the other hand, evidenced greater numbers of plants producing buds and flowers. Seeds should be harvested as soon as the fruits are mature in November, before seed dispersal occurs, as collection delays will reduce their availability. Our results, in addition to being important for understanding the phenological rhythms of this endemic and threatened species, are robust, as they were undertaken for over three years and evaluated four populations and more than 80 individuals, and therefore lay the groundwork for seed collection programs of native Caatinga species and the implementation of reforestation programs.

Appendix. Supplementary data

Supplementary table 1. Mean values of leaf longevity (in months) of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae), in FTSS, state of Pernambuco, Brazil.

	Síte			
Period	Pau ferro	Jutaí	Cristália	Caiçara
1	7.5±0.17 aA	7.6±0.16 aA	7.3±0.15 aA	7.7±0.15 aA
2	7.2±0.25 aA	7.2±0.20 aA	7.7±0.15 aA	7.3±0.15 aA
3	7.4±0.22 aA	7.3±0.26 aA	7.6±0.16 aA	7.7±0.15 aA
CV (%) = 7.86				

CV = coefficient of variation. Means followed by the same letter in the column do not differ significantly by the tukey test ($p < 0.05$), and means followed by the same uppercase letters in the row do not differ statistically by the tukey test ($p < 0.05$). (Mean ± standard error).

Supplementary table 2. Results of Mardia-Watson-Weller (W) test for variation in data of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae), monitored from June of 2019 - May of 2022 in SDTF populations, in Northeastern Brazil.

		Leaf flushing	Young leaf	Mature leaf	Leaf fall	Flower bud	flower	immature fruits	Mature fruits	seed dispersal
Populations		W	W	W	W	W	W	W	W	W
Pau-ferro x Caiçara	Period 1	70.02**	22.54**	15.30**	18.01**	70.06**	62.81**	0.15ns	9.85*	12.44**
	Period 2	39.06**	2.35ns	10.93*	10.11*	1.74ns	13.23**	12.65**	21.09**	27.66**
	Period 3	8.89*	2.47ns	12.24**	3.53ns	14.19**	12.38**	9.07*	2.07ns	11.82*
Pau-ferro x Cristália	Period 1	66.31**	56.52**	1.34ns	12.88**	61.84**	46.19**	0.98ns	2.40ns	6.15*
	Period 2	29.99**	0.36ns	0.27ns	44.53**	1.03ns	17.53**	4.44ns	0.57ns	0.48ns
	Period 3	4.66ns	0.55ns	5.54ns	3.24ns	5.56ns	11.92*	10.32*	4.28ns	0.25ns
Pau-ferro x Jutaí	Period 1	66.27**	65.66**	2.27ns	12.48**	26.17**	18.99**	0.84ns	3.54ns	2.38ns
	Period 2	40.77**	3.57ns	1.53ns	5.05ns	22.42**	15.27**	18.56**	13.23**	7.80*
	Period 3	4.83ns	0.64ns	1.22ns	0.75ns	92.04**	97.50**	8.39*	0.51ns	0.17ns
Caiçara x Cristália	Period 1	14.57**	15.40**	13.09**	12.06**	0.22ns	0.55ns	1.38ns	11.87*	2.43ns
	Period 2	61.87**	2.69ns	12.69**	69.23**	0.09ns	0.01ns	12.40**	17.45**	23.77**

	Period 3	8.09*	9.01*	1.00ns	12.47**	0.40ns	5.05ns	1.59ns	2.53ns	3.80ns
Caiçara x Jutaí	Period 1	3.63ns	10.16*	11.88*	0.32ns	93.91**	88.59**	4.32ns	2.88ns	2.33ns
	Period 2	6.14*	4.04ns	11.70*	21.45**	14.35**	19.46**	12.29**	9.64*	16.09**
	Period 3	7.08*	8.25*	16.59**	6.48*	156.23**	156.23**	0.53ns	1.10ns	3.85ns
Cristália x Jutaí	Period 1	46.68**	16.47**	1.39ns	0.26ns	102.18**	81.88**	0.52ns	2.36ns	1.74ns
	Period 2	41.03**	2.40ns	0.60ns	17.09**	9.07*	12.47**	20.63**	20.60**	2.16ns
	Period 3	1.33ns	2.52ns	8.89*	2.91ns	161.32**	161.32**	1.97ns	4.37ns	3.63ns

(*) $p < 0.05$; (**) $p < 0.01$ indicates statistical differences, using the Mardia-Watson-Wheeler (W) test; (ns) indicates non-significant statistical differences, using the Mardia-Watson-Wheeler (W) test.

Supplementary table 3. Summary of the generalized additive model (GAM) for vegetative and reproductive phenophases of *Handroanthus spongiosus* (Rizzini) S. Grose and abiotic variables of study sites in SDTF, in Northeastern Brazil. Effective degrees of freedom (edf), F-test values, and P-value are provided for each of the following variables: Temperature (°C), Rainfall (mm), and Photoperiod (hours). The coefficient of determination (R2) and the explained deviance (DE) of each model is also shown.

	Smooth terms used	Edf	*F-test*	*P-value*
	Vegetative phenophases			
Leaf flushing - R2 (0.80); DE (0.83)	s(Temperature)	2.81	8.06	0.00003
	s(Rainfall)	2.04	0.69	0.00003
	s(Photoperiod)	4.52	9.12	0.00000
Young leaf - R2 (0.82); DE (0.85)	(Temperature)	1.00	2.48	0.11856
	s(Rainfall)	2.66	2.43	0.00412
	s(Photoperiod)	4.82	22.22	0.00000
Mature leaf - R2 (0.68); DE (0.72)	s(Temperature)	3.59	7.29	0.00001
	s(Rainfall)	2.96	14.15	0.00000
	s(Photoperiod)	3.28	9.57	0.00000
Leaf fall - R2 (0.93); DE (0.95)	(Temperature)	1.00	0.02	0.90553
	s(Rainfall)	3.01	3.97	0.00516
	s(Photoperiod)	8.27	10.12	0.00000
	Reproductive phenophases			
Flower buds - R2 (0.47); DE (0.52)	s(Temperature)	2.28	4.32	0.00485
	(Rainfall)	1.00	0.64	0.42553
	s(Photoperiod)	5.33	0.99	0.00703
Flowers - R2 (0.47); DE (0.52)	s(Temperature)	2.24	4.02	0.00703
	(Rainfall)	1.00	0.88	0.34966
	s(Photoperiod)	5.38	3.09	0.00551
Immature fruits - R2 (0.68); DE (0.72)	s(Temperature)	2.38	2.96	0.03650
	(Rainfall)	1.00	0.02	0.88020
	s(Photoperiod)	8.04	11.87	0.00000
Mature fruits - R2 (0.68); DE (0.72)	s(Temperature)	2.46	2.93	0.03950
	(Rainfall)	1.00	0.09	0.75420
	s(Photoperiod)	7.92	11.10	0.00000
Seed dispersal - R2 (0.72); DE (0.76)	s(Temperature)	2.59	3.85	0.01140
	(Rainfall)	1.00	0.04	0.85010
	s(Photoperiod)	8.14	13.31	0.00000

Edf. = Effective degrees of freedom of smoothed curves; R2= Coefficient of determination; DE = Deviance explained.

6. References

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ARTIGO II - Physiological potential of seeds of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae) determined by the tetrazolium test

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Jailton de Jesus Silva[1], Raquel Araujo Gomes[1], Maria Aparecida Rodrigues Ferreira[1], Claudineia Regina Pelacani[1], Bárbara França Dantas[2,*]

[1] Universidade Estadual de Feira de Santana, Departamento de Ciências Biológicas, Avenida Transnordestina, s/n - Novo Horizonte, 44036-900 Feira de Santana, BA, Brazil.

[2] Embrapa Semiárido, Rodovia BR-428, Km 152, Zona Rural, 56302-970 Petrolina, PE, Brazil.

*Correspondence: Bárbara França Dantas (B.F.D.) (barbara.dantas@embrapa.br)

Physiological potential of seeds of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae) determined by the tetrazolium test

Abstract: The tetrazolium test (TZT) can evaluate the viability and vigor of seeds quickly and in detail. The objective of this study was to determine the optimal conditions for conducting the TZT with seeds of *Handroanthus spongiosus*. For this purpose, seeds from three lots were pre-soaked in water for 16 h, followed by extraction of the tegument and immersion in varying solutions of tetrazolium (0.01-0.1%) for increasing periods (1-4 h) at 30 °C in the dark. The experimental design was com-pletely randomized with a 4 x 4 factorial scheme with 25 seeds per repetition. We applied generalized linear models and the Tukey test for pairwise comparisons of the means at 5% probability. The viability results were compared with those of the germination test at 25 °C using another sample of seeds from the same lots. The immersion of the seeds for 1 h and 4 h did not cause a clear coloration difference. The seeds subjected to all treatment concntrations for 3 h presented average viability greater than 60%, with no difference in germination percentage. The TZT treatment of 0.01% for 3 h was most efficient in assessing the viability of the *Handroanthus spongiosus* seeds.

Keywords: germination; forest seeds; vigor tests; sete-cascas

1. Introduction

Evaluation of the physiological potential of seeds is an important aspect, recommended soon after processing and before storage of seeds. Furthermore, for seeds already stored, it allows distinguishing lots with better quality and choice of parent trees in the field with superior quality for collection of seeds [1, 2].

The germination test and tetrazolium test (TZT) are among the main methods used to assess the physiological potential of seeds [3, 4]. The germination test is used most frequently for official evaluation of viability of seed lots in quality control programs [5]. However, the diagnosis of the seed quality by this test can take several days or even weeks, which can be too long in the case of recalcitrant seeds and seeds with low viability [6]. For example, the evaluation by the standard germination test of seeds of some species of the genus *Handroanthus* can take between 14 to 21 days [7]. This long time lag can hamper decisions about the choice and destination of seed lots [8, 9].

The tetrazolium test (TZT) is an alternative to the germination test to assess the physiological potential of seeds that is fast and enables a detailed analysis of their viability and vigor [10]. Besides this, it also allows diagnosis of the main problems that can affect the quality of seeds in a time frame only slightly longer than 24 hours [11, 12], such as me-chanical damages from collection or processing, damage due to moisture or drying, damages caused by insects and other pests and deterioration during storage [13]. The development of this test is considered one of the main advances in seed testing in the twenti-eth century [6]. However, it depends on tailoring the testing parameters for each species, such as conditions for hydration, concentration of the indicator solution, temperature and conditioning time [14].

The test is based on the action of the enzymes dehydrogenases, which catalyze the glucose reactions and the Krebs cycle, components of the respiratory chain [15]. During the respiration process, these enzymes act as H^+ acceptors and subsequently as H^+ donors, i.e., the transfer H^+ ions released from living tissues to the tetrazolium chloride solution, besides catalyzing the respiration process [15]. When the seed tissues come into contact with the clear TZ solution, a stable and non-diffusible compound is formed with reddish color, called triphenylformazan or just formazan [16]. Since the reaction occurs inside the seed cells and the compound does not diffuse, it is possible to observe separation of colored tissues (which are respiring and thus are alive) and noncolored (dead) tissues [13].

The tissues undergoing deterioration take on a stronger crimson red color due to the greater diffusion intensity of the TZ solution through the damaged cell membranes of those tissues [16].

This test is important in the global seed market, where companies and farmers need rapid and reliable information about the viability and vigor of seed lots to make quick decisions on the marketing and planting of seeds [6]. According to França-Neto and Krzyzanowski (2019), TZT is widely used for quality control of the seeds of many staple crops, forage grasses and fruits/vegetables.

For forest species, TZ testing has become a promising alternative for evaluation of the viability and vigor of seeds based on the same reliability and rapidity of results [9, 17, 18]. Many forest species have seeds with some type of dormancy, so they need long periods to germinate, besides often being recalcitrant [19]. Due to these aspects, the routine use of TZT allows diagnosing the physiological potential quickly and effectively, allowing faster and more accurate decisions.

Various studies have been published attesting to the viability of using this test on species of the Caatinga (shrubland) biome in Brazil [20]. However, few have been focused on the genus *Handroanthus* Mattos [21, 22], and to the best of our knowledge, none have been published on the species *Handroanthus spongiosus* (Rizzini) S. Grose.

H. spongiosus is popularly known by various names: cascudo, ipê-cascudo, sete-cascas, pau-d'arco, pau-d'arco-casca-fina and ipê-amarelo [23]. It is an endangered species according to Brazil's Ministry of the Environment [24]. Studies are scarce of this orthodox species reporting basic aspects, such as seed quality, multiplication, regenera-tion and natural adaptation, as well as conservation [25].

Due to the lack of information about the physiological potential, validation of viability protocols and determination of the quality of this species' seeds, the aim of this study was to determine the optimal conditions for use of the TZT to establish the viability of *Handroanthus spongiosus* seeds rapidly.

2. Materials and methods

2.1. *Plant material*

H. spongiosus fruits were collected in a seasonally dry tropical forest (SDTF) area in the Caatinga in the state of Pernambuco, Brazil. Voucher specimens were deposited with the herbarium of Feira de Santana State University (HUEFS), located in the municipality of Feira de Santana, Bahia state. Two of the collection sites were distributed in the village of Caiçara (9° 07'24.5" S, 40° 23' 16.1" W, 393 m) (HUEFS-252490) and the district of Cristália (8°5'56.3" S, 40°19'27.1" W, 403 m) (HUEFS-259093), both located in the mu-nicipality of Petrolina, Pernambuco. The third collection site was in the village of Jutaí (8° 33' 35.7" S, 40° 12' 1.9" W, 418 m) (HUEFS-259094), located in the municipality of Lagoa Grande, Pernambuco. All three collection sites are located in a region with semiarid climate (Köppen classification of Bsh), with average air temperature between 25 °C and 30 °C throughout the year, and low annual rainfall, between 300 mm and 1000 mm, concentrated from November to April [26, 27].

We collected mature fruits from 15 parent trees at each site, using a trimmer and impermeable tarp. In choosing the parent trees, we considered size, vigor and health, as well as a minimum spacing of 20 m between any two trees. The fruits were collected in the district of Cristália in November 2019, while those in the villages of Caiçara and Jutaí were gathered in November 2021. To conduct the tests, we formed three lots, representing the collection sites and year. After collection, the seeds were stored in a cold chamber (adjusted to 10 ± 3 °C and 60 ± 4% relative humidity) until the start of the experimental in April 2022.

2.2. Water content of the seeds (%)

The moisture content of the seeds was determined using two subsamples of 50 seeds from each of the three lots. These seeds were placed in aluminum capsules, which in turn were placed in an oven at 105 ± 3 °C for 24 h for drying. The weight differences were ex-pressed as percentage of dry weight in relation to the weight of fresh seeds (adapted from Brasil, 2013).

2.3. Germination test (G%)

The germination test was performed with four repetitions of 25 seeds, using three sheets of Germitest® paper moistened with a volume of water equal to the dry weight of the paper multiplied by 2.5. The seeds were placed to germinate in paper rolls, packed in plastic bags and maintained at a temperature of 25 °C in a biochemical oxygen demand (BOD) chamber with photoperiod of 12 hours (adapted from Brasil, 2013). The germination percentage was determined after 14 days by observing the emission of a main root with length of at least 2 mm.

2.4. Tetrazolium test (TZT)

The experimental design was completely randomized with a 4 × 4 factorial scheme (four tetrazolium solution concentrations and four immersion times). We used 100 seeds, subdivided into four repetitions of 25 seeds for each treatment.

We initially evaluated the optimal conditions for the test by adjusting the procedure for removing the tegument (pre-conditioning in water), along with the concentration of the reagent (2,3,5-triphenyl tetrazolium chloride – NEON brand), temperature and exposure time to the solution (data not shown).

The seeds were placed in acrylic germination boxes (gerboxes) measuring 10 x 10 x 5 cm, each on a paper towel sheet moistened with a volume of water equal to the weight of the dry paper multiplied by 2.5, kept on a tabletop in the laboratory (average temperature of 27 °C) for 16 hours. After this pre-soaking period, the tegument was removed from each seed (Figure 1). Then the seeds were immersed for 1, 2, 3 or 4 hours in the solution of 2,3,5-triphenyl tetrazolium chloride at concentrations of 0.01%, 0.05% [28], 0.075% and 0.1% [21], with pH adjusted to 6.5. The seeds in the solutions were then maintained in the dark in a BOD germination chamber adjusted to 30 °C.

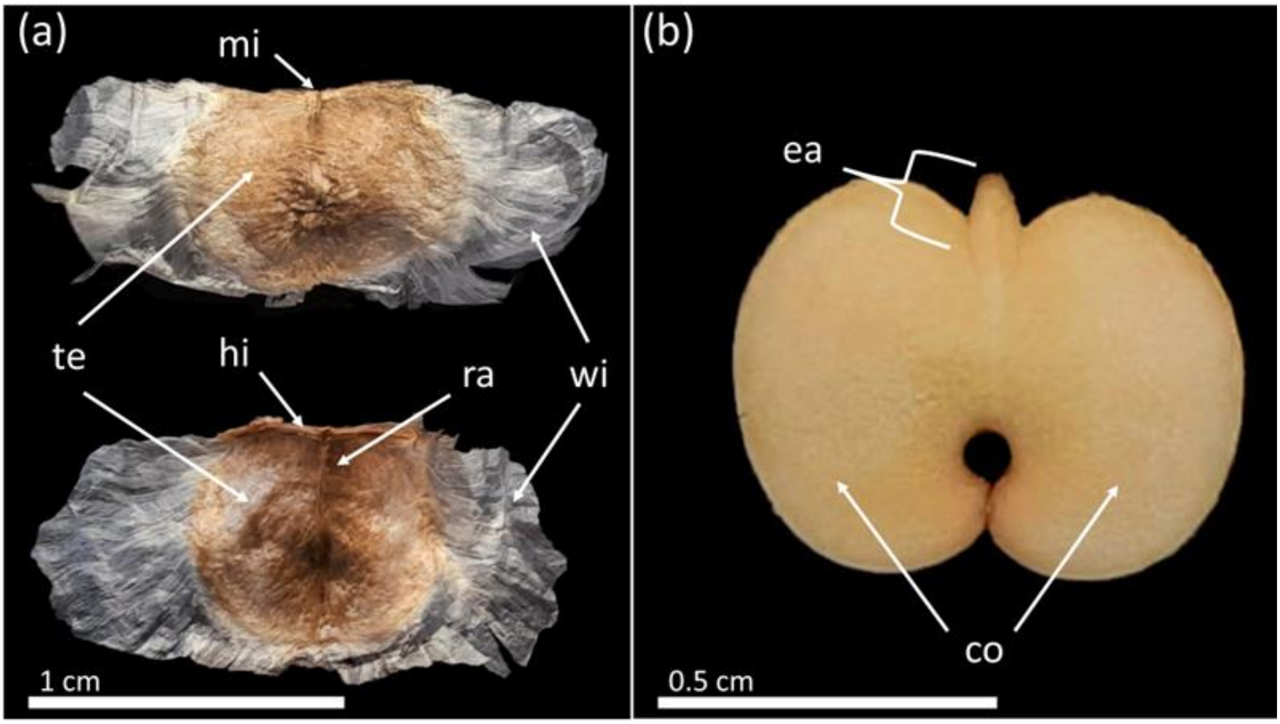

Figure 1. Morphology of the seeds of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae). (a) seed with tegument; (b) seed without tegument; (mi) micropyle; (te) tegument; (hi) hilo; (ra) raphe; (wi) wings; (ea) embryonic axis; (co) cotyledons.

2.5. Analysis of the data

The Shapiro-Wilk test [29] was applied to evaluate the normality of the residuals of the seed viability values, while the Levene test [30] was applied to determine the homogeneity of variances. We then applied generalized linear models (GLMs) to the data because the residuals (errors) of the model had distribution different than normal. After the analysis of the GLMs, we analyzed the significant differences in each factor (solution concentration, im-mersion time and viability percentage) by pairwise comparisons with the post-hoc Tukey test at 5% significance, with adjustment of the means by the Šidák method [31]. To assure the best fit of the methodology, we compared the viability percentage data obtained by the TZT with the germination percentage data obtained by the Dunnett test [32]. All the analyses were carried out with the R software [33].

3. Results and Discussion

The initial water content percentages of the seeds were 5.24%, 5.26% and 5.67% for the Jutaí, Caiçara and Cristália collection sites, respectively. The lots were therefore considered uniform regarding water content, which contributed to diminish the differences in speed during the preconditioning and favored the standardization at the moment of the reaction (staining) of the tissues [15].

The seeds of *H. spongiosus* generally have water content near 5% according to previous studies to evaluate their physiological quality [34, 25]. The seeds are thus classified as dry, and are dispersed in November and December by the wind (anemochory).

The data on percentage of viable seeds obtained by the TZT did not present normality of residuals ($p < 0.05$) and homogeneity of variances ($p < 0.05$) for any of the lots (Table 1). The generalized linear models (GLMs) indicated a significant interaction ($p < 0.01$) between the effects studied for all the lots.

Table 1. Analysis of deviation (ANODEV) for viability (%) of seeds of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae) from different lots, submitted to the tetrazolium test (TZT) with different concentrations and immersion times.

Cristália/2019					
Source of variation	DF	DF Difference	Deviance	Difference of Deviance	p-value
Null		63		1294.25	
Concentration (C)	3	60	87.12	1207.13	< 0.01
Time (T)	3	57	984.26	222.88	< 0.01
C*T	9	48	133.78	89.10	< 0.01
CV (%) = 24.12	W= <0.001	L= <0.001			
Caiçara/2021					
Source of variation	DF	DF Difference	Deviance	Difference of Deviance	p-value
Null		63		1050.95	
Concentration (C)	3	60	56.18	994.77	< 0.01
Time (T)	3	57	935.87	58.90	< 0.01
C*T	9	48	16.74	42.16	< 0.01
CV (%) = 22.22	W= <0.001	L= <0.001			
Jutaí/2021					
Source of variation	DF	DF Difference	Deviance	Difference of Deviance	p-value
Null		63		935.05	
Concentration (C)	3	60	87.15	847.90	< 0.01
Time (T)	3	57	759.97	87.93	< 0.01
C*T	9	48	36.60	51.33	< 0.01
CV (%) = 26.41	W= <0.001	L= <0.001			

Where: DF: degrees of freedom; W: Shapiro-Wilk test; L: Levene test; CV: coefficient of variation.

The percentage of germinated seeds varied between 72 and 90%, represented by the lots collected at the Caiçara and Cristália sites, respectively (Table 2).

Table 2. Results of the viability (%) of seeds of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae), by the tetrazolium test (TZT) at different concentrations and immersion times in the solution.

TZ Concentration (%)	Time (hours)			
	1	2	3	4
Cristália/2019				
G (%) = 90				
0.01	*0 aB	*0 dB	82 aA	88 aA
0.05	*0 aB	*39 cB	94 aA	90 aA
0.075	*0 aC	*71 bB	95 aA	*79 aA
0.1	*0 aB	96 aA	94 aA	*67 aA
Caiçara/2021				
G (%) = 72				
0.01	*0 aD	*6 cC	67 bB	75 bA
0.05	*0 aC	*46 aB	71 aA	77 aA
0.075	*0 aC	68 bB	73 aA	*91 aA
0.1	*0 aC	75 aB	72 aA	*94 aA
Jutaí/2021				
G (%) = 88				
0.01	*0 aB	*0 bB	84 bA	89 bA
0.05	*0 aC	*44 aB	83 bB	*98 aA
0.075	*0 aC	*54 aB	85 aA	*97 aA
0.1	*0 aC	86 aB	89 aA	*97 aA

Where: means followed by (*) (asterisk) indicate difference in germination percentage G (%) by the Dunnett test at 5% probability. Means followed by different lowercase letters in the column differ from each other and means followed by different lowercase letters in the row do not differ from each by the Tukey test at 5% probability. The means were adjusted by the Šidák method. G (%): germination percentage.

By using the TZT, it was possible to determine the classes of the viable (Figure 2) and unviable seeds (Figure 3). The class of viable seeds had nearly uniform crimson red color and the tissues were firm (Figure 2). Some superficial damages were observed, mainly at the edges of the cotyledons, but this did not affect the viability of these seeds (Figure 2k-n). These damages might have been caused during the process of seed collection, processing or tegument removal [35].

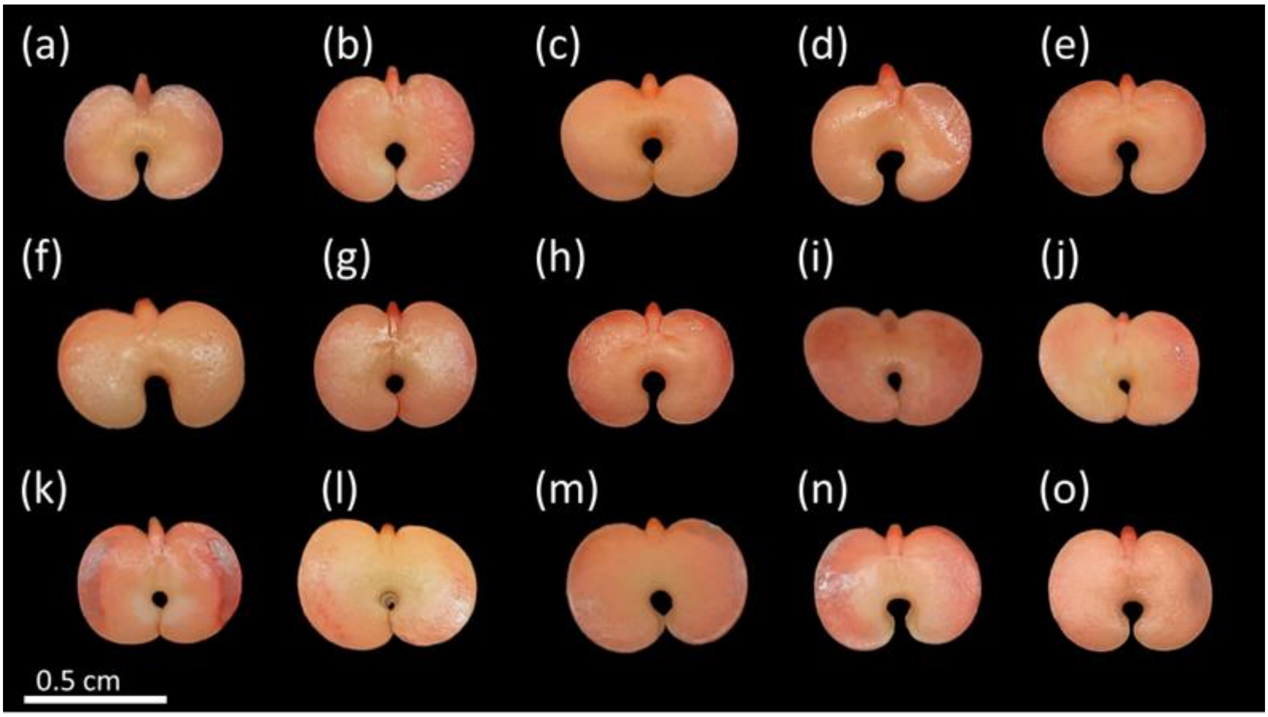

Figure 2. Classes of viability of seeds of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae) after submission to the tetrazolium test (TZT). Class 1: viable seeds; embryo, cotyledons and other regions of the seed with crimson red color and firm tissues, with regions of connection between cotyledons and embryonic axis also strongly colored.

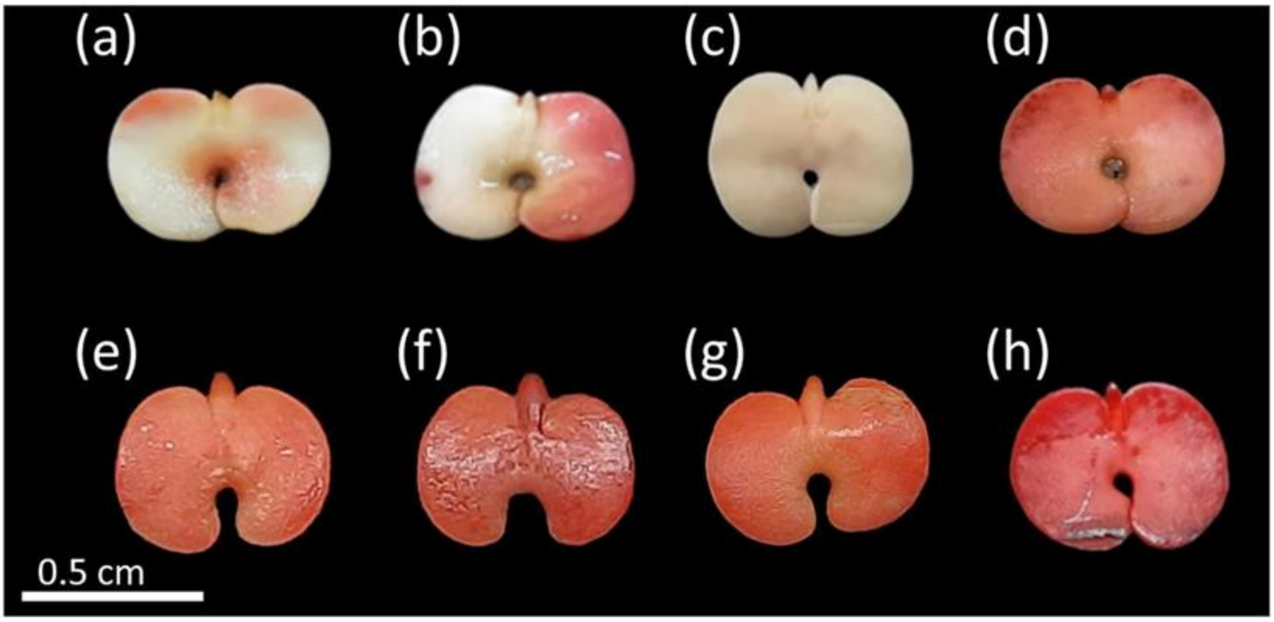

Figure 3. Classes of viability of seeds of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae) after submission to the tetrazolium test (TZT). Class 2: unviable seeds; more than 50% of the cotyledons uncolored (a and b); embryonic axis and cotyledons without staining; (c); damaged embryo and cotyledons with intense crimson red color (d, e, f, g and h).

The seeds classified as unviable were those with large portions of tissues without staining, mainly affecting the cotyledons and embryonic axis (Figure 3a-b), as well as the presence of structures without any staining (Figure 3c) or with damaged cell membranes (more intense color) (Figure 3d-h), also accompanied by dead tissues (without staining) at the lower end of the embryonic axis (Figures 3d and 3h). Figures 3e and 3f show tissues with flaccid texture and weak resistance to touch.

The immersion of the seeds for 1 h, at any concentration of the TZ solution, was not sufficient to reduce the TZ salt in the tissues, and there was no staining, making distinction between viable and deteriorating tissues impossible (Table 2). Similar results were reported in *Libidibia ferrea* (Mart. ex Tul.) LP Queiroz var. ferrea seeds, in which the time of 1 h was insufficient to color the tissues [36].

The period of immersion of the seeds in the TZ solutions should be evaluated with caution. The use of solutions with low salt concentrations and insufficient exposure periods can cause underestimation of the quality indicated by the test and consequently cause mistaken interpretation of the physiological quality of the seeds [9].

The seeds immersed for 2 h in the 0.05% tetrazolium solution had different germination percentage for all the lots, while the seeds immersed in the 0.1% solution were the only ones without difference in the germination percentage (Table 2). Seeds immersed for 3 h at all the concentrations did not present significant differences of viability percentage and germination percentage in any of the lots studied.

The seeds immersed for 4 h had uniformly intense staining, making it impossible to distinguish the live and dead or damaged tissues (Figure 3). This was observed in all the lots at concentrations of 0.075% and 0.1%, which differed from the germination test (Table 2). The immersion of the seeds for 4 h was efficient to evaluate the viability of the seeds only at the lowest concentration tested (0.01%).

Some researchers have reported that more intense staining of seeds after the TZT is associated with greater difficulty in differentiating tissues and identifying lesions, making it possible to confuse highly vigorous tissues with those having weak vigor [37, 38, 39, 40].

During the period of pre-conditioning adjustment of the seeds in distilled water, it was possible to observe the development of dark spots along the total length of the seeds, possibly due to excessive entry of water when the seeds were soaked for longer than 18 h, which could have caused damages to the tissues (Figure 4). Besides this, there was rapid absorption of water in the first 16 h. The establishment of a limit on pre-conditioning in distilled water serves to start measuring the metabolic activity, and consequently the validation of the test with adequate staining of the tissues.

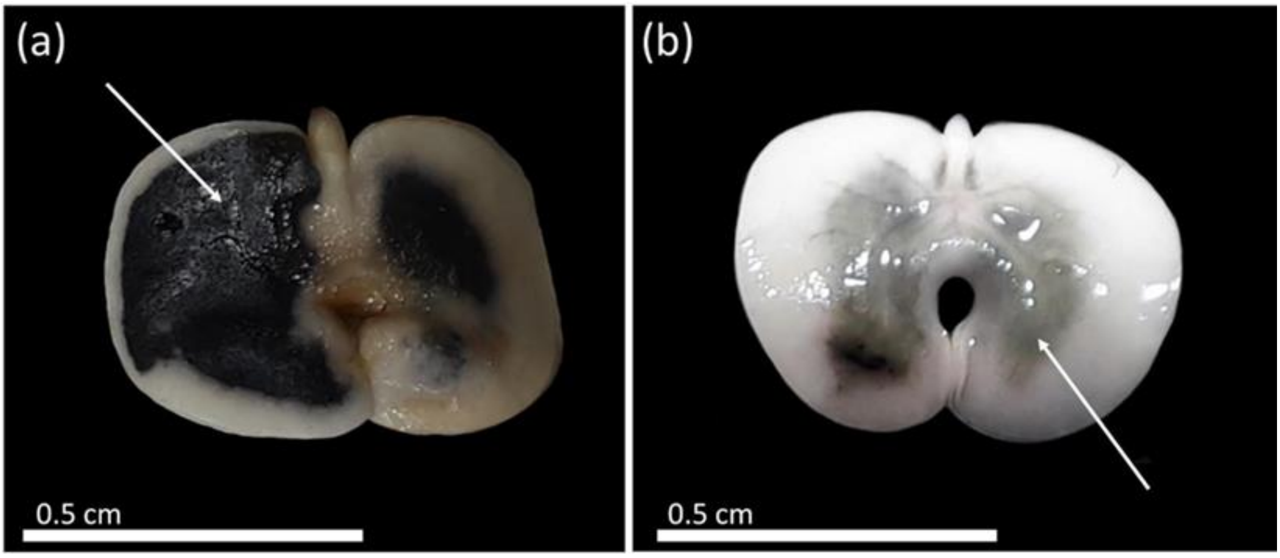

Figure 4. Formation of dark spots in seeds of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae) after soaking for 18 h in distilled water. (a) and (b) dark spots after soaking in distilled water for 18 h.

The ideal temperature for soaking of some species of the genera *Handroanthus* and *Tabebuia* can vary between 25 and 30 °C, while the soaking time varies between 12 and 24 h [21, 41, 28, 9]. These parameters indicate the time necessary for the water to hydrate all the seed tissues, overcoming the resistance levels imposed, which are variable among species.

The preliminary test results indicated that the pre-conditioning of the *H. spongiosus* seeds in distilled water for 16 h favored the removal of the tegument and was sufficient to facilitate the gradual entry of the tetrazolium solution in the tissues, as can be noted by the effective staining (Figure 2 and Table 2).

As observed in this study, the living tissues had a light red to bright crimson color (Figure 2), in contrast to the tissues in advanced stage of deterioration, which had an intense red color, bordering on purple, caused by the rapid diffusion of the solution through the damaged cell membranes (Figure 3d-h). In these damaged tissues, the cell membranes cannot resist the flow and diffusion of the solutions, which can easily react in the target tissue. The dead tissues, in turn, did not have final coloring by the TZT, indicated by the presence of milky white areas (Figure 3a-c).

Some authors recommend tetrazolium solutions in concentrations ranging from 0.5% to 1.0% with staining times ranging from 6 to 24 hours for agricultural species in general [13]. Specifically for some forest species, the concentrations can vary between 0.05% and 1%, with staining times ranging from 1 to 48 hours [20]. These concentrations and staining times should be adjusted for each species, due to the wide variety of seed shapes and compositions.

In other studies, the greatest efficiency in detecting the viability of seeds of ipê trees (*Handroanthus* spp. and *Tabebuia* spp.) was obtained with a 0.05% TZ solution after 4 h at 40 °C, for seeds of *T. aurea*, and after 24 h at 36 °C for *T. roseoalba* [28, 9]. The *H. spongiosus* seeds in this study needed shorter times and lower concentrations (Table 2), due to the smaller size and easier exposure of cotyledons and embryo to the solution.

In this work, the best combinations of immersion time and concentration of tetrazolium solution that presented uniformity, visual clarity in distinguishing living and dead tissues and coherence with the results of the germination test of the *H. spongiosus* were 2 h with 0.1% concentration and 3 h with any concentration studied, at 30 °C in the dark.

Since the intention of using the TZT is as an alternative to the use of germination and vigor tests, with faster results [5], we recommend using the shortest time and lowest solution concentration tested, to obtain time savings and lower costs of reagents [18]. However, special attention should be paid to the immersion time, to avoid underestimating the physiological quality of the seeds [35, 9].

4. Conclusions

The comparison between the tetrazolium test and the germination test of *H. spongiosus* seeds showed that the TZT is an efficient and reliable method to assess the viability of these seeds. The optimal conditions are removal of the tegument followed immediately by immersion in the solution of 2,3,5-triphenyl tetrazolium chloride at a concentration of 0.01% during 3 hours, to reduce time and costs.

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ARTIGO III - Conservation and physiological quality of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae) seeds

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Jailton de Jesus Silva[1], Sara de Souza Alencar[2], Raquel Araujo Gomes[2], Janete Rodrigues Matias[2], Claudinéia Regina Pelacani[1], Bárbara França Dantas[2*]

[1] Universidade Estadual de Feira de Santana, Departamento de Ciências Biológicas, 44036-900, Feira de Santana, BA, Brasil.

[2] Embrapa Semiárido, 56302-970, Petrolina, PE, Brasil.

* Corresponding author: barbara.dantas@embrapa.br

Conservation and physiological quality of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae) seeds

Abstract: *Handroanthus spongiosus* (Rizzini) S. Grose is an endangered tree species. However, its seed quality, storage, and conservation strategies are issues still unexplored. The objective of this work was to evaluate the physiological quality of *H. spongiosus* seeds subjected to different storage times, packaging, and environments for their conservation. A completely randomized experimental design was used, in a double factorial arrangement with an additional treatment (recently-harvested seeds), consisted of five storage times (up to 24 months) and six storage conditions, combining packaging types (permeable and impermeable) and environments (room, cold chamber, freezer, and liquid nitrogen conditions). Seed germination percentage and normal seedling percentage, shoot length, root length, and root to shoot dry weight ratio were evaluated. The seed germination and normal seedling percentages of *H. spongiosus* seeds conserved under room conditions decreased over the storage time. Normal seedling percentages decreased from the 12th month of storage onwards. Low and ultralow temperatures are recommended for short and medium-term conservation of *H. spongiosus* seeds, since they did not affect the growth of seedlings.

Keywords: Caatinga; dry forest; germination; longevity; storage.

1. Introduction

Anthropogenic actions have contributed for the degradation of the Caatinga biome through practices characterized by the exploratory process of this region, such as the removal and burning of native vegetation and agricultural and livestock activities (Ferreira et al., 2014). In addition, many plant species have been threatened around the world due to aggression to ecosystems and impoverishment of biodiversity caused by these actions (Voronkova et al., 2018).

Thus, conservationist strategies for threatened species have been developed and applied as alternatives for ex situ conservation through seed or seedling production in different times of the year (Shen et al., 2015). Therefore, determining conservation strategies for seeds of threatened wild species is needed and involves the maintenance of their viability and physiological potential during storage (Araújo et al., 2017).

The storage environment and packaging type affect the maintenance of viability and vigor of seeds in the short-, medium-, and long-terms. Successfully conserving forest seeds require previous information on physiological characteristics, since seeds from different species require different conditions for conservation (Vitis et al., 2020; Walters and Pence, 2021).

Determining ideal environmental conditions, such as relative air humidity and temperature, for the conservation of seeds is needed to maintain their physiological quality during storage (Veiga-Barbosa et al., 2013; Tonetto et al., 2015). An adequate storage environment can minimize the speed deterioration, allowing the maintenance of viability of seeds for a longer period than that obtained under natural non-controlled conditions (Torres et al., 2020). In addition, initial seed water content and storage packaging affect the maintenance of the seed physiological quality due to gas exchanges between seeds and the environment (Reis et al., 2012; Lúcio et al., 2016; Gomes et al., 2018; Ribeiro et al., 2018).

The plant species *Handroanthus spongiosus* (Rizzini) S. Grose, popularly known in Brazil as *cascudo, ipê-cascudo* or *sete-cascas*, belongs to the Bignoniaceae family; it is endemic to the Caatinga biome and is classified as an endangered species, according to the official national list of threatened flora species (Lohmann et al., 2013; Lohmann, 2020).

Seeds from plants of the *Handroanthus* genus present a relatively short natural viability period, which hinders the conservation and, consequently, the production of seedlings for these species (Cabral et al., 2003). Researchers have developed and published works involving the storage of different ipe species over the lats years due to the importance of these species (Shibata et al., 2012; Abbade and Takaki, 2014; Martins and Pinto, 2014; Tonetto et al., 2015; Maciel et al., 2020; Araújo et al., 2021). However, information on longevity, storage, or conservation is not found for *H. spongiosus* seeds.

Moreover, no information on the quality of *H. spongiosus* seeds stored under room, low, and ultralow temperatures is found. Thus, the objective of this work was to evaluate the physiological quality of *H. spongiosus* seeds subjected to different storage times, packaging, and environments for their conservation.

2. Materials and methods

2.1 Seed collection

Handroanthus spongiosus (Rizzini) S. Grose (HUEFS-259093) seeds were obtained by harvesting mature fruits (brownish) at the seed dispersion stage from nine plants in Lagoa Grande, PE, Brazil (8°34'4"S, 40°10'18"W), in December 2017. The fruits were processed to remove branches, leaves, damaged seeds, and other impurities, generating the recently-harvested seed lot.

2.2 Seed storage

The experiment was conducted in a completely randomized experimental design, in a double factorial arrangement, with an additional treatment (5×6+1). The factors consisted of storage times (6, 9, 12, 18, and 24 months) and storage environmental conditions, considering the packaging (permeable or impermeable) and environments (room, cold chamber, freezer, and liquid nitrogen conditions).

Recently-harvested seeds were placed in polyethylene bags (PB) and cotton bags (CB) for room condition storage (RC), with mean temperature of 25±4 °C and 45±3% relative air humidity, or in cold chamber (CC), set to 10±3 °C and 60±4% relative air humidity. Seeds were placed in PB for the freezer storage (FS; -20 °C and 66% relative air humidity). The seeds stored in liquid nitrogen (LN) (-196 °C) were kept in polyethylene cryogenic tubes. Thus, six storage conditions were tested: polyethylene bag packaging and room condition storage (PB-RC), cotton bag packaging and room condition storage (CB-RC), polyethylene bag packaging and cold chamber storage (PB-CC), cotton bag packaging and cold chamber storage (CB-CC), polyethylene bag packaging and freezer storage (FS), and cryotube packaging and liquid nitrogen storage (LN).

The recently-harvested seeds and seeds stored for 6, 9, 12, 18, and 24 months were evaluated for physiological quality (seed water content, germination, and vigor). The seeds stored in FS and LN were subjected to thawing (4 hours in a refrigerator and 1 hour under room temperature) before evaluating the seed quality (Alencar et al., 2018).

2.3 Seed physiological quality evaluations

The water contents of recently-harvested and stored seeds were obtained by the oven method at 105±3 °C for 24 hours using two samples of 50 seeds (Brasil, 2013).

The physiological quality of recently-harvested and stored seeds were evaluated through germination tests carried out using four replications of 50 seeds for each treatment. The seeds were distributed over three Germitest paper sheets, moistened with distilled water at the proportion of 2.5-fold the dry paper weight, individually placed in polyethylene bags, and incubated in a BOD (biochemical oxygen demand) chamber at constant temperature of 25±1 °C and photoperiod of 12 hours (adapted from Brasil, 2013) for 14 days. Then, the germination percentage (%G) and normal seedling percentage (%NS) were obtained, considering seeds with radicle lengths equal to or higher than 2.5 mm as germinated.

The seed vigor was evaluated by the performance of ten normal seedlings of each replication, considering the root and shoot lengths (cm) and root and shoot dry weights (mg), according to Nakagawa (2020).

2.4 Statistical analysis of the data

The data were analyzed to verify the assumptions of analysis of variance through the normality of residues and homogeneity of variances by the Shapiro-Wilk (Shapiro and Wilk, 1965) and Levene (1960) tests, respectively, at 0.05 probability level. The data did not meet the assumptions of ANOVA and it was chosen not to use the angular transformation for the dependent variable; thus, they were fitted to Generalized Linear Models (GLM). The GLM were analyzed and the significant differences within each storage time, storage condition, and variables studied were analyzed through comparisons of means pairs by the post-hoc Tukey's test at 5% significance. The means were fitted by the method of Šidák (1967). The results found for the stored and recently-harvested seeds were compared by the Dunnett test (Dunnett, 1955) at 0.05 probability level. The analyses were carried out using the R program (R Core Team, 2020).

3. Results and Discussion

The data of germination percentage (%G) ($p = 0.0814$) and percentage of normal seedlings (%NS) ($p = 0.6037$) presented normality of residues by the Shapiro-Wilk test, but only %NS presented homogeneous variance ($p = 0.0504$) by the Levene test. The GLM showed that the interaction between the studied factors was significant for all variables, except for shoot length ($p = 0.2473$), for which only the effects of isolated factors were significant.

The recently-harvested seeds presented initial water content of 5.67%, which varied from 3.18% to 8.94% in the different storage conditions, mainly considering the packaging used (Table 1). Different seed structures presented different water levels; the water content obtained represents the mean of the whole seed (McDonald et al., 1994; Bewley et al., 2013). Water levels lower than 10% represent the water responsible for maintaining the structural integrity and property of macromolecules and are affected by the seed chemical composition and temperature (Vertucci, 1993; Bewley et al., 2013; Marcos-Filho, 2015). Oscillations in these levels depend on the species and storage environment, which can affect the cell physiological status, including the conformation of proteins and organic compounds consisted of polymers of amino acids, which are water sorption sites (Bewley et al., 2013; Marcos-Filho, 2015).

Table 1. Water contents (%) in *Handroanthus spongiosus* seeds subjected to different storage conditions and times.

		Storage time (months)				
RHS	5.67					
Storage conditions		6	9	12	18	24
CB-RC		*4.90	*4.84	5.59	*8.94	*6.32
PB-RC		5.49	5.09	*4.98	*4.33	*4.33
CB-CC		*6.52	*6.96	*6.32	*8.75	*3.18
PB-CC		5.91	*6.38	*6.37	*8.84	5.35
FS		*6.34	5.99	5.59	*7.59	5.11
LN		*7.06	*8.52	*7.82	*8.24	*7.18

*Seed water contents significantly different from recently-harvested seeds (RHS) by the Dunnett test at 0.05 probability level. CB-RC = seeds placed in cotton bags and stored under room conditions; PB-RC = seeds placed in polyethylene bags and stored under room conditions; CB-CC = seeds placed in cotton bags and stored in cold chamber; PB-CC = seeds placed in polyethylene bags and stored in cold chamber; FS = seeds stored in a freezer; LN = seeds stored in liquid nitrogen (-196 °C); RHS = recently-harvested seeds. n= 100.

Seeds conserved under CB-RC presented a lower water content than the recently-harvested seed lot up to the 12nd month, followed by a significant increase of 3.27% in the 18th, and 0.65% in the 24th month of storage, different from the polyethylene bag packaging and room condition storage (PB-RC), which presented a 1.34% decrease in these two last times. In the cold chamber (CC) and freezer storage (FS) environments the seed water content decreased only in the 20th month; the cotton bag packaging and cold chamber storage (CB-CC) stood out with a 2.49% decrease. However, the liquid nitrogen storage (LN) kept the water content above that of the recently-harvested seed lot over the storage time (Table 1).

Regarding the physiological quality of seeds, the cryostorage in liquid nitrogen (LN) was the only condition that prevented the deterioration of *H. spongiosus* seeds up to 24 months, presenting similar results or superior results to those of recently-harvested seeds. However, the storage in permeable packaging and room temperature (CB-RC) presented the greatest seed deterioration after 24 months, with lower seed germination and seedling performance (Table 2). This is due the non-controlled conditions in the CB-RC, with seeds exposed to oscillations of temperature and relative air humidity, that can increase deterioration and loss of integrity of membranes, compromising RNA and protein syntheses, causing degradation of RNA and even disintegration of cell nuclei (Corbineau, 2012; Jyoti and Malik, 2013; Demidchik, 2015; Capilheira et al., 2019).

Table 2. Physiological quality of *Handroanthus spongiosus* seeds subjected to different storage conditions and times.

Storage times (months)		Storage conditions					
		CB-RC	PB-RC	CB-CC	PB-CC	FS	LN
		Seed germination (%)					
0	90						
6		*73.5 aB	87.5 aA	88.0 aA	86.0 abA	86.0 bA	91.0 aA
9		80.0 aB	87.0 aA	90.0 aA	91.5 aA	91.5 aA	88.0 bA
12		*49.5 bC	*78.0 aB	89.5 aA	90.5 aA	90.5 aA	87.0 bA
18		*22.5 cC	*46.0 bB	87.0 aA	94.0 aA	94.0 aA	92.0 aA
24		*4.0 dD	*32.0 cC	*64.0 bB	*76.5 bB	*76.5 cB	91.5 aA
CV%	18.1						
		Normal seedlings (%)					
0	73.5						
6		*28.0 bC	68.0 abB	82.5 aA	80.5 aA	67.0 bB	75.0 aA
9		67.0 aB	79.5 aA	80.0 aA	84.0 aA	*86.5 aA	75.0 aB
12		*20.0 bE	*59.5 bC	80.5 aA	83.5 aA	77.5 abA	74.0 aA
18		*10.0 cC	*31.0 cB	71.5 abA	80.0 aA	76.0 abA	75.5 aA
24		*2.0 dD	*18.5 dC	*46.0 cB	*58.5 bB	78.0 abA	74.0 aA
CV%	19.8						
		Shoot length (cm)					
0	2.42						
6		2.72 aA	2.84 aA	2.80 aA	*3.50 aA	2.85 aA	2.90 aA
9		*1.83 abA	*1.89 abA	2.33 aA	2.23 aA	2.12 aA	2.54 aA
12		*1.86 abA	*1.75 abA	2.75 aA	2.16 aA	2.19 aA	2.26 abA
18		*1.56 bcA	*1.90 abA	2.48 aA	2.25 aA	2.23 aA	2.58 abA
24		*1.02 cB	*1.27 bB	2.27 aA	2.20 aA	2.42 aA	2.46 abA
CV%	23.4						
		Main root length (cm)					
0	2.73						

6		*3.00 aB	*3.56 aA	*3.80 aA	*3.23 aB	2.97 aB	*3.24 aB
9		2.12 aB	2.32 bB	2.51 aB	2.84 aA	2.85 aA	2.51 aB
12		*1.85 bB	2.03 bB	2.67 aA	2.60 aA	2.84 aA	2.66 aA
18		*1.11 bC	*1.79 bC	2.69 aB	*3.63 aA	*3.63 aA	*3.64 aA
24		*1.02 cB	*1.00 cB	2.90 aA	*4.34 aA	*3.26 aA	*3.45 aA
CV%	28.7						
Root to shoot dry weight ratio							
0	0.22						
6		*3.04 aA	0.52 aC	*1.81 aB	*1.54 bB	0.16 cC	0.20 aC
9		0.19 of	0.25 bA	0.26 bA	0.29 cA	0.27 bA	0.21 aA
12		*1.57 bB	0.24 bC	0.25 bC	*2.04 aA	0.24 bC	0.28 aC
18		*0.64 cA	0.16 bA	0.26 bA	0.28 cA	0.26 bA	0.24 aA
24		0.25 dB	*0.07 dC	0.19 cB	0.26 cB	*1.04 aA	0.26 aB
CV%	29.3						

Means fitted by the Šidák method followed by different letters lowercase in the columns are significantly different from each other and means followed by the same uppercase letters in the rows are not statistically different from each other by the Tukey's test at 0.05 of probability. * Root to shoot dry weight ratio significantly different by the Dunnett test at 0.05 probability level. CB-RC = seeds placed in cotton bags and stored under room conditions; PB-RC = seeds placed in polyethylene bags and stored under room conditions; CB-CC = seeds placed in cotton bags and stored in cold chamber; PB-CC = seeds placed in polyethylene bags and stored in cold chamber; FS = seeds stored in a freezer; LN = seeds stored in liquid nitrogen (-196 °C).

After 12 months of storage, decreases in %G and %NS of seeds stored in permeable packaging was higher than 50%. However, the packaging did not affect the quality of seeds stored in cold chamber (Table 2).

Seeds conserved under low temperatures in CC and FS presented germinations above 80% until the 18th month of storage, and did not differ from recently-harvested seeds. In addition, the %NS and performance (shoot and root lengths) of seedlings from seeds stored in these conditions were statistically equal to or better than those of recently-harvested seeds (Table 2).

The results found for root to shoot dry weight ratio, calculated using the dry biomass of seedlings, where similar those found for the other evaluated variables. The root to shoot dry weight ratio of seedlings from seeds stored in LN for up to 24 months were similar to that of recently-harvested seeds and, according to the seed deterioration, it was higher, denoting a higher investment in roots by the seedlings. The cold storage (CC and FS) presented intermediate responses and the CB-RC condition resulted in higher deterioration of seeds and higher root to shoot dry weight ratio than the other treatments (Table 2).

Seeds can be classified by their tolerance to desiccation and low-temperature storage into three groups, orthodox, intermediate, and recalcitrant (Walters, 2015). Seeds of several species of the *Handroanthus* and *Tabebuia* genera are orthodox, they can disperse and be dried to water contents from 14.17% to 5.6% (Martins et al., 2009a; Silva et al., 2011; Guedes et al., 2012; Martins and

Pinto, 2014; Gonçalves et al., 2015; Alencar et al., 2018), which allows their storage under low (-20 °C) and ultralow (-196 °C) temperatures, maintaining them viable for many years (Walters et al., 2013; Ballesteros et al., 2021).

Seed water content is one of the most important factors for seed storage in LN; high water contents in the cells can disrupt membranes during freezing (Panis et al., 2005). *H. spongiosus* seeds with water content of 7.18% stored in liquid nitrogen for two years maintained a high physiological quality, which was a similar result to that of recently-harvested seeds (Table 2). This denotes an advantage of this species, since not all seeds tolerate temperatures below zero, as found for *Handroanthus impetiginosus* (Mart. ex DC) (= *T. impetiginosus*) seeds with 4.2% water content stored in liquid nitrogen, whose physiological quality decrease after 360 days (Martins et al. 2009b).

Seeds of some species of the Caatinga biome tolerate liquid nitrogen storage for periods above 24 months without losing their physiological quality, as is the case of *Amburana cearensis* (Allemão) AC Sm. (Araújo et al., 2017). This is probably because ultralow temperatures practically cease cell metabolism (Garcia et al., 2014). The storage of seeds in LN is an alternative for medium- and long-term conservation, since it is possible to maintain the seed viability and vigor for several years under low temperatures (-196 °C), although this process has a high cost (Kaviani, 2011; Walters et al., 2013).

Despite the water content of *H. spongiosus* seeds conserved in LN increase after six months, no loss in seed or seedling quality was found. This increase in seed water content after the storage period in low temperatures (CC, FS, and LN) was caused by the water vapor condensation process that occurs between the seed contact surface (lower temperature) and the surrounding air at thawing (higher temperature) when the seeds were removed from the cold storage (Delouche, 1968). These seeds are hygroscopic, they absorb or lose water to the environment until a balance is established between the seed water content and relative air humidity (Delouche, 1968; Oliveira et al., 2014); the period between the thawing and weighing of seeds was enough for them to absorb the water.

A test for measuring water content of cryo-conserved seeds was carried out using two replications of 50 seeds, which were directly placed in the oven after the LN or subjected to a thawing process. The water contents were similar to those obtained during the storage: 7.15% for seeds directly placed in the oven and 7.24% for those subjected to a thawing process, confirming that they absorb water soon after their removal from the LN.

Despite some species of the Caatinga biome conserve their seed physiological quality for more than 12 months under room conditions (>25 °C) (Lúcio et al., 2016; Gomes et al., 2018; Ribeiro et al., 2018), in general, storing under temperatures above 20 °C and relative air humidity higher than 70% are not recommended, as they compromise the seed physiological quality and promote the action of microorganisms and insects (Carvalho and Nakagawa, 2012). The combination of high temperatures with great water contents (usually above 12%) accelerates cell respiration, which leads to the consumption of the reserve material, oxidation of cell membranes, and degeneration of biological systems. Thus, the seeds rapidly lose their vigor and viability (Smaniotto et al., 2014).

The storage of seeds under room conditions (RC) presented the lowest seedling sizes, regardless of the type of packaging, denoting that this environment favored the maintenance of their respiratory metabolism and consumption of reserves, affecting the vigor and formation of seedlings (Dias et al., 2016; Araujo et al., 2021). This combination of factors involving continuous and not controlled vapor exchange between the medium and the seed with high temperatures can increase the respiratory rate and affect the development of structures and biomass allocation, even when the water contents do not oscillate. Similar results were found for *H. chrysotrichus* seeds, which could not be stored at room temperature for periods longer than 30 days, whereas *H. impetiginosus* and *Handroanthus serratifolius* (Vahl) S. Grose seeds did not germinate after four and nine months of storage, respectively (Silva et al., 2011; Maciel et al., 2020; Araújo et al., 2021).

Handroanthus heptaphyllus (Vell.) Mattos and *H. impetiginosus* seeds presented germination above 70% after 6 months of storage in CC and refrigerator, respectively (Maciel et al., 2020; Araújo et al., 2021), and *Handroanthus chrysotrichus* (Mart. ex dc.) stored in FS presented 54% germination after 10 months (Tonetto et al., 2015). *H. spongiosus* seeds presented germination of approximately 90% when stored in CC and FS after 12 months (Table 2), higher than those obtained for other species of the same genus that present very similar morphological characteristics. These environments (CC and FS) can be used by seedling and plant growers as viable strategies for ex situ conservation of seeds, since they are more accessible.

The root system can be significantly compromised depending on the storage conditions and time, with negative consequences to seed quality and seedling vigor (Table 2). It was reported by Mucha et al. (2015), who found that the seed storage temperature affected the root anatomy of *Populus nigra* L plants and reported that the storage at high temperatures decreased the proportion of roots with absorptive function (with primary development). Seedlings with higher root systems can explore a greater soil volume and have greater potential to absorb water and promote nutrient cycling and absorption (Finér et al., 2007; Betegón-Putze et al., 2019; Thorup-Kristensen et al., 2020).

The *H. spongiosus* seedlings presented higher dry matter allocation in the shoots during the post-seeding development. This can be attributed to higher investment of seedlings in thin roots at this initial stage of development as a strategy to explore a greater volume of the substrate and maximize water absorption for their development (Gransee and Führs, 2013; Yuan et al., 2016). However, these thinner roots little contribute to the root to shoot dry weight ratio. Species adapted to seasonally dry environments present a genetic trend of allocating greater biomass in the root system as a form to reach deeper soil waters faster and efficiently while using the variable water from rainfall events (Markesteijn and Poorter, 2009; Tomlinson et al., 2012; Qi et al., 2019), but it was not found for *H. spongiosus* at the seedling stage.

Seeds with low water content (lower than 10%) placed in impermeable packaging and stored at low temperatures (< 10 ^oC) presented higher longevity, as also found for *Tabebuia aurea* (Silva Manso) Benth & Hook ex S. Moore, *Tabebuia caraiba* (Mart.) Bureau, *H. chrysotrichus* (= *T. chrysotricha*), *H. impetiginosus* (= *T. impetiginosus*), and *Handroanthus umbellatus* (Sond.) Mattos (Martins et al., 2009a; Guedes et al., 2012; Martins and Pinto, 2014; Neves et al., 2014; Araújo et al., 2021). In these conditions, there is a resistance of the packaging to water vapor exchanges between the seeds and the medium, and a low promotion of development of microorganisms that produce heat through their many metabolic reactions, thus avoiding energetic losses (Cardoso et al., 2012; Lopes and Lima, 2015).

The conservation of physiological quality of *H. spongiosus* seeds during the storage is connected to the conditions used; environments with low temperatures can conserve seed viability and vigor for longer periods. This information could be used to subsidize the development of appropriate strategies for ex situ seed conservation for *H. spongiosus* and other related species from different origins.

4. Conclusion

Storing *Handroanthus spongiosus* seeds under room conditions is not recommended, since it cause losses in seed germination and vigor. The most adequate conditions for their conservation are provided by cold chamber, freezer, and liquid nitrogen environments. The use of impermeable packaging and low temperatures is the most indicated method for the maintenance of the physiological quality of *H. spongiosus* seeds.

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ARTIGO IV - Production of high-quality seedlings of an endangered species using goat manure and biochar as substrates

Artigo Submetido à Journal of Forestry Research

Jailton de Jesus Silva[a*], Claudineia Regina Pelacani[a], Raquel Araujo Gomes[a], Diana Signor[b], Bárbara França Dantas[b]

[a]Universidade Estadual de Feira de Santana, Departamento de Ciências Biológicas, Avenida Transnordestina, s/n - Novo Horizonte, 44036-900 Feira de Santana, BA, Brazil

[b]Embrapa Semiárido, Rodovia BR-428, Km 152, Zona Rural, 56302-970 Petrolina, PE, Brazil

*Corresponding author: Jailton de Jesus Silva (jj.jailton@outlook.com)

Production of high-quality seedlings of an endangered species using goat manure and biochar as substrates

Abstract: Studies with the use of alternative substrates have enabled producing seedlings of various species with high quality and low cost. The objective of this work was to evaluate the best cultivation conditions for production of *Handroanthus spongiosus* seedlings. The seeds were collected from five different populations in the state of Pernambuco, Brazil, and cultivated in six substrates: soil, sand, soil + vermiculite, soil + goat manure, soil + vermiculite + goat manure, and soil + goat manure + 30% biochar. We evaluated the emergence, water content, germination percentage, shoot and root length, shoot and root dry mass, and Dickson quality index. The substrates with addition of organic matter favored increased shoot length, stem diameter and dry mass. The addition of goat manure and biochar in the formulation of the substrates can improve the development of *H. spongiosus* seedlings and increase the economic benefits. In addition to growing conditions, determining the best locations for seed collection can facilitate decisions on the implementation of nurseries for the production of high-quality seedlings.

Keywords: Ipê . Semiarid . *Handroanthus* . Waste . Organic matter

1. Introduction

The strategies for production of high-quality seedlings of native species occurring in seasonally dry tropical forests (SDTF) are still incipient. The main obstacles to this production are finding adequate substrate formulations that favor development of seedlings (Shalizi et al. 2019) the variability of the emergence responses in relation to the origin of the seeds (Khurana et al. 2001) and the different spatial scales between and within populations (Mitchell et al. 2017; Vargas-Figueroa and Torres-González 2018). This variability can also serve as an important positive characteristic, because the plants produced may have better resistance to various environmental factors, a situation little explored for the species analyzed here.

The use of alternative substrates as inputs from existing processes of farms, as well as the byproducts of the timber industry, can increase the autonomy of farmers and make their activity more sustainable (Madrid-Aispuro et al. 2020). The inclusion of animal manure in substrates can supply the majority of nutrients necessary for good seedling development (Vukobratović et al. 2018). However, there is no single substrate that is ideal for all species (Ribeiro et al. 2007; Cáceres et al. 2013; Madrid-Aispuro et al. 2020).

The Northeast region of Brazil concentrates 94.5% of the national production of goat products (Viana et al. 2022), meaning a plentiful supply of goat manure exists for use as input to formulate substrates for production of seedlings. Another input found widely in this region and that can potentially be used to prepare or enrich substrates for seedling production is the waste from the production of wine and other grape derivatives. The Vale do São Francisco region accounts for the country's second largest wine production, with approximately 500 hectares of vineyards, producing millions of liters of wine annually (almost 15% of national output), only behind the state of Rio Grande do Sul (Pereira et al. 2015).

Therefore, developing strategies are important to favor the production of seedlings of native species at low cost, accessible to nursery owners and smallholders. Among these native species is *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae), endemic to the Caatinga and variously known by the common names "ipê-cascudo", "ipê-amarelo", "sete-cascas" or "pau-d'arco" (Gentry 1992; Lohmann et al. 2020). It occurs mainly in sandy soils and can reach height of 10 meters (Gentry 1992; Lohmann et al. 2020). It is on the IUCN red list of threatened species (SIBBR 2021) as well as the corresponding list of the Brazilian Ministry of the Environment (Brasil 2021). It can be used for recomposition of native vegetation in degraded areas and for urban afforestation (Gentry 1992; Lohmann et al. 2020; Brasil 2021). We did not find any previous studies on strategies and protocols to produce *H. spongiosus* seedlings.

Based on the endangered conservation status of the species, the strong anthropization of dry tropical forests, especially in the Caatinga, and the lack of information on the emergence and quality of seedlings, we formulated the following two research questions: (i) Do *H. spongiosus* seeds have different behavior when collected from different tree stands and cultivated in substrates with different formulations? (ii) What is the best cultivation condition for recruitment and production of high-quality seedlings of *H. spongiosus*?

2. Materials and methods

2.1 Description of the study area

The *H. spongiosus* seeds were collected in a SDTF area in the state of Pernambuco – Brazil in November 2019, and voucher specimens of the botanical materials were deposited in the herbarium of Feira de Santana State University (HUEFS), located in the municipality of Feira de Santana, Bahia State. The collection sites were located in the villages of Pau ferro (8°55’57.4” S, 40°43’19.8” W, 431 m) (HUEFS - 243297) and Caiçara (9° 07’24.5” S, 40° 23’ 16.1” W, 393 m) (HUEFS - 252490), the experimental farm of Embrapa Semiárid research unit (Embrapa Semiárido) (9° 8’ 8.9” S, 40° 18’ 33.6” W, 365 m) (HUEFS - 259090), and the district of Cristália (8°5’56.3” S, 40°19’27.1”W, 403 m) (HUEFS - 259093), all located in the municipality of Petrolina, Pernambuco. The fifth collection site was located in the village of Jutaí (8° 33’ 35.7” S, 40° 12’ 1.9” W, 418 m) (HUEFS - 259094) in the municipality of Lagoa Grande, Pernambuco (Fig. 1).

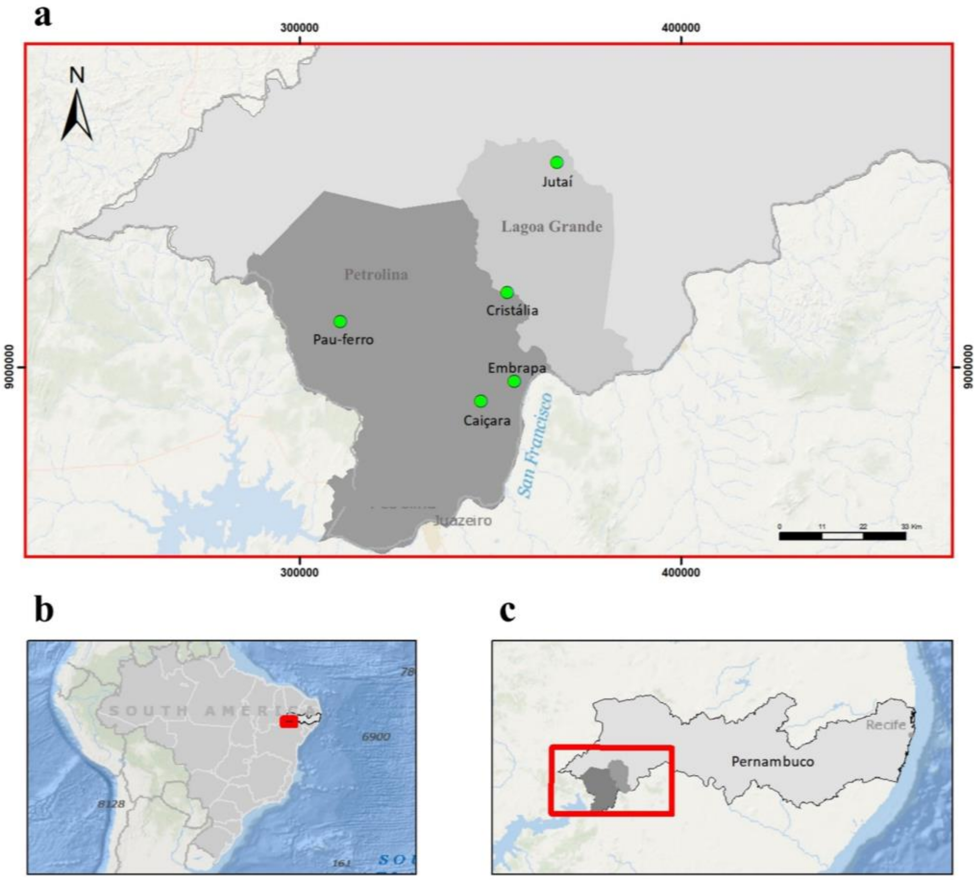

Fig. 1. Location of the study area. (a, b e c) Sites of collection of seeds of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae) in the state of Pernambuco, Brazil.

The predominant vegetation in this region is anthropized Caatinga remnant, with the presence of semideciduous xerophytic plants. The region has low rainfall, with yearly average of 435 mm, along with high rates of potential evapotranspiration (1520.9 mm), average annual temperature of 26 °C and relative humidity of 60% (Leal et al. 2005). The climate is classified as BSh (Alvares et al. 2013), with the presence of a strong rainy season in the summer (November to April) and a dry season from May to September. The soils of the Caatinga are characterized as shallow, stony and poor in organic matter on their surface (Queiroz et al. 2017).

2.2. Collection of seeds and water content measurement

At each collection site we selected eight parent trees for collection of the fruits with seeds, with the aid of a trimmer and tarp. The selection of the parent trees was based on size, vigor and health, with a minimum distance between the trees of 20 m. The seeds were obtained from ripe fruits (brown color), since the process of seed dispersion starts at the moment of physiological maturity. After opening the fruits, the seeds were gathered to form a single lot representing the site of origin. Then the water content was determined by oven drying the seeds at 105 ± 3 °C for 24 h using two subsamples of 50 seeds for each sampling site (Brasil 2013). Quiescent seeds were stored in a cold chamber at 10 ± 3 °C and relative humidity of 60 ± 4% until conduction of the experiment (Silva et al. 2022).

2.3. Germination and vigor seeds

The seed germination percentage was evaluated with subsamples of four repetitions of 50 seeds collected from each sampling site. The seeds were distributed between three sheets of Germitest paper, moistened with distilled water in a proportion of 2.5 times the weight of the dry paper, placed individually in polyethylene bags and incubated in a BOD chamber (biochemical oxygen demand) at constant temperature of 25 ± 1 °C and 12 h photoperiod for 14 days (Brasil 2013). Seeds with radicle length greater than or equal to 2.5 mm were considered germinated for calculation of the germination percentage (%G).

The seeds vigor (SV) was determined by electrical conductivity test with four repetitions of 50 seeds. The seeds were placed in beakers with 75 mL of distilled water and then incubated at a constant temperature of 30 ± 1 °C for 24 h (Ferreira et al. 2017). After this interval, the electrical conductivity was read (expressed in $\mu S.cm^{-1}.g^{-1}$) in a solution containing the seeds with a Digimed model DM-31 benchtop conductivity meter for aqueous solutions, with a sensor (electrode) (Vieira et al. 1999).

2.4. Production of seedlings

The experiment was structured in a 6 x 5 factorial scheme (6 cultivation conditions x 5 collection sites) in a completely randomized design with four repetitions of 25 seeds per treatment, under a sunscreen (50% light interception) in the nursery sector of the Embrapa Semiárido research unit (09° 04’ 16.4” S, 40° 19’, 5.37” W, 379 m), during the period from December 2020 to May 2021.

Five seeds were sown in each polyethylene bag (15 x 20 cm) at a depth of 1.0 cm, filled with six substrate types (v/v): soil (So); sand (SD); soil + vermiculite (1:1, SoV); soil + goat manure (1:1, SoM); soil + vermiculite + goat manure (1:1:1, SoVM); and soil + goat manure + 30% biochar (1:1:1, SoMB). The substrates in all the bags were irrigated daily at intervals of 1 hour for 1 minute with an automatic spraying mechanism.

The soil used was classified as Ultisol (Argissolo Amarelo, according to Brazilian Soil System Classification), collected in the upper layer (0-20 cm) in the experimental field of Embrapa Semiárido. Before incorporation into the substrates, the soil and sand were sieved to remove impurities (twigs and leaves). We also used in the substrate formulation containing coarse “E” expanded vermiculite, biochar and goat manure. Biochar was produced from grape juice production residues. The goat manure used was obtained from the semiarid Embrapa experimental farm, left for a week under irrigation to remove excess ammonia.

2.5. Physical and chemical properties of the substrates

The physical and chemical characteristics of all substrates including the biochar were determined by the method described by (Embrapa 2009). All the analyses were carried out in the Laboratory for Analysis of Soil, Water and Plants of Embrapa Semiárido in Petrolina, Pernambuco (PE). The total porosity and density of the soil were determined, respectively, by the stress table (stress of 6 kPa) and volumetric ring methods, and the granulometry was measured by the sieve-pipette and textural triangle method (Embrapa 2009). The chemical analyses consisted of determining the electrical conductivity (EC) in saturation paste; pH in water at soil-water ratio of 1:2.5; contents of Al, Ca and Mg (exchangeable, extracted with KCl 1 mol.L^{-1}, analyzed by titrimetry); P, K and Na (Mehlich 1 extraction); Cu, Fe, Mn and Zn (Mehlich 1 extraction and reading by atomic-absorption spectrophotometry); sum of bases (SB); and cation exchange capacity (CEC) (calcium acetate extraction), in all cases according to Embrapa (2009).

The substrates and/or seedlings were irrigated daily, during the 180 days of trail, at intervals of 1 hour for 1 minute with an automatic spraying mechanism.

2.6. Emergence and growth of seedlings

The final emergence percentage (%E) was calculated 14 days after sowing (DAS). The morphological characteristics were evaluated at 180 DAS involving measurement of shoot length (SL, cm) and root length (RL, cm) with a ruler (cm) and stem base diameter (SBD, mm) with a digital pachymeter (0.01 mm). Then the seedlings were washed in tap water to remove substrate clumps and cut at the base of the stem and separated into organs (roots and shoots) to obtain the dry mass. The shoots and roots were placed in labeled brown paper bags and arranged in a forced-air oven at temperature of 65 °C for 72 h. The shoot dry mass (SDM) and root dry mass (RDM) were determined by weighing with an analytic balance (precision of 0.0001 g) and the results were expressed in grams (Nakagawa 2020). The total dry mass (TDM) was obtained by the sum of SDM and RDM.

Based on these morphological characteristics, we evaluated the quality of the seedlings according to the collection site and substrate types by calculating the Dickson quality index (DQI) (Dickson et al. 1960), according to Equation: DQI = (TDM (g))/((SL (cm))/(SBD (mm))+(SDM (g))/(RL (g))).

2.7. Statistical analyses

Initially we evaluated the normal distribution of residuals and homogeneity of variances by the Shapiro-Wilk test (Shapiro and Wilk 1965) and Levene test (Levene 1960), respectively, at probability of 0.05. Then we analyzed the data by applying generalized linear models (GLM) (Carvalho et al. 2018). After the GLM analysis, we investigated significant differences within the principal effects (substrates and collection sites) by pairwise comparison of the means using the post-hoc Tukey test ($P < 0.05$). The means were adjusted by the method of Šidák (Šidák 1967).

To evaluate the relationship of the different cultivation conditions, seed collection sites and morphological characteristics of the seedlings, we applied multiple factor analysis (MFA) utilizing the "*FactoMineR*" package (Lê et al. 2008) of the R software (R Core Team 2020). The MFA is used to analyze related multivariate characteristics and thus extract key information from the original dataset. The choice of the principal components was based on the quantity of variance explained, with a threshold of at least 70% of the total variance of the original dataset (Rencher and Christensen 2012). All the analyses were performed with the R software (R Core Team 2020).

3. Results and Discussion

3.1. Germination (G%) and vigor (SV) of seeds

The upper and lower water content (WC) values were observed for the seeds gathered at Cristália (6.90%) and Embrapa (5.20%), respectively (Table 1). There was a statistically significant difference ($P < 0.05$) between the collection sites for germination percentage (%G) and electrical conductivity test for seed vigor (SV). The highest values of %G were found for the seeds collected at Cristália

(91.5 ± 2.22) and the lowest value in Caiçara (75.0 ± 3.70). The SV is related to the integrity of the cell membranes and is measured to determine the physiological quality of seeds, where low values denote low quantities of leachates (ions, sugars, organic acids, amino acids) in the absorbed water (Lima et al. 2015). The seeds collected at Cristália, Embrapa and Pau ferro had the best physiological quality.

Table 1. Water content (WC), germination percentage (%G) and electrical conductivity of seeds leachate - seed vigor (SV) of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae).

Sites	WC (%)	%G	SV ($\mu S.cm^{-1}.g^{-1}$)
Pau ferro	5.27	84.0 ±3.16 b	129 ± 16.93 b
Caiçara	5.26	75.0 ± 3.70 c	272 ± 22.23 a
Embrapa	5.20	84.5 ± 4.35 b	126 ± 5.62 b
Cristália	6.90	91.5 ± 2.22 a	114 ± 3.78 b
Jutaí	5.24	82.0 ± 4.24 b	229 ± 21.63 a
SW (*P*)		0.979 (0.862)	0.866 (0.014)
L (*P*)		0.475 (0.754)	1.914 (0.160)
CV%		8.68	18.49

Means followed by different lowercase letters in the columns differ statistically by the Tukey test at probability of 0.05. *WC%*, water content; *SV*, seed vigor - electrical conductivity of leachate; *SW*, Shapiro-Wilk test; *L*, Levene test; *CV%*, coefficient of variation; *P*-values between parentheses. Mean (± standard error).

3.2. Physical and chemical properties of the substrates

The lowest EC values of the solution (water + substrates), pH, CEC and base saturation (V%) were obtained in the substrates composed of SD, So and SoV (Table 2). The EC and pH results allowed inferring the concentration of soluble salts and input of available nutrients in the substrates (Antunes et al. 2022). According to Minami et al. (2010), EC values between 0.15 and 0.49 $mS.cm^{-1}$ are considered low, while those greater than 3.4 $mS.cm^{-1}$ are considered very high. Therefore, among the substrates tested in our experiments, those with the presence of organic matter (goat manure) were the richest in nutrients. However, pH values below 5.8 can increase the availability of iron and manganese and reduce the availability of nitrogen, potassium, calcium and magnesium (Stöcker et al. 2016), while values greater than 6.5 can cause unavailability of phosphorus, iron, zinc and copper (Noulas et al. 2018). Only the soil and SoV substrates had pH values below 6.5.

Table 2. Physical-chemical properties of the substrates produced and chemical properties of the biochar produced based on grape juice processing residues.

Parameters	SD	So	SoM	SoV	SoVM	SoMB	Biochar
EC ($mS.cm^{-1}$)	0.21	5.63	12.45	4.55	6.87	8.58	0.82
pH	6.90	6.40	7.60	6.40	7.60	7.50	5.40
P ($mg.dm^{-3}$)	1.43	4.88	55.30	6.61	221.80	167.86	730.23
K^+ ($mg.dm^{-3}$)	0.04	1.40	1.10	2.00	2.10	1.80	0.95
Na^{2+} ($mg.dm^{-3}$)	0.11	0.46	0.56	0.48	0.62	0.47	0.02
Ca^{2+} ($mg.dm^{-3}$)	0.60	2.50	4.90	2.40	5.00	6.50	3.00
Mg^{2+} ($mg.dm^{-3}$)	0.25	1.30	2.60	1.30	2.80	3.60	1.70
H + Al ($cmol_c dm^{-3}$)	0.20	1.20	0.00	1.00	0.00	0.00	13.00
SB ($cmol_c dm^{-3}$)	1.00	5.70	9.20	6.20	10.50	12.40	5.70
CEC ($cmol_c dm^{-3}$)	1.20	6.90	9.20	7.10	10.50	12.40	18.60
V (%)	80.60	82.50	100.00	86.60	100.00	100.00	30.40
Cu ($mg.dm^{-3}$)	0.59	0.76	0.99	0.80	1.05	0.78	ND
Fe ($mg.dm^{-3}$)	90.23	14.04	23.88	34.69	68.64	54.60	ND
Mn ($mg.dm^{-3}$)	10.64	27.36	41.60	54.75	60.10	33.54	ND
Zn ($mg.dm^{-3}$)	1.22	1.26	4.86	7.24	7.40	1.36	ND
Soil density ($mg.dm^{-3}$)	1.69	1.41	1.24	1.38	1.21	1.23	ND
Particle density ($mg.dm^{-3}$)	2.68	2.47	2.53	2.53	2.38	2.35	ND
Total porosity (%)	37.03	44.57	49.73	45.72	49.24	47.53	ND
Total sand ($g.kg^{-1}$)	971.00	627.00	615.00	571.00	522.00	608.00	ND
Silt ($g.kg^{-1}$)	28.00	302.00	348.00	357.00	426.00	325.00	ND
Clay ($g.kg^{-1}$)	0.88	71.18	35.88	72.58	52.18	66.98	ND
Ash (%)	ND	ND	ND	ND	ND	ND	8.37
C (%)	ND	ND	ND	ND	ND	ND	55.65
N (%)	ND	ND	ND	ND	ND	ND	1.80
H (%)	ND	ND	ND	ND	ND	ND	5.76

SD, sand; *So*, soil; *SoM*, soil + cured goat manure; *SoV*, soil + vermiculite; *SoVM*, soil + vermiculite + goat manure; *SoMB*, soil + goat manure + 30% biochar; *EC*, electrical conductivity; *pH*, potential of hydrogen determined in water; *P*, phosphorus content; *Mg^{2+}*, magnesium content; *Ca^{2+}*, calcium content; *Na*, sodium content; *K^+*, exchangeable potassium; *H + Al*, potential acidity; *SB*, sum of bases (Ca^{+2} + Mg^{+2} + Na^{+1} + K^{+1}); *CEC*, cation exchange capacity (H + Al + SB); *V%*, saturation of bases (SB/CEC)*100; *Cu*, copper; *Fe*, iron; *Mg*, magnesium; *Zn*, zinc; *ND*, not determined

The low values of sum of bases (SB) in the substrates without addition of organic matter indicated that the cation exchange sites were filled, mainly by hydrogen cations (H^{+1}) and aluminum cations (Al^{+3}), which could have caused nutritional deficiency (Almeida and Sánchez 2015).

The addition of organic matter increased the quantity of magnesium (Mg^{2+}), an element in the composition of chlorophyll molecules, active in photosynthesis, respiration and synthesis of carbohydrates (Peng et al. 2019). There also was an increase in the availability of phosphorus (P), contributing to cell growth, energy transfer in root cells, photosynthesis and cell respiration (Berti et al. 2017). Finally, there were increases in the levels of calcium (Ca^{2+}), a nutrient essential for the development of the roots, maintenance of cell integrity and membrane permeability (Antunes et al. 2022), as well as potassium (K^+), sodium (Na^+), manganese (Mn) and zinc (Zn).

The soil density values reflect the compaction of the substrate; indeed, it is the most direct measure of compaction (Gao 2017). According to Andrade et al. (2018), compacted substrates hinder the uptake of water and nutrients by the roots and cause deficient oxygenation.

3.3. Emergence and growth of the seedlings

The morphological variables that presented normal distribution of the residuals by the Shapiro-Wilk test were emergence percentage (%E) ($P < 0.346$) and stem diameter (SBD) ($P < 0.077$), while regarding homogeneity of variances, only DQI presented homogeneous variances ($P < 0.802$) (Table 3). The GLM analysis indicated significant interaction ($P < 0.01$) of the factors evaluated with all the morphological characteristics.

Table 3. Analysis of deviance (ANODEV) of emergence percentage (%E), shoot length (SL, cm), root length (RL, cm), shoot dry mass (SDM, g), root dry mass (RDM, g), stem base diameter (SBD, mm) and Dickson quality index (DQI) of seedlings of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae).

Source of variation	Analysis of Deviance (ANODEV)							
	Df	%E	SL	RL	SDM	RDM	SBD	DQI
Location (L)	4	56.37**	3.187**	3.214**	17.232**	38.016**	0.687**	4.2142**
Substrate (S)	5	366.42**	11.283**	7.039**	49.107**	60.417**	2.735**	6.1032**
L*S	20	77.22*	5.082**	7.032**	40.749**	70.254*	2.057**	8.6011**
Shapiro-Wilk		0.346	< 0.001	< 0.001	< 0.001	< 0.001	0.077	< 0.001
Levene		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.802
CV%		21.70	19.27	22.54	17.23	22.35	27.04	16.12

Df, degrees of freedom; *%E*, emergence percentage; *RL*, root length; *SL*, shoot length; *RDM*, root dry mass; *SDM*, shoot dry mass; *SBD*, stem base diameter; *CV%*, coefficient of variation; *DQI*, Dickson quality index. Significant differences were determined as $P < 0.05$ * and $P < 0.01$ ** by the F-test.

The sand substrate was the cultivation condition that most favored %E of the radicle, with values between 46% (Jutaí) and 70% (Pau ferro) (Table 4). This higher %E in sand can be related to the lower resistance to emergence and initial development of the seedlings' organs and the better supply of water and oxygen necessary for emergence (Pedrosa et al. 2020). In this phase, the nutrient reserves of the seeds contribute sufficiently to the emergence, without the need for external sources of nutrients (Mayer 1963; Bewley and Black 1994). The need for nutritional support from the substrate occurs with the expansion of the cotyledon, formation of the first true leaf and roots (Corte et al. 2006). Sand alone does not provide this support, so it tends to produce seedlings with low biomass increment (Abreu et al. 2022) and this was observed in our results.

Table 4. Average morphological responses observed for emergence percentage (%E), shoot length (SL, cm), root length (RL, cm) and stem base diameter (SBD, mm) in seedlings of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae).

	Substrates					
	So	SD	SoV	SoM	SoVM	SoMB
Local	Emergence (%)					
Jutaí	7 bD	46 bA	7 aD	18 bC	12 cC	32 aB
Cristália	19 aC	56 bA	7 aC	21 bB	24 bB	33 aB
Caiçara	4 cC	49 bA	8 aC	19 bB	21 bB	14 bC
Embrapa	34 aB	66 aA	4 aD	38 aB	50 aB	20 bC
Pau ferro	24 aC	70 aA	11 aC	26 aC	34 bB	15 bC
	Shoot Length (cm)					
Jutaí	7.12 aC	5.24 aD	5.87 aD	14.90 aA	8.81 aC	11.77 aB
Cristália	4.86 aC	4.48 aC	5.94 aC	15.88 aA	6.40 aC	11.94 aB
Caiçara	4.43 aC	5.01 aB	5.63 aB	4.42 cC	6.30 aB	8.05 aA
Embrapa	6.97 aC	4.50 aD	5.78 aD	12.67 bB	8.76 aC	13.32 aA
Pau ferro	5.30 aC	4.10 aC	6.77 aB	7.26 cB	8.90 aB	11.56 aA
	Stem base Diameter (mm)					
Jutaí	1.71 bC	1.84 aB	1.86 aB	2.59 aA	2.39 aB	2.13 bB
Cristália	1.74 bC	1.57 aC	2.07 aB	2.91 aA	2.45 aA	2.69 bA
Caiçara	1.36 bC	1.57 aC	1.78 bB	2.65 aB	2.46 aA	1.91 cB
Embrapa	2.29 aA	1.60 aB	1.72 bB	2.24 bA	2.10 aA	2.18 bA
Pau ferro	2.43 aB	1.85 aB	2.11 aB	1.92 bB	2.53 aB	3.63 aA
	Root Length (cm)					
Jutaí	19.48 bC	23.43 aB	28.95 bA	22.44 aB	19.02 aC	14.12 cD
Cristália	28.33 aB	26.14 aB	36.97 aA	22.66 aB	13.29 bC	17.61 bC
Caiçara	15.22 cB	28.88 aA	17.38 cB	7.67 cC	9.50 cC	9.65 cC
Embrapa	26.00 aA	23.03 aB	29.73 bA	17.70 bB	15.75 aC	19.57 bB
Pau ferro	22.77 bA	22.65 aA	23.97 bA	12.04 bB	18.31 aA	22.58 aA

SD, sand; *So*, soil; *SoM*, soil + goat manure; *SoV*, soil + vermiculite; *SoVM*, soil + vermiculite + goat manure; *SoMB*, soil + goat manure + 30% biochar. Means followed by different lowercase letters in the columns and different uppercase letters in the rows do not differ statistically by the Tukey test at probability of 0.05.

During the experiment (december/2020-may/2021), we observed the death of the seedlings cultivated in sand and soil alone. This may have occurred due to the low relative humidity (67%), low levels of rainfall (244 mm) and high temperatures (27 °C) at the experiment site, which caused rapid drying and an increase in the temperature of the substrates after irrigation. Moreover, the soil substrate suffered from compaction in the initial days of the experiment, hampering the emergence and growth of the seedlings.

The addition of goat manure and biochar to the substrates favors the growth of the shoot (SL) and stem diameter (SBD) of the seedlings. The highest SL values were observed in the seedlings from seeds collected at Cristália (15.88 cm) and Jutaí (14.90 cm) cultivated in SoM (Table 4; Fig. 2). On the other hand, the largest SBD values were found in seedlings from seeds gathered at Pau ferro (3.63 mm) and Cristália (2.91 mm), cultivated in SoMB and SoM, respectively. The root lengths (RL) were higher on seedlings grown in the soil, sand and SoV substrates, with the standouts being the seedlings grown in SoV from seeds collected at Cristália (36.97 cm), Embrapa (29.73 cm) and Jutaí (28.95 cm). However, it is noticed that the volume of roots is smaller in these substrates in relation to substrates with the addition of manure and biochar.

Fig. 2. Seedlings of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae) formed from seeds collected in november 2019 at Pau ferro and 180 days after sowing (DAS) in different cultivation conditions.

The dry mass production increased with addition of manure (Table 5), associated with the accumulation of nutrients extracted from these substrates during the development of the plants, since manure is a good source of macro and micronutrients (Antunes et al. 2022).

Table 5. Average morphological responses observed for shoot dry mass (SDM, g), root dry mass (RDM, g) and Dickson quality index (DQI) of seedlings of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae).

	Substrates					
	So	SD	SoV	SoM	SoMV	SoMB
Location	Shoot Dry Mass (g)					
Jutaí	0.158 bC	0.388 aC	0.103 cC	1.415 bB	1.227 bB	2.943 aA
Cristália	0.299 bC	0.428 aC	0.463 bC	1.979 bA	1.125 bB	2.104 bA
Caiçara	0.944 aA	0.353 aB	0.186 cC	0.234 cB	0.452 cB	0.356 cB
Embrapa	0.604 aB	0.320 aC	0.642 aB	2.725 aA	2.741 aA	2.066 bA
Pau ferro	0.170 bC	0.257 aC	0.384 bC	2.325 aB	3.220 aA	2.397 aB
	Root Dry Mass (g)					
Jutaí	0.295 cC	1.399 aB	0.460 cC	1.436 cB	1.851 cB	3.326 aA
Cristália	0.616 bC	0.984 bC	2.319 aB	3.279 aA	2.318 bB	2.893 aB
Caiçara	1.292 aA	0.794 bB	0.940 bA	0.242 dC	0.212 dC	0.295 bC
Embrapa	1.702 aC	0.875 bD	0.952 bD	2.849 aB	3.317 aA	3.629 aA
Pau ferro	0.427 bD	0.634 cC	0.693 cC	2.130 bB	4.291 aA	2.942 aB
	Dickson Quality Index					
Jutaí	0.205 bC	0.525 aB	0.168 cD	0.372 bC	0.810 aA	0.948 aA
Cristália	0.278 bC	0.388 bB	0.915 aA	0.905 aA	0.983 aA	0.984 aA
Caiçara	0.625 aA	0.323 bB	0.335 bB	0.172 cC	0.140 bC	0.105 cC
Embrapa	0.675 aB	0.350 bC	0.425 bC	0.770 aB	0.991 aA	0.885 aA
Pau ferro	0.222 bC	0.360 bB	0.290 bC	0.458 bB	0.988 aA	0.996 aA

SD, sand; *So*, soil; *SoM*, soil + goat manure; *SoV*, soil + vermiculite; *SoVM*, soil + vermiculite + goat manure; *SoMB*, soil + goat manure + 30% biochar. Means followed by different lowercase letters in the columns differ statistically and means followed by different uppercase letters in the rows do not differ statistically by the Tukey test at probability of 0.05.

Definition of the time for seedlings to remain in nurseries generally depends only on shoot length and stem diameter, without paying attention to the size of the roots (Güney et al. 2020). However, it is intuitive that well-developed roots favor the formation of healthy seedlings, by fortifying the entire structure, increasing the chances for growth and development in the field, especially in arid and semiarid regions where erratic rainfall is among the most restrictive factors for establishment (Schenk and Jackson 2002).

The Dickson quality index (DQI) is considered a good indicator of seedling quality because it reflects the balance in distribution of dry biomass and growth between the shoots and roots, enabling the choice of seedlings better able to adapt to adverse field conditions (Güney et al. 2020). Higher values of this index are associated with greater chance of survival and establishment of seedlings in the field (Santos et al. 2020; Zuffo et al. 2021). The greatest Dickson quality indices were obtained for the seedlings originating from seeds collected at Pau ferro and cultivated in SoMB (0.996) and those from seeds gathered at Embrapa and cultivated in SoVM (0.991).

The relationship of the seed collection sites, cultivation conditions and morphological characteristics of the seeds could be observed from the results of the multiple factor analysis (MFA) (Fig. 3). Principal component 1 (PC1) explained 56.43% of the variability of the data while principal component 2 (PC2) explained 17.24%, for a total of 73.67%. Fig. 3a shows that the characteristics which most contributed to the construction of PC1 were SL, SBD, SDM, RDM and DQI, in contrast to PC2, which received the greatest contributions from RL and %E. The seed collection sites Pau ferro, Embrapa and Cristália together with the cultivation conditions SoM, SoVM and SoMB were positively associated with PC1. This suggests a similar performance of these three substrates, as can be seen in Fig. 3b. On the other hand, the sites Jutaí and Caiçara along with the cultivation conditions So, SD and SoV were positively associated with PC2 (Fig. 3b).

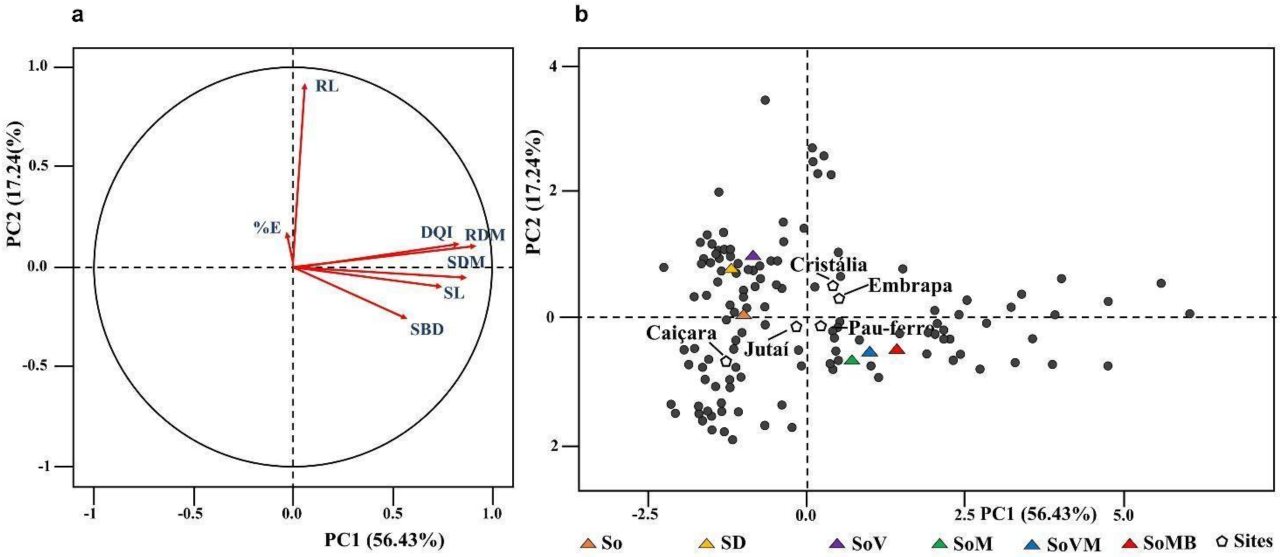

Fig. 3. Multiple factor analysis related to %E and morphological characteristics of seedlings of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae) cultivated in different substrates from seeds collected in november 2019 at Pau ferro, Caiçara, Embrapa, Cristália and Jutaí, Pernambuco, Brazil. Circle of correlation between the morphological responses (a); relation between seed collection locations and substrates (b). *SD*, sand; *So*, soil; *SoM*, soil + goat manure; *SoV*, soil + vermiculite; *SoVM*, soil + vermiculite + goat manure; *SoMB*, soil + goat manure + 30% biochar. The pentagons correspond to the seed collection locations; the triangles correspond to the cultivation conditions. *%E*, emergence percentage; *SL*, shoot length; *RL*, shoot length; *SBD*, stem base diameter; *SDM*, shoot dry mass; *RDM*, root dry mass; *DQI*, Dickson quality index.

These results corroborate those found in the univariate analysis, in which the substrates with addition of organic matter generally had the best physical-chemical attributes, promoting better morphological characteristics of the seedlings. The use of this type of analysis complements univariate analysis, enabling an overview of the interplay of the cultivation conditions, seed collection sites and morphological characteristics of the seedlings.

Therefore, the employment of agricultural wastes, such as goat manure and biochar, can favor farmers with small and medium-sized operations because these residues are easily available at zero or low cost.

The seedlings from seeds gathered at Caiçara presented different results from the others since the best yields were obtained with use of soil and sand alone. These results confirmed our hypothesis that the collection site and type of substrate used would influence the quality of the *H. spongiosus* seeds.

The addition of goat manure to the soil provided better results in the development and formation of *H. spongiosus* seedlings. Biochar has an important effect of increasing water retention in the soil or in substrates and this can be important in regions with a semiarid climate characterized by high temperatures and low rainfall concentrated in some months of the year.

In environments where there is no formation of organic matter on the surface that favors the emergence and recruitment of seedlings, as is the case of the Caatinga, an alternative would be the production of seedlings in a nursery using goat manure in the preparation of the substrate.

Direct sowing in a substrate with only sand allows rapid emergence, however it produces smaller seedlings with a low increase in biomass. Substrates with goat manure, despite not favoring a quick emergence, produce larger seedlings with a greater increase in biomass and with better distribution between shoots and roots.

We did not evaluate the performance of the seedlings after transplantation in the field, only at nursery area, so more studies are necessary focused on the survival and growth of *H. spongiosus* in field conditions.

4. Conclusion

The results presented here can help support the production of seedlings for restoration of degraded seasonal dry tropical forests and can be used as a basis for production of seedlings of other species. The addition of goat manure and biochar in the formulation of the substrates can improve the development of *H. spongiosus* seedlings and increase the economic benefits in nursery operations, because these inputs are locally abundant. The determination of the best places to collect seeds will facilitate decisions on the implementation of *H. spongiosus* nurseries for production of high-quality seedling.

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Considerações finais

Os resultados desse estudo permitiram entender como populações de *Handroanthus spongiosus* em florestas tropicais sazonalmente secas (FTSS) no Domínio Caatinga respondem às condições ambientais sazonais. As informações obtidas poderão auxiliar programas de colheita de sementes para restauração ecológica dessa espécie, principalmente em populações com presença de árvores-matrizes de maior produção de sementes e com qualidade fisiológica.

Foi possível desenvolver protocolos para determinar a qualidade fisiológica das sementes de forma rápida e confiável por meio do teste de tetrazólio, o que contribui para o avanço do conhecimento na área de tecnologia de sementes, em especial para espécies arbóreas ameaçadas. Foram desenvolvidas estratégias de armazenamento para as sementes utilizando diferentes condições (embalagens x temperatura de armazenamento), onde observou-se que temperaturas ultrabaixas são favoráveis à manutenção da qualidade fisiológica das sementes de *H. spongiosus* por até 24 meses.

Os melhores substratos para produção de mudas de qualidade de *H. spongiosus* e aptas às condições adversas de campo são aqueles com adição de matéria orgânica (esterco caprino) + biocarvão em sua formulação, pois proporcionam maiores mudas e incremento de massa seca.