UNIVERSIDADE DE BRASÍLIA
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
DEPARTAMENTO DE BOTÂNICA

O PAPEL DO BANCO DE SEMENTES E DE ESPÉCIES NATIVAS NA RECUPERAÇÃO DA FLORA DA ILHA DA TRINDADE – BRASIL

BRUNO DOS SANTOS RABELO

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Orientador: Fabian Borghetti

Tese apresentada ao Programa de Pós Graduação em Botânica da Universidade de Brasília, como parte dos requisitos necessários para obtenção do título de Doutor em Botânica, sob a orientação do Prof. Fabian Borghetti.

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´´ *If I have seen further it is by* ***Standing on the shoulders of Giants***.`` Isaac Newton

Sumário

RESUMO

O banco de sementes é a reserva de sementes viáveis e não germinadas depositadas no solo, as quais estão constantemente sujeitas às pressões do ambiente no qual se encontram. Os processos responsáveis pela sua dinâmica, tais quais a viabilidade destas sementes e processos como dormência, germinação e recrutamento de novos indivíduos, são regidos por fatores como disponibilidade hídrica e temperatura do solo. Logo, condições ambientais são agentes determinantes de influência sobre a distribuição e história de vida de espécies vegetais. Este é um fator fundamental não só para a manutenção e expansão da distribuição das espécies, mas principalmente para a sucessão secundária em comunidades vegetais em áreas que sofreram algum tipo de perturbação. A Ilha da Trindade é um ambiente que passou por uma grande perda de biodiversidade vegetal após a introdução de animais exóticos. Como consequência, vieram a extinção de espécies nativas, ocorrência de erosão de solos e o desaparecimento de parte dos cursos d’água. Este trabalho teve como objetivo investigar o potencial de regeneração natural da vegetação de áreas da Ilha da Trindade e qual seria o papel do banco de sementes do solo e de espécies nativas neste processo. As respostas aqui buscadas poderiam ser utilizadas num futuro plano de restauração da vegetação da Ilha, visando a recuperação de áreas degradadas e a contenção de processos erosivos. No capítulo 1, foi feita a caracterização abiótica de áreas com e sem cobertura vegetal selecionadas para o estudo, afim de conhecer as condições de temperatura e disponibilidade hídrica às quais as sementes do banco estão sujeitas. Investigamos ainda a composição de espécies dos bancos de sementes e se haveria diferença entre áreas cobertas por vegetação e de solo exposto. Por último, simulando em laboratório as condições abióticas de temperatura e disponibilidade hídrica de ambas as áreas, testamos sua influência sobre a mortalidade de sementes das espécies identificadas no banco de sementes do solo. Esta etapa teve como objetivo explicar a diferença na densidade de sementes entre as áreas, como resultado da pressão abiótica mais intensa exercida por parte dos ambientes de solo descoberto. Tais resultados poderiam determinar a capacidade destas espécies em compor o banco de sementes, e consequentemente, seu potencial em favorecer a revegetação das áreas descobertas. Medidas abióticas mostraram uma diferença significativa entre áreas de solo exposto e as áreas com vegetação, sendo que, na ausência de vegetação as condições se tornam mais extremas, com maiores temperaturas e redução da disponibilidade hídrica. O banco de sementes é composto por três espécies, *Bulbostylis nesiotis*, *Cyperus atlanticu*s e *Cyperus* *sp.* (espécie ainda não identificada), com uma expressiva redução na densidade de sementes viáveis nas de áreas desprovidas de vegetação, quando comparadas às áreas com vegetação. Por último, simulações dos padrões de temperaturas mostraram alta mortalidade de sementes sob condições de solo descoberto, o que explicaria a menor densidade de sementes encontradas. Entretanto, mesmo nestas condições, parte das sementes de *Bulbostylis nesiotis* manteve sua viabilidade, indicando uma vantagem desta espécie sobre as demais no processo de recolonização. No capítulo 2, investigamos a porcentagem de germinação e o tempo médio para germinação como resposta a um gradiente de déficit hídrico e a temperaturas simuladas das áreas de solo exposto e áreas com vegetação. Estes experimentos foram realizados com sementes frescas e repetidos após seis e doze meses utilizando-se do mesmo lote de sementes. Com isso seria possível identificar a influência dos efeitos abióticos sobre sementes com diferentes tempos de dispersão. Sementes de *B. nesiotis* germinaram sob ambas as simulações de temperaturas, mas as condições de solo descoberto reduziram a porcentagem de germinação e aumentaram o tempo médio para tal. O tempo pós dispersão apresentou baixa influência sobre as sementes, confirmando a aptidão de *B. nesiotis* em favorecer a composição do banco. Apesar dos altos níveis de germinação na simulação de áreas com vegetação, nenhuma das espécies de *Cyperus* apresentou germinação sob as condições de solo descoberto. Além disso, o tempo pós dispersão apresentou ligeira influência sobre estas espécies, ao se considerar os parâmetros analisados. Concluímos então que as áreas perturbadas na Ilha da Trindade podem mostrar uma habilidade natural de regeneração através do banco de sementes do solo. Entretanto, as condições abióticas do solo descoberto influenciaram a germinação das espécies estudadas. Logo, com apenas *B. nesiotis* e *Cyperus* como agentes da recolonização, este pode ser um processo lento. Seria necessário um plano de restauração que acelerasse este processo de regeneração, visando prioritariamente a contensão dos atuais processos erosivos, através do estabelecimento de novos indivíduos, respeitando a composição de espécies nativas e a sucessão ecológica e evitando os efeitos das pressões abióticas desfavoráveis. Novos estudos seriam então necessários para enriquecer a lista de espécies, entre nativas e endêmicas, adequadas para uso na recuperação das áreas ainda prejudicadas.

Palavras – chave: Banco de sementes, Fatores abióticos, Mortalidade de sementes, Germinação, Temperatura do solo, Estresse hídrico, Ilha da Trindade, sucessão secundária

ABSTRACT

Seed banks are the reserve of viable and non-germinated seeds in the soil, which are constantly subjected to environment pressures. The process that are responsible by their dynamic, such as seed viability, dormancy, germination and recruitment of new seedlings, are driven by factors like soil temperature and water availability. Therefore, environment conditions are often the main agents of influence on the distribution and life history of plant species. This is a fundamental factor nor only for the maintenance and expansion of species distribution, but mainly for the restructuring of plant communities in areas that have suffered some kind of disturbance. The Trindade Island is an environment that has undergone a great loss of biodiversity after the introduction of exotic animals. As a consequence, came the extinction of native plant species, occurrence of soil erosion and the disappearance of part of the water courses. The objective of this work was to investigate the potential of natural regeneration of the vegetation of areas on Trindade Island and what would be the role of the soil seed bank and native species in this process. The responses sought here could be used in a future vegetation restoration plan of the Island, aiming the recovery of degraded areas and the containment of erosive processes. In Chapter 1, we performed the abiotic characterization of areas with and without vegetation cover selected for the study, in order to know the temperature and water availability conditions to which the seeds were subjected after their insertion in the seed bank. We also investigated the species composition of the seed banks and whether there would be a difference between vegetated and bare soil. Finally, simulating the abiotic conditions of temperature and water availability in both areas, we tested their influence on the Seed Viability Loss (SVL) of seeds of the species identified in the soil seed bank. The objective of this stage was to explain the difference in seed density between the areas as a result of the more intense abiotic pressure exerted by bare soil environments. These results could determine the ability of these species to compose the seed bank, and consequently, their potential to favor the revegetation of areas of bare soil. Abiotic measurements showed a significant difference between bare soil and vegetated areas, being more extreme in the absence of vegetation. The research in the seed bank reached *Bulbostylis nesiotis*, *Cyperus atlanticus* and *Cyperus sp.* (specie not yet identified), with a significant reduction in seed density in bare soil areas, when compared to vegetated areas. Finally, simulations of temperature patterns showed high SVL under bare soil conditions, which could explain the lower seed density found. However, even under these conditions, part of the *Bulbostylis nesiotis* seeds maintained its viability, indicating an advantage of this species over the others in the recolonization process. In Chapter 2, we investigated the germination percentage and the mean time for germination as responses to a gradient of water stress and to simulated temperatures of the bare soil and vegetated areas. These experiments were performed with fresh seeds and then repeated after six and twelve months using seeds from the same collect. This way it would be possible to identify the influence of abiotic effects on seeds with different dispersion times. *B. nesiotis* seeds germinated under both temperature simulations, but bare soil conditions reduced the germination percentage and increased the mean time for germination. Post-dispersion time presented low influence over the seeds, confirming the ability of *B. nesiotis* to favor the seed bank composition. Despite the high levels of germination in the simulation of vegetated areas, none of the Cyperus species showed germination under the conditions of bare soil. In addition, the time after dispersion had a slight influence on these species, considering the parameters analyzed. We conclude that disturbed areas on Trindade Island may show a natural regeneration ability through the soil seed bank. However, the abiotic conditions of the bare soil were able to interfere in the full germinative performance of the species studied. Therefore, with only B. nesiotis and Cyperus as agents of recolonization, this would be a slow process. A restoration plan would be needed to accelerate this regeneration process, aiming primarily to contain the current erosive processes, by establishing new individuals, respecting the composition of native species and avoiding the effects of unfavorable abiotic pressures. New studies would then be needed to enrich the native and endemic list of species, which are suitable for use in the recovery of areas still disturbed.

Key – words: Soil seed banks, abiotic factors, seed viability loss, germination, soil temperature, water stress, Trindade Island

INTRODUÇÃO GERAL

Banco de sementes: características e influência sobre a dinâmica da vegetação

O banco de sementes é a reserva de sementes viáveis e não germinadas depositadas no solo. Trata-se de um sistema dinâmico constantemente sujeito a entrada e saída de sementes, cuja composição é resultado da influência de fatores bióticos (dispersão de sementes, dormência e germinação) e abióticos (temperatura, umidade, luminosidade, déficit hídrico, pluviosidade) (Leck *et al*, 1989; Fenner & Thompson, 2005; Baskin & Baskin, 2014).

Um fator crucial na distinção entre tipos de banco de sementes é a longevidade, ou seja, por quanto tempo as sementes do banco são capazes de se manterem viáveis pós dispersão e inserção no sistema. Neste contexto, bancos de sementes podem ser classificados como dois tipos principais, os transientes ou os persistentes. Segundo Simpson e colaboradores (1989) bancos transientes apresentam sementes caracterizadas pela baixa longevidade, as quais geralmente mantém sua viabilidade apenas até a temporada de dispersão subsequente. Bancos de sementes transientes têm traços funcionais que estão diretamente voltadas à rápida germinação pós-dispersão, como sementes de grandes dimensões e/ou estruturas que facilitem a fixação no solo, ausência de mecanismos de dormência, capacidade de germinação na ausência de luz e em uma alta gama de temperaturas (Thompson & Grime, 1979). Já no caso de bancos persistentes, geralmente compostos por sementes pequenas, há espécies onde uma pequena parcela das sementes pode até apresentar germinação logo após a dispersão, tal qual ocorre no banco transiente, entretanto, sua maioria é composta por sementes com características que promovem a manutenção de sua viabilidade por longos períodos, como dormência e dependência do estímulo da luz para germinarem. Isto as leva permanecer viáveis por períodos que vão além de uma estação de dispersão (Thompson & Grime, 1979).

Outra importante abordagem na pesquisa de banco de sementes é a similaridade entre a composição vegetal acima do solo (vegetação estabelecida) e abaixo dele (sementes depositadas no banco), que fornece informações sobre a dinâmica da comunidade vegetal. É possível encontrar os mais variados níveis de similaridade entre banco e a vegetação devido às diferentes estratégias das espécies para sobrevivência das sementes no solo, bem como para sua germinação e recrutamento de plântulas (Thompson

& Grime, 1979; Dessaint, 1997). Hopfensperger (2007) elaborou um levantamento com diversos trabalhos voltados para a similaridade entre banco de sementes e vegetação estabelecida, os quais mostraram um padrão geral em que a similaridade entre o banco e a vegetação presente diminui conforme os estágios de sucessão ecológica avançam.

Um dos principais fatores responsáveis por este padrão são as diferentes estratégias de colonização de espécies pioneiras e tardias. Em áreas com pouca cobertura vegetal ou com vegetação em estágios iniciais de sucessão, observa-se em sua maioria a presença de espécies pioneiras, geralmente gramíneas e herbáceas, que tem por característica a dispersão de sementes em curtas distâncias, conferindo alta similaridade entre a vegetação e o banco (Dessaint *et al*, 1997). Em pastagens na região do Mar Mediterrâneo, Levassor, Ortega e Peco (1990) encontraram uma similaridade de cerca de 81% entre a vegetação estabelecida e o banco de sementes. Seguindo este mesmo padrão de resposta, em uma área de pastagem do estado do Novo México – Estados Unidos, Henderson, Pederson e Redak (1988) encontraram uma correlação de quase 89% entre a composição de espécies da vegetação estabelecida e o banco de sementes. Apesar disso, sementes de gramíneas e herbáceas conseguem se manter viáveis também em ambientes de estágios de sucessão mais tardia, como florestas temperadas e tropicais (Oosting & Humpreys, 1940; Putz & Appanah, 1987), só que neste caso estas sementes ficam dependentes da ocorrência de janelas de oportunidades que as favoreçam, ou seja, eventos que possibilitem a germinação e estabelecimento de novos indivíduos, como a abertura de clareiras em meio à vegetação, possibilitando a incidência solar direta no solo.

Por outro lado, espécies arbóreas, as quais se caracterizam por comporem ambientes já mais estabelecidos ou de sucessão tardia, geralmente apresentam uma estratégia de estabelecimento diferente – o banco de plântulas – caracterizado por sementes que germinam rapidamente após a dispersão, mas que, em condição de plântula, apresentam taxas de desenvolvimento extremamente baixas, até que haja disponibilidade de recursos (como uma fonte luminosa) suficiente para se desenvolverem (Parker *et al*, 1989). Assim, em áreas de sucessão tardia, enquanto o banco de sementes se mantém composto por espécies pioneiras, como gramíneas e herbáceas, a comunidade é composta principalmente por espécies arbóreas, evidenciando a baixa similaridade entre o banco de sementes e a vegetação.

Assim, o banco de sementes se torna um fator fundamental não só para a manutenção e expansão da distribuição das espécies, mas principalmente para a reestruturação da comunidade vegetal em áreas que sofreram algum tipo de perturbação, além de também possibilitar a identificação da composição das comunidades vegetais em tempos passados (van der Valk & Pederson, 1989; Hopfensperger, 2007).

Considerando então a importância do banco de sementes na restauração e estabelecimento de novas comunidades vegetais em áreas degradadas ou que sofreram algum distúrbio (Baskin & Baskin, 2014; van der Valk & Pederson, 1989), o estudo do papel do banco de sementes na dinâmica da vegetação envolve três pontos fundamentais: (1) conhecer a composição de espécies do banco de sementes, (2) conhecer as características biológicas das sementes nele inseridas, tais como o tipo de dormência, tolerância ao déficit hídrico do solo, à perda d'água por parte das sementes e à condição de temperatura do local e (3) avaliar o impacto de fatores abióticos no banco de sementes, tais como temperatura atmosférica e do solo, pluviosidade, disponibilidade hídrica e luminosidade (Leck *et al*, 1989).

Fatores abióticos e sua influência sobre o banco de sementes

Sementes do banco de sementes do solo estão constantemente sujeitas às influências do meio abiótico no qual estão inseridas. A temperatura do solo e sua disponibilidade hídrica são dois dos mais importantes fatores de influência sobre a viabilidade destas sementes e processos como dormência, germinação e recrutamento de novos indivíduos (Parker *et al*, 1989; Baskin & Baskin, 2014; Ooi *et al*, 2014; Liyanage & Ooi, 2017).

Tais características abióticas envolvidas com o banco de sementes, propiciam a formação de microclimas que exercem constante influência, positiva ou negativa, sobre o banco de sementes. Conhecê-las permite uma predição mais apurada da composição de sementes no solo e, conseqüentemente, do potencial de regeneração da vegetação (van der Valk & Pederson, 1989; Breshears *et al*, 1998; Weerasinghe *et al*, 2008; Ooi *et al*, 2012).

A temperatura do solo desempenha um papel de grande importância sobre a distribuição da vegetação, influenciando tanto a viabilidade e dormência de sementes do banco quanto os indivíduos jovens (Labouriau, 1983; Parker *et al*, 1989; Borghetti, 2004; Ooi *et al*, 2012, Lyianage & Ooi, 2017). Esta influência age diretamente sobre a dinâmica do banco de sementes, onde flutuações de temperatura servem como indicador das condições do ambiente e poderão inibir ou promover a ocorrência ou não da germinação (Leck et al, 1989). Áreas cobertas por vegetação tendem a apresentar temperaturas mais amenas, uma vez que a cobertura vegetal propicia a formação de um microclima que favorece a germinação e estabelecimento de indivíduos juvenis. Já áreas desprovidas de cobertura vegetal apresentam condições climáticas mais extremas, como altas temperaturas e baixa umidade ou disponibilidade hídrica, e dependem de espécies bastante tolerantes a condições adversas para sua restauração (Harte *et al,* 1995; Pierson & Wight, 1991; Breshears *et al* 1998; Flerchinger & Hardegree, 2004). Janelas de oportunidade são uma alternativa neste processo e podem favorecer o surgimento de novos indivíduos, quando eventos como chuvas tornam o ambiente favorável a germinação e estabelecimento de novos indivíduos em áreas abertas (Gioria *et al*, 2016). Há de se ressaltar também que, mesmo antes de favorecer a germinação das sementes do banco, a chuva já desempenha um papel fundamental para a dinâmica da vegetação ao promover a movimentação das sementes pelo ambiente tanto lateralmente, agindo como dispersor secundário, quanto verticalmente no solo, seja soterrando ou trazendo sementes para camadas mais superficiais (Benvenuti, 2007; Marthews *et al*, 2008).

Outra característica diretamente responsável pela manutenção da viabilidade de sementes no banco e ao sucesso de germinação e recrutamento é a disponibilidade hídrica no solo. Espécies oriundas de ambientes com condições climáticas de baixa previsibilidade, como regimes de chuvas não uniformes, são caracterizadas por diversas estratégias para prevenir os efeitos de flutuações de condições abióticas, tais como produção de mucilagem, dormência e resistência a períodos de seca, que podem ser prejudiciais às sementes e plântulas (Baskin & Baskin, 1982; Ooi *et al*, 2007; Barbosa & Garcia, 2014). Áreas de solo nu tendem a apresentar maior perda d’água por parte do solo, em comparação a áreas com cobertura vegetal, que se mostram com maior potencial de retenção de umidade, evidenciando a influência direta da cobertura vegetal sobre as características microclimáticas às quais o banco de sementes estará submetido (Breshears *et al*, 1998; Royer *et al,* 2011). Logo, a interação entre temperatura e disponibilidade hídrica do ambiente será um processo chave para o futuro das sementes do banco. A temperatura atua como um agente mais ligado à quebra de dormência (Ooi *et al*, 2012; Lyianage & Ooi, 2017), enquanto que a disponibilidade hídrica determinará o sucesso ou não da germinação e recrutamento (Fenner & Thompson, 2005).

A luz é um importante agente de influência sobre a germinação, principalmente de sementes pequenas e com pouca reserva, portanto o soterramento deste tipo de sementes se torna um fator inibidor da germinação, visto que a luz só é capaz de penetrar até o primeiro centímetro de profundidade do solo (Bliss & Smith, 1985; Tester & Morris, 1987; Baskin & Baskin, 2014). Com isso, sementes pequenas e soterradas dependem de eventos aleatórios que levem a distribuição das sementes ao longo de diferentes camadas do solo, favorecendo a germinação daquelas que se encontram na superfície ou a poucos milímetros de profundidade (Baider *et al*, 1999; Tobe *et al*, 2005), e a formação do banco de sementes por parte daquelas soterradas, dispersando a germinação ao longo do tempo (Leck *et al*, 1989; Parker *et al*, 1989).

Fatores abióticos são comumente apontados como os principais agentes de influência sobre a distribuição e história de vida de espécies vegetais (Lu *et al*, 2014; Frosini *et al*, 2012; Grant & Grant, 1993), mas distúrbios resultantes da ação antrópica e ocupação de ambientes por espécies exóticas, invasoras ou introduzidas também são responsáveis por grande parte da redução da distribuição e até da extinção de inúmeras espécies ao redor do mundo, principalmente as endêmicas (Wilcove *et* al, 1998; Sax & Gaines, 2008; Kueffer *et al*, 2009).

Dentre os exemplos de como espécies exóticas podem ser prejudiciais à biodiversidade de ambientes com alto grau de endemismo estão os ambientes insulares (Sax & Gaines, 2008; Weerasinghe *et al*, 2008; Carvalho-Silva *et al*, 2013), onde ocorre cerca de um terço das espécies vegetais conhecidamente ameaçadas (Groombridge, 1992). Estes são locais de grande importância ecológica pelo seu potencial em proporcionar eventos de especiação, e consequentemente, configurarem ambientes com alto grau de endemismo (Caujapé-Castells *et al*, 2010; Whittacker & Fernández-Palacios, 2007; Myers, 2000). Estes eventos ocorrem principalmente em decorrência do isolamento geográfico aos quais tais espécies estão submetidas, e à consequente necessidade das mesmas em se adaptarem às condições ambientais locais (Sax & Gaines, 2008; Serafini, 2010). Os processos de especiação e a diversidade de grupos de seres vivos resultantes deles, dependem não só das condições do habitat, mas inclusive do tipo de evento que as originou. Ilhas resultantes da movimentação de placas tectônicas ou elevação do nível dos oceanos, como Madagascar e Tasmânia respectivamente, geralmente apresentam espécies endêmicas resultantes de populações que já ocorriam na região anteriormente à sua separação do continente. Nestas regiões é maior a diversidade de mamíferos e anfíbios, quando comparadas às ilhas oceânicas. Ilhas oceânicas, que por sua vez resultam principalmente de erupções vulcânicas, portanto, nunca estiveram conectadas às massas continentais, apresentam maior diversidade de grupos com capacidade em alcançá-las, como aves marinhas, peixes e plantas, por exemplo (Groombridge, 1992).

Num contexto global, se considerarmos a proporção entre área emersa e endemismo, ambientes insulares promovem uma contribuição significativamente maior à biodiversidade vegetal do planeta, quando comparadas às massas continentais. Ilhas abrigam quase 25% de toda biodiversidade vegetal conhecida, apesar de representarem apenas 5% da cobertura de terra do planeta. (Bramwell & Caujapé-Castells, 2011)

Por outro lado, tal restrição tanto territorial quanto ecológica, faz com que estes ambientes se mostrem suscetíveis a condições adversas, como o estabelecimento de espécies exóticas ou introduzidas (Kier *et al,* 2009; Caujapé-Castells *et al*, 2010; Serafini 2010). No caso de espécies vegetais endêmicas, o impacto da chegada de exóticas, sejam elas animais ou vegetais, se torna ainda mais acentuado em ilhas onde sua riqueza e abundância são baixas (Kueffer, 2009).

Espécies de mamíferos herbívoros foram historicamente introduzidas em ilhas e seus impactos vem da pressão direta exercida pelas altas taxas de herbivoria. Partindo do princípio exposto por Groombridge (1992) - em que a diversidade de espécies em ilhas oceânicas é maior para aves marinhas, peixes e plantas, enquanto ilhas continentais é para anfíbios e mamíferos, a introdução de mamíferos herbívoros se torna especialmente nociva a ilhas oceânicas como é o caso de Trindade, visto que uma barreira natural restringindo a chegada de mamíferos, levou a espécies vegetais endêmicas a possuir poucos ou nenhum mecanismo de defesa contra a herbivoria. Além disso, resultados indiretos também são relatados e o principal deles é que ambientes degradados pela herbivoria se tornam propícios à colonização por plantas invasoras, as quais são geralmente dispersas pelos próprios herbívoros e tem maiores capacidades adaptativas em ambientes degradados, acabando por colonizar áreas antes nativas (Diong, 1982; Aplet *et al,* 1991; Siemann *et al*, 2009; Busby, 2010. Oduor *et al*, 2010.; Cole *et al*, 2012).

Espécies endêmicas de ambientes insulares podem se mostrar suscetíveis à introdução de espécies não nativas, uma vez que estas espécies de ampla distribuição apresentam maior capacidade competitiva e adaptativa, podendo se tornar uma ameaça à biodiversidade de tais ambientes (Sax & Gaines, 2008). Em Galápagos, por exemplo, a presença da invasora *Cinchona pubescens* Vahl (*Rubiaceae*) foi responsável não só pela diminuição da diversidade de espécies da ilha em mais de 50% nas áreas onde ocorre, sendo as endêmicas as mais afetadas, tal situação propiciou a chegada de outra espécie invasora, *Stachys agraria* Schltdl. & Cham., que colonizou áreas de solo nu resultantes da presença de *C. pubescens* (Jager *et al*, 2009).

Influência de agente bióticos e abióticos sobre a dinâmica da vegetação dentro do contexto de ocupações da Ilha da Trindade – Brasil.

No Brasil, um exemplo de ambiente insular que passou por redução de sua biodiversidade através da ação de espécies introduzidas é a Ilha da Trindade (Figura 1), que passou por uma grande transformação em sua composição florística após a introdução de animais exóticos, como porcos (*Sus scrofa* L.) e cabras (*Capra hircus* L.), que foram introduzidas no século XVII com o intuito de favorecer a ocupação e permanência humana na ilha (Lobo, 1918; Barth, 1958; Carvalho-Silva *et al*, 2013).

Figura 1 Visão da face leste da Ilha da Trindade (foto: Simone Marinho)

Tais autores apontam a herbivoria praticada por estas espécies como responsável por grande parte da perda da riqueza de espécies da Ilha. Relatos dão conta de que, antes das cabras, espécies arbóreas eram predominantes ao longo de quase toda superfície da ilha, sendo *Colubrina glandulosa* subsp. reitzii (M.C.Johnst.) Borhidi (Rhamnaceae) a mais abundante delas (Lobo, 1918; Eyde & Olson, 1983; Martins & Alves, 2007), mas com outros exemplares arbóreos de grande porte, como *Paratecoma peroba* (Record) Kuhlm, que teve sua presença recém relatada para a Ilha (Witovisk *et al*, 2018). Hoje, espécies de grande porte se restringem quase que exclusivamente às amendoeiras (*Terminalia catappa* L.) introduzidas (Figura 2. a) e a pontos isolados como a floresta das samambaias gigantes (*Cyathea delgadii* Sternb.) (Figura 2. b) e o Pico do Desejado, onde *Myrsine umbellata* R. Br. (Primulaceae), é a espécie arbórea dominante (Figura 2. c) (Barth, 1958; Alves, 1998; Silva & Alves, 2011; Carvalho-Silva *et al*, 2013).

Figura 2 Perfis da vegetação da ilha da Trindade. (a) Amendoeiras próximas à Praia dos Portugueses (b) Floresta de Samambaias Gigantes (c) Arbóreas no Pico do Desejado com predominância de *Myrsine umbellata* (d) Visão geral da Ilha evidenciando a predominância de espécies de baixo porte, como *Cyperus atlanticus*

Com isso, alguns pontos da ilha passaram a não ter cobertura vegetal ou contar apenas com a presença de espécies herbáceo-arbustivas (Faria *et al,* 2012), com a predominância de áreas cobertas apenas por vegetação de baixo porte (Figura 2.d), tendo como espécies mais abundantes *Bulbostylis nesiotis* (Hemsl.) C.B.Clarke (Cyperaceae) e *Cyperus atlanticus* Hemsl. (Cyperaceae). Como conseqüências vieram, a extinção de espécies nativas, ocorrência de erosão de solos e o desaparecimento de grande parte dos cursos d'água (Barth, 1958; Silva & Alves *et al*, 2011).

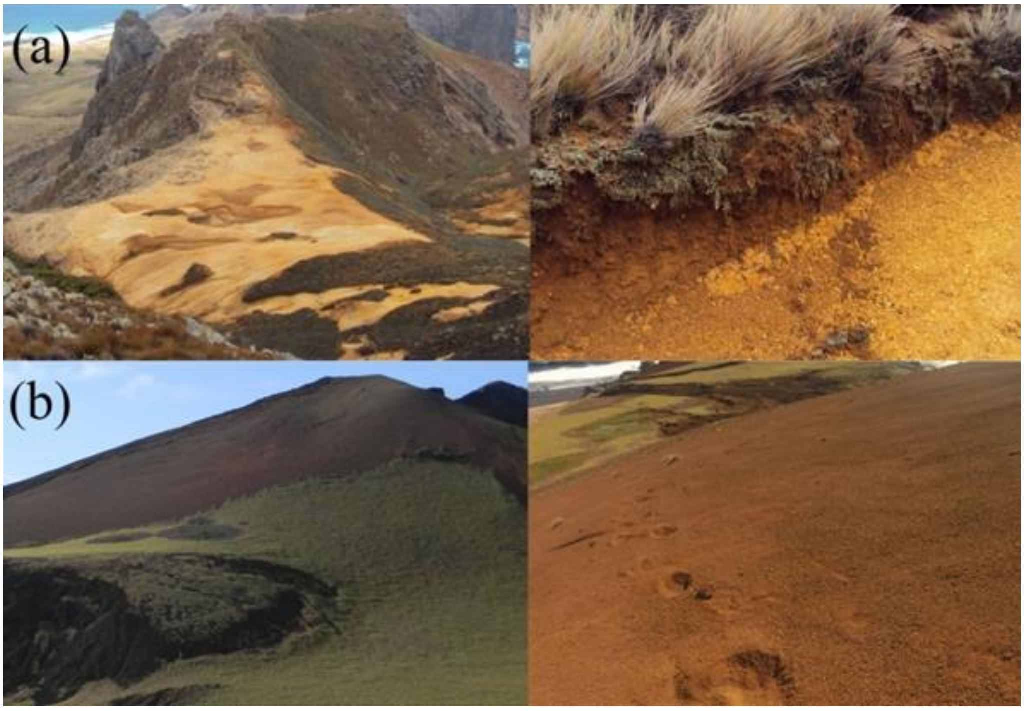

Figura 3 Perfil de duas das áreas desprovidas de vegetação e detalhe de seus respectivos solos. (a) Região próxima ao platô do Pico do Desejado (b) Morro do Paredão. (fotos: Bruno Rabelo)

Esta soma de fatores negativos indicou a necessidade de um plano de recuperação da vegetação da ilha, sendo que a retirada das cabras foi uma das providências tomadas. Em 2005 ocorreu sua total erradicação, tendo sido talvez a medida mais relevante para a contenção do processo de degradação (Clemente *et al*, 2012; Silva & Alves, 2011; Carvalho-Silva *et al*, 2013).

Cerca de dez anos após os primeiros esforços de retirada das espécies introduzidas, a ilha já apresentava indícios não só de recuperação da vegetação (Figura 4) , com aumento da riqueza e abundância de espécies em locais que tiveram sua cobertura vegetal quase que totalmente suprimida, como também do reaparecimento de alguns cursos d'água. (Alves & Martins, 2004; Martins & Alves, 2007). A partir desta iniciativa, a Ilha vem passando por um momento de transição, de um ambiente quase que desprovido de vegetação, para um ambiente em recuperação. Como exemplo, em expedição à ilha em 1918, o pesquisador Bruno Lobo catalogou apenas 26 espécies de plantas, e hoje esse número já chega a mais de 130 espécies de plantas, sendo 11 espécies endêmicas (Alves, 1998), duas das quais representam a maior cobertura vegetal da ilha (*Bulbostylis nesiotis, Cyperus atlanticus*), e recentemente uma nova gramínea foi identificada (*Sporobolus nesiotioides* Longhi-Wagner, R.J.V. Alves & Nilber) (Longhi-Wagner *et al*, 2013).

Atualmente, algumas espécies endêmicas que chegaram a ser consideradas extintas pela ação predatória das cabras, já foram novamente registradas e têm mostrado alta capacidade de regeneração, tais como *Plantago trinitatis, Asplenium beckeri* e *Elaphoglossum beckeri* Brade (Dryopteridaceae) (Alves, 1998; Silva *et al,* 2013). A recuperação natural por parte destas espécies pode ter sido desencadeada tanto pelo banco de sementes do solo que se manteve até a retirada das cabras quanto por populações estabelecidas em áreas não alcançadas pelos herbívoros (Martins & Alves, 2007; Alves *et al*, 2011; Silva *et al*, 2013).

Figura 4 Recuperação da vegetação. A primeira foto é datada de 1994, quando a llha ainda era habitada por rebanhos de cabras. À direita, já em 2009, foto do mesmo local quase cinco anos após a erradicação dos caprinos (foto: Alves & Silva, 2013).

Estudos em ilhas como Havaí, Guadalupe (México), Pinta (Galápagos) e Santa Catalina (México), apontaram para a recuperação vegetal de ambientes antes degradados pela ação predatória de mamíferos introduzidos. A partir da retirada destes animais, espécies endêmicas e nativas em todos os locais citados, passaram a ocupar áreas antes degradadas, auxiliando assim, na recomposição de espécies e prevenção de erosões e perda de solo (Aguirre-Muñoz *et al,* 2008; Cole *et al,* 2012; Hamann, 1979; Knapp, 2014). Esta recuperação pode ocorrer de forma rápida e eficaz em casos de espécies que têm por característica a chuva de sementes nas proximidades da planta mãe, acarretando o avanço da comunidade vegetal sobre ambientes desprovidos de vegetação e deixando ainda uma outra parte deste conjunto de sementes armazenada no solo (Olano *et al*, 2012).

Assim, este trabalho investigou o papel do banco de sementes na recomposição florística das áreas ainda desprovidas de vegetação (Figura 3), as quais, devido a esta condição, se mostram suscetíveis a eventos de erosão, já observados em outros pontos da ilha. Para isso, além da análise do banco, estudou-se as respostas das sementes de espécies da Ilha, por meio da germinação, a simulações em laboratório de condições ambientais encontradas nas áreas de estudo da Ilha. Tais respostas poderão então auxiliar na compreensão do potencial das áreas de estudo em se recuperarem de forma espontânea e qual seria o papel das espécies alvo na recuperação das áreas.

HIPÓTESES

- Em comparação às áreas desprovidas de vegetação, áreas com presença de cobertura vegetal propiciam condições abióticas mais favoráveis à viabilidade das sementes, levando a uma maior riqueza e abundância no banco.

- Os diferentes padrões abióticos encontrados exercem influências de distintas proporções sobre a germinação das espécies encontradas no banco de sementes.

- Áreas de solo desnudo são locais em que um menor número de espécies é capaz de colonizar. Entretanto, as espécies presentes nas áreas adjacentes apresentam capacidade de germinação, mesmo sob condições ambientais mais extremas, o que torna o banco de sementes das áreas expostas hábil a favorecer sua regeneração natural.

- A revegetação da Ilha é um processo que pode ser determinado tanto pela expansão das populações já estabelecidas quanto pela recolonização de áreas atualmente desprovidas de cobertura vegetal.

OBJETIVOS

Objetivo Geral

- Investigar o papel do banco de sementes do solo como potencial agente na regeneração natural da vegetação de áreas da Ilha da Trindade.

Objetivos específicos

- Caracterizar cada um dos ambientes selecionados quanto a condições abióticas (temperatura do solo e disponibilidade hídrica do solo) com o objetivo de investigar qual o seu impacto na viabilidade das sementes no solo.
- Caracterizar a composição específica e as características dos bancos de sementes em cada uma das áreas de estudo da Ilha.
- Investigar a capacidade de germinação de espécies do banco, a partir de experimentos em laboratório que simulem as condições de campo (temperatura do solo, déficit hídrico), de modo a reconhecer seu potencial para atuar na recomposição vegetal da área estudada.
- Analisar a influência do tempo de armazenamento sobre a viabilidade das sementes das espécies alvo.

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CAPÍTULO 1

Soil temperature, but not water stress, have a strong potential to affect abundance of endemic species in soil seed bank

Soil temperature, but not water stress, have a strong potential to affect abundance of endemic species in soil seed bank

Abstract

Oceanic islands have a global conservation value due to the high levels of endemism and range restriction of their species composition. However, the biodiversity of many of these environments have been threatened due to the introduction of exotic species over the last centuries. On Trindade Island, the decrease of biodiversity, in particular endemic species has been caused by introduced goats. After their eradication, the island has shown potential for regeneration, but some areas still have little evidence for potential vegetation recovery. The lack of vegetation cover can influence the dynamics of a plant community by causing an increase of soil temperatures and reducing soil water availability. In this study, we set out to identify whether the presence of a seed bank of the native species can be a key to recovery of the vegetation and to determine the capacity for continued recovery of bare patches. Measurements were taken to compare abiotic patterns of bare and vegetated patches and these patterns were used to identify Seed Viability Loss (SVL), through laboratory experiments. Seeds of only three species were found in the soil samples (*Bulbostylis nesiotis*, *Cyperus atlanticus* and *Cyperus sp.*). Abiotic measurements showed a significant difference between bare and vegetated patches. Laboratory-simulated bare soil temperature patterns caused the Seed Viability Loss of *C. atlanticus* and *Cyperus sp.* seeds. Therefore, only *B. nesiotis* may be able to germinate on bare patches, which may lead to a slow recovery process. This approach contributes to a better understanding of how the association between functional traits of endemic species and environmental conditions can influence the recovery of disturbed native areas.

Keywords: soil seed bank, *Cyperaceae*, temperature, water stress, seed viability loss

Introduction

Oceanic islands have a global conservation value due to the elevated levels of endemism and range restriction of their species composition (Myers *et al*, 2000; Kier et al, 2009). They have a significant contribution to the Earth’s biodiversity, since their representation of land coverage is only about 5%, while their plant biodiversity reaches

25% of all known biodiversity (Whittacker & Fernández-Palacios, 2006; Bramwell & Caujapé-Castells, 2011). These islands are subject of disturbance due to human intervention, which has placed the persistence of native ecosystems under pressure (Brockie et al., 1988; Cole *et al,*2012).

One of the most common disturbances result from the introduction of domestic herbivores, such as goats and pigs (MacPhee & Flemming 1999; Campbell & Donlan, 2004). Insular vegetation, composed of mainly endemics that have lost their area because of the presence of herbivores on islands around the world, is particularly susceptible to grazing due the natural absence of large herbivores in these environments originally. Overgrazing of such species leads to loss of vegetation cover and biodiversity, causing soil erosion and decrease of seed bank richness (Groombridge, 1992; Weerasinghe, 2008, Clemente *et al*, 2009; Clemente *et al,* 2012; Zhu *et al*, 2015). Fifty years ago, having goats on the islands was common for several reasons causing 26% of plant species to be threatened by increased levels of herbivory (Lucas & Synge, 1978). Currently, goats are being removed to avoid extinction of insular species.

Removal of feral herbivores from oceanic islands is a powerful tool for conservation and can lead to insular vegetation recovery (Brockie *et* al, 1988; Campbell & Donlan, 2005; Aguirre-Muñoz *et al,* 2008; Cole *et al,*2012; Hamann, 1979; Knapp, 2014). This recovery can occur mainly by the regrowth of small populations unreached by herbivores, therefore, seedbanks are considered a fundamental driver of vegetation recovery processes in disturbed areas (van der Valk & Pederson, 1989; Hopfensperger, 2007).

On oceanic islands, particularly those that are far from the mainland, are more intensively damaged by introduced species. In these cases, the role of seed banks in vegetation recovery may be even more important, due geographic isolation and the limited number of endemic species that can lead to recovery (Weerasinghe, 2008; Tanaka *et al,* 2010; Cross *et al*, 2015; Ortiz-Alcaráz, 2016). The seeds in the soil are constantly subjected to abiotic influences that are positive or negative. Temperature and moisture are arguably among the most important of these factors (Baskin & Baskin, 2014). In seed banks, these factors control process such dormancy, maintenance of viability, germination and seedling recruitment (Parker *et al*, 1989; Ooi *et al*, 2014; Liyanage & Ooi, 2017). Understanding seed bank dynamics and their drivers, in combination with assessing seed bank composition in addition to seeds traits, is essential for informed management and restoration decisions aimed at ecosystem recovery.

In Trindade, a Brazilian oceanic island located in the South Atlantic Ocean, the main cause of decreased plant and animal biodiversity was the herbivory of introduced domestic mammals, especially those of goats and pigs (Barth, 1958; Alves, 1998). Since the eradication of goats and pigs in 2005, the island has shown signs of vegetation recovery with the reappearance of some endemic species that were thought to be extinct as a result of herbivory. The natural recovery of these endemic species was driven by persistence in the soil seed bank or small relict populations on unreached regions (Martins & Alves, 2007; Alves *et al*, 2011; Silva *et al*, 2013).

Thirteen years after the eradication of introduced herbivores, the island has shown potential for regeneration by recruitment of new seedlings, but these observations began a few years after the goats withdraw (Sylvestre, 2007; Alves, 2011), but several areas on the island continue to maintain bare soil patches, with little evidence for vegetation recovery. The overexposure of these areas to solar irradiation can drive changes in the microclimate patterns of these locations. These changes influence the dynamics of a plant community by both increasing soil temperatures and reducing soil water availability (Harte *et al,* 1995; Pierson & Wright, 1991; Breshears *et al* 1998; Flerchinger & Hardegree, 2004). Some of the consequences already reported of these bare patches are decreased species richness and reduction of soil layers caused by erosion (Clemente *et al*, 2009; 2012).

In this study, our goal was to describe abiotic patterns of bare and covered soil areas and also identify the presence of a seed bank of native species, which could possibly be the key for recovery of the vegetation of Trindade Island. Furthermore, by studying the response of the same species under different abiotic patterns that replicate the conditions of bare and covered soils on the Island, we aimed to determine the capacity for continued recovery of bare patches. Our approach offers a better understanding of how the association of functional traits of endemic species and environmental conditions can influence the recovery of disturbed native areas.

Materials and methods

Study site

The site of the present study located on Trindade (20° 30' S, 29° 22' W), a volcanic Island located in the South Atlantic Ocean, 1.200 km from the Brazilian coast. The island has a land area of 10 km², and a peak altitude of 600 meters. The mean temperature is 27,7°C during the hottest month (February) and 22,9°C during the coldest month (August), with an annual average of 25°Cn (Figure 5). The annual average rainfall is 1476 mm distributed through the year, with no defined rainy and dry seasons, which is classified by Koppen as Tropical with no dry season - '*Af*' - (Tropical climate, with no winter season and high precipitation levels with no dry season).

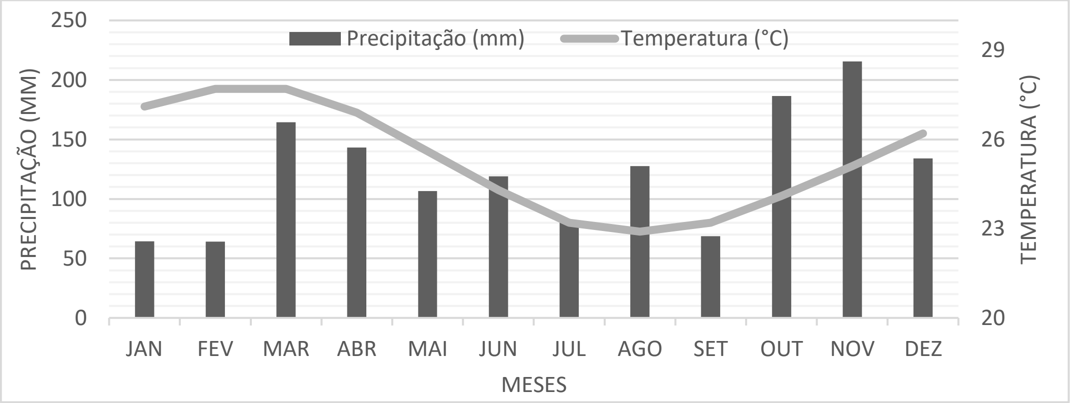

Figure 5 Mean rainfall levels and air temperature throughout the year.

The island was subjected to heavy grazing by goats for more than 300 years prior to their eradication in 2005. After their elimination, natural recovery of the vegetation was observed in some areas, but large areas remain as bare soil and are subject to processes of damage such as erosions. Today, the largest part of the island vegetation is composed of herbaceous and sedges species like *Bulbostylis nesiotis* (Hemsl.) C.B.Clarke (Cyperaceae) and *Cyperus atlanticus* Hemsl. (Cyperaceae). Tree species are basically restricted to introduced ones such as *Terminalia catappa L.* and a few other species on the higher peaks, such as *Cyathea delgadii*, which composes a fern forest (Barth, 1958; Alves, 1998; Silva & Alves, 2011; Carvalho-Silva *et al*, 2013).

Two hills, Paredão (20°31'10''S, 29°18'15''O) and Andradas (20°30'47''S, 29°18'37''O), were selected to be the focus of this study. This selection was done due the similarity of coverage and vegetation composition between them. Though the vegetation become scarcer from the bottom to the top of each hill, with bare soil areas occurring close the tops, sedges predominate as the main vegetation cover of the soils up the hills.

For this study these hills were subdivided into vegetated (VEG) and bare soil (BARE) locations, resulting in four plots (Par.veg, Par.bare, And.veg, And.bare). Soil samples were collected, and abiotic measurements were made in each plot.

Abiotic assessment of bare soil and vegetated areas

Abiotic factors were measured ten times over three months for each plot. In each case, both temperature and soil water availability were measured hourly to assess the pattern of variation of these factors in each of the four plots. For soil temperature, three points were randomly selected in each plot and soil temperature was recorded at three soil depths (1 cm, 3 cm and 5 cm) at each point (Thermometer Instrutherm TE 400). The measurements started at 5:30am and finished at 3:30pm, the period of direct solar incidence. This allowed for the assessment of the variation in temperature throughout the day and at different depths. For available moisture measurements, soil samples were collected using plastic cups at 5:30am, 10:30am and 3:30pm, at the same locations as the soil temperature assessment. These cups were closed, separately by samples, and taken to a laboratory for the measurements of water availability of the soil, which were done, at the end of the day, using a dew point hygrometer (WP4C - Decagon). The thermal and hydrological patterns recorded in vegetated and bare soil areas were later used to establish the germination experiments parameters.

Seed bank assessment

To compare the density of germinated seeds and the species composition of the seed banks, in January of 2016, 100 soil samples were collected through the whole area of each of the four sites (two hills with two areas each) resulting on 400 samples. Soil sample dimensions were 11 cm x 11 cm x 3.5 cm (for more details see Weerasinghe *et al*, 2008). These 100 soil samples from each plot were combined in the field and transported to the laboratory for a six months seed emergence experiment.

Each treatment was divided into 15 plastic trays. To avoid the influence of burial on seed germination, the samples were spread out on trays that were 2 cm deep and was turned after three months. Trays were placed outside of the laboratory (University of Brasilia), where the mean temperature was 25°C. Germination was checked daily and scored on seedling emergence by treatment. Trays where watered every day for a consistent amount of moisture in each tray.

Emerged seeds were separately recorded by area and the final result was a general sum of the 100 soil samples of each area. After conclusion of the experiment, some individuals were transplanted to separate pots to grow for later identification. The sieving method to search seeds was firstly used but no seeds were found, then, due the small size of the seeds, the emergence method was chosen.

Seed viability loss* (SVL) *under different soil temperatures and water stress simulations

This experiment was conducted to test whether the measured abiotic patterns were able to cause some influence over the seed density of soil seed bank of covered areas and bare soil areas. Here we aimed to identify levels of seed viability loss under differente temperature patterns. Based on the results of the previous seed bank experiment, seeds were collected, from dispersing plants (not from the soil seed banks), from both hills, in January of 2016. Seeds found in the bare soil samples were the same of the covered soil areas. The species used for the SVL experiment were *Bulbostylis nesiotis* (Hemsl.) C.B.Clarke (*Cyperaceae*), *Cyperus atlanticus* Hemsl. (*Cyperaceae*), and *Cyperus sp.*(*Cyperaceae).*

To determinate whether the temperature pattern recorded in the field influence seed viability, two different germination chambers were used, one simulating the temperature patterns for vegetated plots (VEG), and the other for bare plots (BARE). The VEG treatment consisted of a daily variation of temperatures starting at 21°C at 6 am for one hour, which was progressively increased to 30°C from 7am to 11am, staying constant at this temperature for one hour. From noon to 6-am of the next day, the temperature was decreased constantly from 30°C to 21°C, after which the cycle was repeated. For the BARE treatment, the temperature also started at 21°C for the 6 – 7am period. However, from 7 - 11am, the temperature was progressively increased to 55°C, staying constant for one hour, from 11 – noon, and decreased to 30°C during the noon – 6 pm interval. From 6 pm to 6 am, the temperature was decreased to 21°C, and the cycle then restarted. In each chamber, 60 seeds of each species were divided into four petri dishes lined with moistened filter paper, which were watered whenever necessary. Germination was checked daily and the germinated seeds were recorded and removed from the dishes. The experiment was carried out for 2 months and at the end of this period, the tetrazolium test (1%, by 24 hours) was used to assess whether any of the non-germinated seeds were still viable.

To assess if the seeds were able to maintain their viability under conditions of water stress, seeds of the three species were subjected to a gradient of PEG 6000 solutions. This is a very common solute used to simulate water stress conditions by being a chemically inert solute and by do not show toxicity upon the seeds. The PEG solutions used were -0.2; -0.4; -0.6; -0.8 and -1.0 MPa, widely used concentrations which are able to indicate seed capacity to keep their viability under water stress (Villela *et al*, 1991). In a chamber set to a 12 hours photoperiod and a constant temperature of 25°C, 60 seeds of each species were divided among four dishes lined with moistened filter paper, using the PEG concentrations for the treatments and distilled water as control. All the dishes were sealed with parafilm. Germination was checked daily, and germinated seeds were recorded and removed from the dishes. The experiment was carried out over 2 months and at the end of this period, a tetrazolium test was used to assess whether any of the non-germinated seeds were still viable. Seed Viability Loss was considered by the seeds that were not viable at the Tetrazolium test.

Analysis

The analysis of abiotic patterns (soil temperature and water potential) were done using ANOVA followed by a Tukey test, with soil depths levels (1cm, 3cm, and 5cm) and presence or absence of vegetation as factors of influence over the temperature patterns and soil water potential. This analysis allows to identify the influence of soil cover by vegetation over the thermal patterns and moisture availability in the soil. To determine the soil temperature fluctuations, we considered only the surface temperatures of both areas (presence and absence of vegetation), analyzing differences between hours of the day. This stage provides maximum and minimum temperatures throughout the day in the different areas. To compare the abundance of seeds of each species found in the seed bank, we used a General Linear Model (GLM) and log function transformation, with a Poisson error distribution. A quasi-poisson model was used if data were over dispersed. For analysis of Seed Viability Loss (SVL) under temperature patterns, we used Analysis of Variance (ANOVA) followed by a Tukey test, comparing SVL responses of the three species and two different patterns of temperature. The final number of dead seeds were estimated by the difference between the initial amount of seeds and the sum of the number germinated and the number that produced a positive tetrazolium response. The data analysis of SVL under the gradient of water potential were done by species, separately. Each specie responses to the water stress were analyzed using an ANOVA test followed by Tukey test with the PEG solution concentration as main factor. Significance for all statistical tests was set at $p < 0.05$. All the analysis was done using program R Studio Version 1.0.143 and the packages readxl and lsmeans (Lenth, 2016; Wickham & Bryan, 2017).

Results

Abiotic assessment of bare soil and vegetated areas

At the bare soil plots, temperatures nearer the surface were significantly higher than at any other depths ($p<0.01$), reaching a maximum of 60°C and minimum of 21.4°C (Figure 6). Despite deeper layers (3 and 5 centimeters) have produced more moderate values, where the maximum and minimum were 35°C and 23°C, respectively, their difference between them was also significant ($p < 0.01$). Vegetated plots showed a smaller diurnal range of temperatures across all layers, however, as observed in bare, surface layer showed higher temperature values, by being different when compared to 3 cm ($p = 0.03$) and 5 cm ($p < 0.01$), otherwise, there was no influence between the deeper layers on the temperature patterns ($p = 0.41$) (21.4°C and 28.5°C on the surface and 25°C to 28°C in deeper layers.

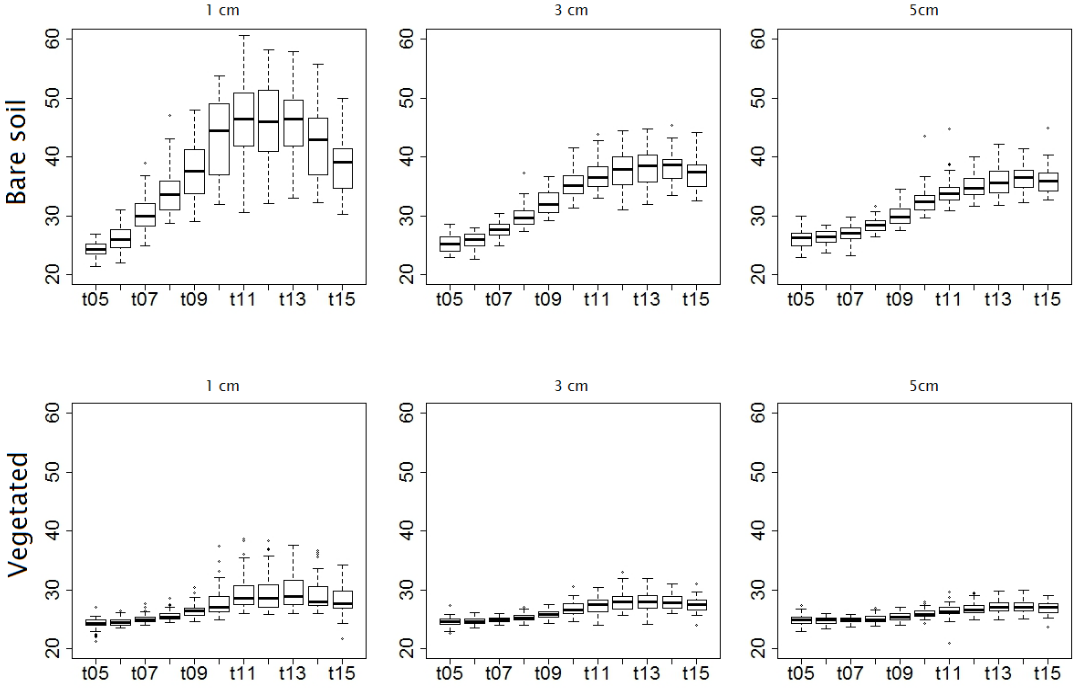

Figure 6 Temperature (°C) patterns recorded in bare soil and vegetated soil at 1, 3, and 5cm depth and different times of the day: 5:30 am (t05) to 15:30 pm (t15). Data expressed as hour throughout the day.

Vegetation cover still being the main factor of influence over the patterns of water availability in the soil (*p* > 0.01) and the surface of both patches showed more stressful conditions than their respective deeper layers (Table 1).

In bare soil plots, water potential was affected by depth, with differences occurring between the 3cm and 5cm layers, compared to the surface (*p* < 0.01), while the deeper layers showed similar values (*p* = 0.21). Near the surface, the average water potential was -50.21 ± 3.12 MPa, while at 3 cm and 5cm, this reduced to - 5.10 ± 0.78 MPa, and - 0.85 ± 0.16 MPa, respectively (Table 1). At the vegetated plots, there was no influence of depth on the moisture availability (*p* > 0.92). At these plots, the averages were of -1.93 ± 0.51 MPa on the surface, -0.16 ± 0.04 MPa in 3 cm layer and -0,10 ± 0.02 MPa in 5cm layer (Table 1).

Table 1. Mean soil water potential (MPa) (±SE) of the four areas: Bare soil and Vegetated Soil on Andradas Hill and Paredão Hill. Different letters in the rows indicate significant differences (*p* < 0.05).

		Paredão Hill		Andradas Hill	
Hour	Depth	Bare	Vegetated	Bare	Vegetated
5	1	- 22 ± 2.79^{a}	- 1.79 ± 0.81^{b}	- 12.9 ± 2.77^{a}	- 1.15 ± 0.51^{b}
	3	- 7.86 ± 1.42^{a}	- 0.13 ± 0.04^{b}	- 2.73 ± 1.15^{b}	- 0.3 ± 0.19^{b}
	5	- 2.18 ± 0.70^{a}	- 0.08 ± 0.04^{b}	- 0.93 ± 0.40^{ab}	- 0.16 ± 0.09^{b}
10	1	- 81 ± 5.24^{a}	- 1.64 ± 0.94^{c}	- 50.93 ± 8.08^{b}	- 0.72 ± 0.54^{c}
	3	- 9.26 ± 2.95^{a}	- 0.1 ± 0.05^{b}	- 4.67 ± 2.30^{ab}	- 0.09 ± 0.05^{b}
	5	- 0.59 ± 0.31^{a}	- 0.05 ± 0.02^{a}	- 0.64 ± 0.22^{a}	- 0.07 ± 0.04^{a}
15	1	- 74.0 ± 7.11^{a}	- 4.13 ± 2.03^{b}	- 60.75 ± 8.4^{a}	- 2.16 ± 1.73^{b}
	3	- 1.35 ± 0.47^{b*}	- 0.15 ± 0.05^{b}	- 4.75 ± 1.87^{a*}	- 0.17 ± 0.15^{b}
	5	- 0.22 ± 0.22^{a}	- 0.05 ± 0.02^{a}	- 0.51 ± 0.19^{a}	- 0.14 ± 0.12^{a}

Seed banks assessment

After six months, a total of 5,628 emerged seedlings were observed in the soil samples of the four studied areas of vegetated and bare plots on both hills (Table 2).

Results showed that the square meter of vegetated areas had an expressive higher number of viable seeds when compared to bare soil. The low seed density in uncovered sites can be a signal of two possible events: surrounding species are able to disperse their seeds to the bare areas, otherwise these seeds are influenced by abiotic conditions that might being leading to their decrease of viability.

These emerged seedlings were divided into two groups based on their morphological characteristics: (1) The *Cyperus* group (*C. atlanticus and C. species*) and (2) the *Bulbostylis* group (*B. nesiotis)*. The main considered aspects to distinguish the seedlings groups was leaf width and the amount of leaves per seedlings (Figure 7), where *Cyperus* group (Figure 7.A) shows seedlings with lower leaf number but wider leaves, when compared with *Bulbostylis* group (Figure 7.B).

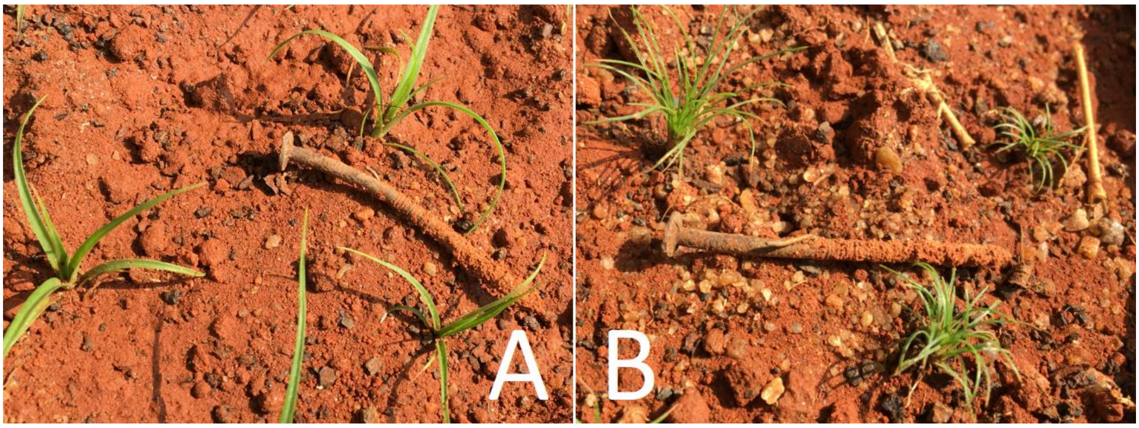

Figure 7 Seedlings of both found groups in seedbank investigation. A = *Cyperus* group, B = *Bulbostylis* group.

We found no correlation between the hills where soil samples were collected and the seeds density, once *Cyperus* density by square meter is higher in Andradas, while *Bulbostylis* is more abundant in Paredão Hill (Table 2).,

Table 2. Number of emerged seedlings per square meter of the four areas: Bare soil and Vegetated Soil on Andradas Hill (And.Bare and And.veg), and Bare Soil and Vegetated soil on Paredão Hill (Par.Bare and Par.Veg).

Group	And.Bare	And.Veg	Par.Bare	Par.Veg
(1) *Cyperus sp.p*	350	2880	273	1967
(2) *Bulbostylis nesiotis*	0	12	36	110

Seedlings identification

Cyperus atlanticus and *Bulbostylis nesiotis:* perennial herbs, both able to vegetative reproduction, pollinated and dispersed by the wind. Endemics from the Archipelago of Trindade and Martim Vaz. Their capacity to occur in a wide range of habitats throughout the island make these species responsible of the largest plant cover on the Island and with a good capacity to drive recovery processes of degraded areas (Martins & Alves, 2007). However, they are also described as “Vulnerable” due the restrict occupation area (less than 20km²) and potential threats, such as burning and human exploitation, with the possibility of being included in the category ´´Critically endangered`` or even ´´Extinct`` in a very short time (CNCFlora, 2012).

Cyperus sp: Species similar to *C. atlanticus* (morphologically), however, during many expeditions to the study sites, a population (presumed to be *Cyperus* atlanticus) was observed occurring at some sites on the lower areas of Trindade, but with some differences when compared to *C. atlanticus*. Currently, an investigation is on course to answer if this is a different Cyperus species or not. Therefore, this work is considering that the emerged seedlings can present two *Cyperus* species, considering that is not possible to identify between *Cyperus* species by seeds or young seedlings.

Despite the similarities, is possible to distinguish the species by genus (*Bulbostylis* and *Cyperus*) through the seedling morphology, as indicated above. In addition, after the collect at the field, the seeds morphology also supported the species classification (Table 3).

Table 3. Morphologic informations of the studied species. (SI = Size: Ti = tiny. L = length (mm). W = width (mm). SH = seed shape: Ob = obovate, El = Elliptic. CS = cross section shape: Tr = triangle. ST = Stylopodium: Pr = present, Ab = absent. A = apex shape: Con = concave, Api = apiculate. B = Base shape: Na = narrower, Sp = spitiform. Bold characters indicate differences among genus. Measurements were done using program ImageJ.

	SI	**L (mm)**	**W**	**SH**	CS	**ST**	**A**	**B**
B. nesiotis	Ti	**0.9**	**0.55**	**Ob**	Tr	**Pr**	**Con**	**Ce**
C. atlanticus	Ti	**1.4**	**0.8**	**El**	Tr	**Ab**	**Api**	**Sp**
C. sp.	Ti	**1.4**	**0.79**	**El**	Tr	**Ab**	**Api**	**Sp**

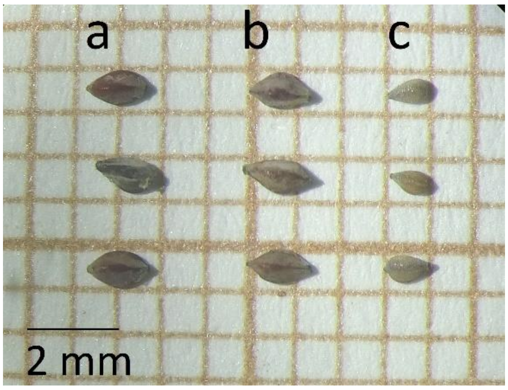

Figure 8 Seeds of (a) Cyperus atlanticus, (b) Cyperus sp and (c) Bulbostylis nesiotis

Seed Viability Loss (SVL) under different simulations of soil temperatures

The three species showed similar responses to the different temperature treatments (VEG) (20/30°C) and BARE (20/55°C), presenting high levels of SVL at the BARE-simulated temperatures and low levels of SVL at the VEG-simulated temperature, showing a significative difference among treatments for all species ($p < 0.01$). There was no loss of seed viability recorded for *B. nesiotis* seeds under VEG treatment, with 100% of germination for their seeds, while seeds under BARE conditions showed 72 ± 5.8 % of SVL (Figure 9). Both *Cyperus* species showed complete loss of viability when exposed to the BARE temperature treatment, and only negligible SVL under the cooler VEG temperatures (*C. atlanticus* = 3.35 ± 3.35; and *Cyperus sp.* = 6.67 ± 4.71), with no statistical differences among them (*p* = 0.98) (Figure 9). These results can show a comparable ability of the species to maintain seed viability on VEG treatment and only *B. nesiotis* being able to survive under the extreme temperatures of BARE.

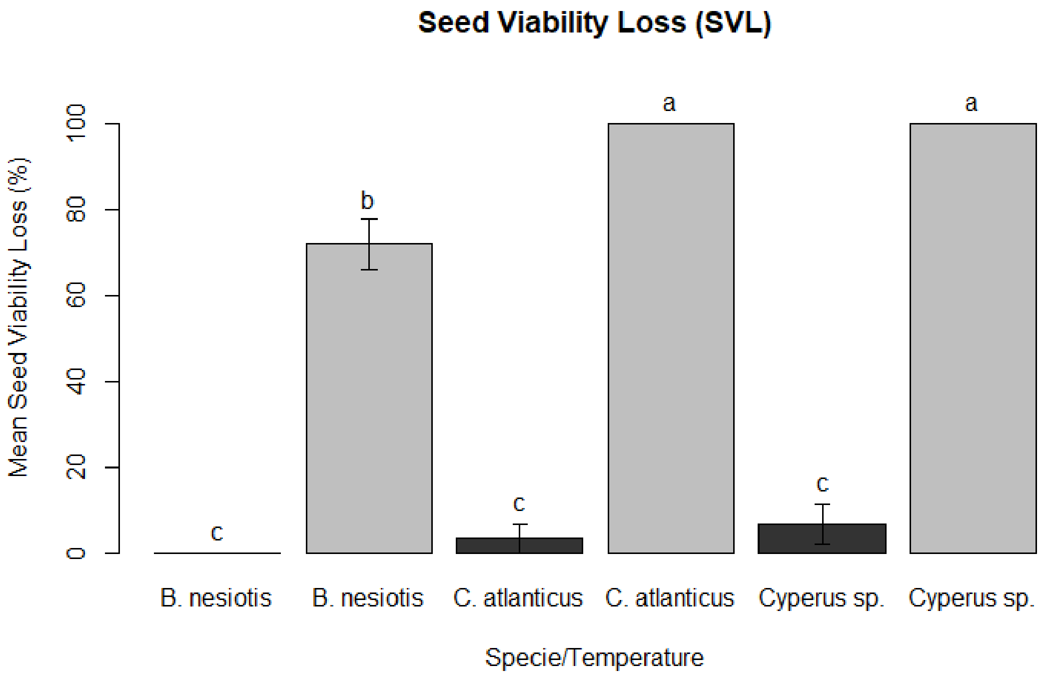

Figure 9 Mean Seed Viability Loss (SVL) under simulated temperature patterns. (■)VEG and (■) BARE indicates a 20/30°C and 20/55°C temperature range, respectively. Different letters indicate significant differences between treatments and species responses (P < 0.05).

Seed Viability Loss (SVL) under different simulations of water stress

Water stress had no influence on SVL for any species (Figures 10, 11 and 12).

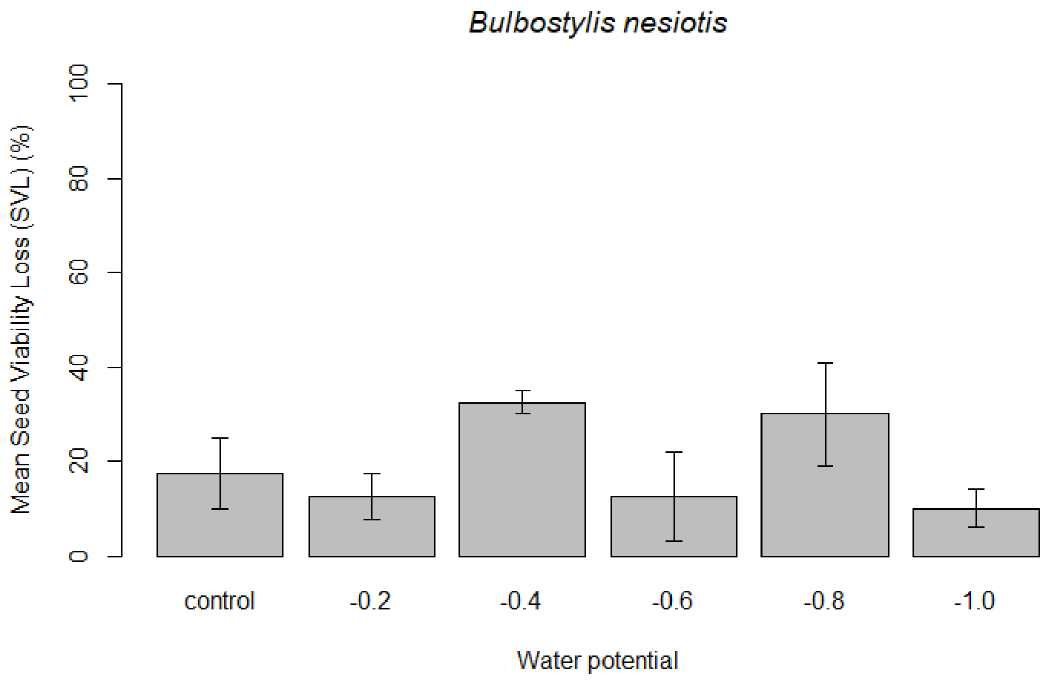

Figure 10 Mean (SE) Seed Viability Loss (SVL) of B. nesiotis seed under a gradient of water stress.

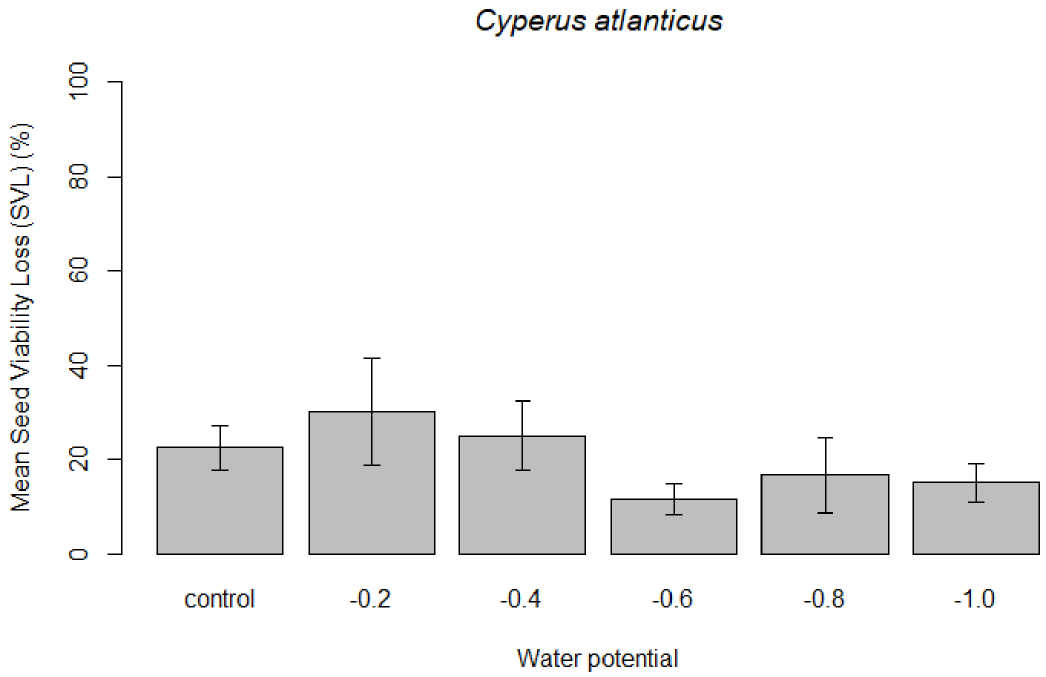

Figure 11 Mean (SE) Seed Viability Loss (SVL) of C. atlanticus seeds under a gradient of water stress.

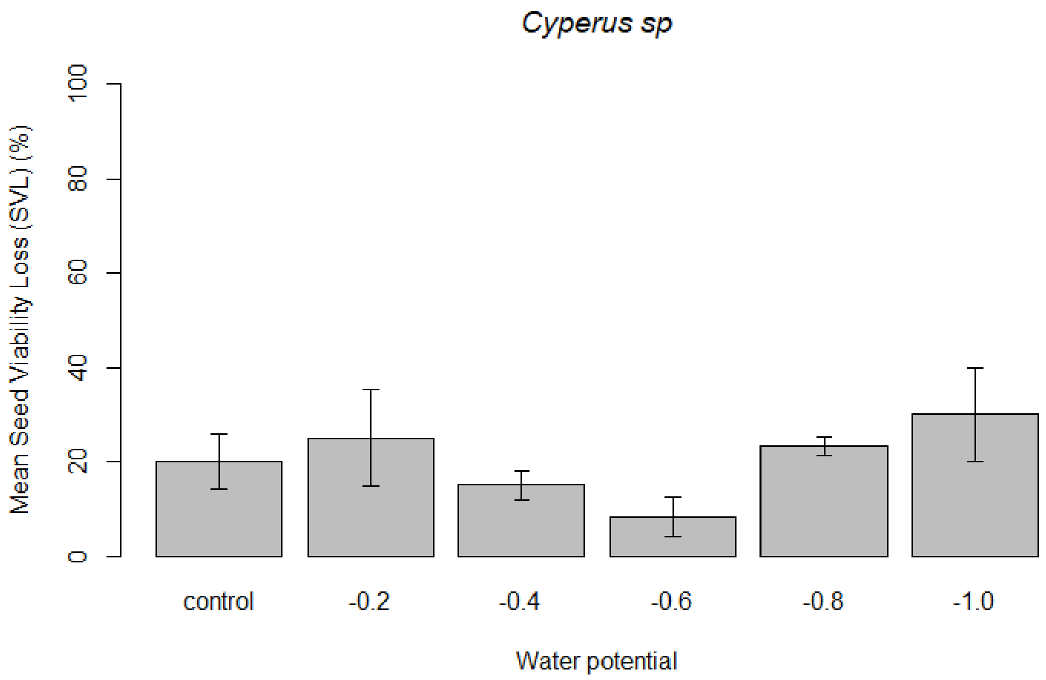

Figure 12 Mean (SE) Seed Viability Loss (SVL) of C. sp seeds under a gradient of water stress.

Discussion

Vegetation restoration and management by soil seed banks, as reported by van der Valk & Pederson (1989), require three basic features to be successful: (1) suitable abiotic conditions for seeds maintenance in the soil, (2) availability of native and endemic seeds, and (3) absence of non-native seeds in the seed banks. Our study had tested all these factors and found both positive and negative responses.

The difference in soil temperature and water stress patterns recorded on both studied hills emphasizes the strong influence that vegetation cover might have abiotic factors at relatively small scales. This can be an important factor preventing seeds and seedlings from the effects of stressful conditions on unsuitable environments (Breshears *et al*, 1998; Wang *et al*, 2012). In contrast, temperature patterns in bare soils provide a clear identification of a mechanism that has the potential to cause the lower diversity and abundance of seeds found in these areas (Ooi *et al*, 2012). In addition to the unfavorable abiotic conditions of bare soil areas, another fact that can explain the low specie richness in the seedbanks from the studied areas: surrounding vegetation shows low diversity of species, those able to help on the revegetation by seed dispersion or vegetative growth (Weerasinghe, 2008).

Temperature had a stronger effect on seed viability, germination and seedling recruitment of seeds more broadly (Labouriau, 1983; Parker *et al*, 1989; Ooi *et al*, 2012, Lyianage & Ooi, 2016). Subsequently, soil temperature direct affect soil seed bank dynamics. Tropical seed species require fluctuations of temperature as a signal of vegetation gaps for germination. This is an important environmental signal for suitable conditions of light and temperature availability and to their dormancy brake (Leck et al, 1989). Despite this requirement of low and high temperatures, too high temperatures can produce the opposite effect, inhibiting germination and even causing seed viability loss. No germination occurred under bare soil temperature, showing that *Cyperus* species are not able to maintain viability on bare soil surface. However, some seeds can be found in the seed banks of these areas, which means that the temperature at deeper layers might be more suitable for seeds. It is likely that some Cyperus seeds survived for long enough to become incorporated into the soil seed bank at deeper levels, and this contributes to the much lower densities of such seeds in bare compared to vegetated soil samples. *B. nesiotis* seeds stayed viable at surface temperatures of bare sites, however these areas have lower seed densities, compared with covered places. Our results are in agreement with field studies where the specie is apparently the only one to occur in bare areas. Indeed, on *Morro Vermelho*, another area that suffered a severe damaged by goats on Trindade Island, Martins & Alves (2007) reported seedlings and young individuals of *B. nesiotis* and *C. atlanticus* leading the vegetation recovery on bare patches few years after the complete withdraw of the goats. The same authors argue that the tussocks of these species can to provide microclimatic conditions, such as lower soil temperatures, allowing another species to recruit from seeds on those damaged areas. In our case, Turtle Hill and Andradas Hill are located at lower altitudes (75 meters), when compared to Morro Vermelho (505 meters), showing more extreme abiotic patterns such as higher soil and air temperatures and fewer rain regimes. A high range of altitude is a strong factor that increase the diversity of habitats on Oceanic Islands by differences on levels of rain and wind incidence, which results a variety of microclimate features (Whittaker & Fernández-Palacios, 2007; Hamann, 2011). Factors such as shade, vegetation cover and water (water streams and rain) can be favorable for seed by reducing soil temperature and making them good for the maintenance of seed viability in the seed bank and regulating germination (van der Valk & Pederson, 1989). This viability maintenance in the soil can be important not only to replacement of stand vegetation, but to species recovery from soil seed bank. On Trindade, the endemics *Plantago trinitatis* Rahn (Plantaginaceae), *Asplenium beckeri*

Brade (Aspleniaceae), were considered extinct by the herbivores once that their stand population had disappeared due the herbivory (Alves, 1998; Silva *et al*, 2013).

Our study showed that the seeds kept high viability over a considerable gradient of water potential, and that low water potential has minimal effect on the viability of seeds. For several germination tests, is a common way to consider that the lack of seed germination under water stress conditions is a negative response (Demir & Mavi, 2008; Zhang *et* al, 2010, Cochrane *et* al, 2014; Moral *et al*, 2015; Flores *et al*, 2017). Indeed, moisture levels can determine germination success, hence, influencing the dynamic of the firsts plant stages, as germination and recruitment (Fenner & Thompson, 2005; Ribeiro & Borghetti, 2013). On other hand, despite no germination, the results can show that the simulated water deficit tested were not too intense to reduce seed viability. In disturbed areas, the remnant seeds that stay viable under these conditions of water stress can be determinant leading to the vegetation recovery (Baskin & Baskin, 1982; Green, 1989; Leck *et al*, 1989; Weerasinghe *et al*, 2008). Both found results (presence of seeds in the soil seed bank and their ability to keep those viability under water stress simulations) are in agreement to the field observations of some natural recovery signals. Otherwise, seeds responses to the temperature simulations shows the *Cyperus* seeds dependence to stay under the soil surface to be prevented of stressful temperature conditions and waiting windows of opportunities with suitable conditions for their germination and stablishment, while *B. nesiotis* seeds are able to this even on bare soil conditions, as long they have minimum moisture resources to support them.

Vegetation recovery on insular environments can occur naturally, however, it can be a hard and slow process, and the damage by herbivory is not the only factor to contribute for this. Some studies showed that even after the removal of introduced mammals, secondary processes can still be occurring besides the disturbances caused by herbivory. Larger amounts of non-native seeds in the relitual seedbank, compared to endemic/native seeds, is an example. On Nakoudojima (Japan), an island severely damaged by goats, Weerasinghe (2008) showed that alien plant species were dominant in the seedbanks of damaged areas. The authors concluded that the vegetation recovery would lead to a community of non-native species over native areas, replacing the endemics and increasing their possibility of extinction. On the Hawaiian territory, herbivory by feral pigs resulted on impacts over native community by soil disturbance and promoted the occurrence and spread of non-native plants (Diong, 1982; Siemann *et* *al*, 2009; Oduor *et al*, 2010). Further, even reproduction patterns were altered, with herbivory inhibiting seedling establishment, vegetative propagation became the main regeneration strategy (Busby *et at*, 2010). Finally, the withdraw of the feral pigs resulted on strong increase on density of native woody plants and stem density of young tree, but also promoted an increase in density of non-native species ferns (Cole *et al*, 2012), as a result of the non-native seedbank formed, as reported above by Weerasinghe *et al* (2008) on the Japanese Island. In contrast with those studies, regeneration of the vegetation on Trindade Island after removal of introduced herbivores, while not particularly diverse, displays a capacity for recovery from a persistent seed bank which is composed only by endemic species. Currently, some endemic species, as well as several presumed to be extinct, have successfully reestablished during this time, such as *Plantago trinitatis*, *Asplenium beckeri* and *Elaphoglossum beckeri* Brade (Dryopteridaceae) (Alves, 1998; Silva *et al*, 2013). The areas of the island most impacted by goats, and the subject of our study, have naturally regenerated via the establishment of sedges species, but our results have shown that recovery of these bare areas is likely to be slow because of the effects that abiotic features that occur in such areas have on seeds and their ability to persist.

Finally, our study showed low species richness in the seed banks and only *B. nesiotis* being able to lead the natural recovery process, while many studies on vegetation recovery to prevent soil erosion concluded that restoration efforts may be more effective when use a mixture of species than monocultures due the variety of root systems and their traits to anchor the soil (Reubens *et al*, 2007; Zhu *et al*, 2015).

Conclusions

Disturbed sites on Trindade may show a natural ability to regenerate through the soil seed banks. Despite the limitations of some species, the three main requirements to a successful vegetation restoration and management where observed at the study areas. First, the environmental conditions, although their extreme condition on the top layer of the bare soil, did not reduce the seed viability of all species, as long *B. nesiotis* presented ability both to survive and germinate under those conditions. Second, even with a low richness of species, seeds of endemic sedges were found in the seed bank and are candidates to promote the revegetation, recovering those areas with the same presumed species that lived before the goats and pig’s arrivals. In addition, the observed vegetative propagation of these species might to support this recovery. Finally, despite the presence of exotic plant species on the island, another positive factor was the absence of non-native species in the seed banks, once they could inhibit the endemic recovery.

However, it might be a slow process due the amount of viable seeds and their dependence of windows of opportunities to germinate, once that, under the abiotic conditions tested of the bare patches, only *B. nesiotis* seems to be able to recruit new seedlings. Therefore, these areas will be staying subjected to erosions and their consequences, such as loss of the soil layer and increase of bare patches.

It will be necessary a restoration plan that supports a faster regeneration, avoiding the negative consequences of the introduced herbivores, which still happening even after they have been removed. New studies are now required to enrich the list of species, among native and endemic, those better fitted to a future recovery project.

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CAPÍTULO 2

Germination responses to temperature, water stress and time after seed dispersion as indicative of regeneration capacity of disturbed areas by seed bank species.

Germination responses to temperature, water stress and time after seed dispersion as indicative of regeneration capacity of disturbed areas by seed bank species.

Abstract

Seed banks play a key role in vegetation recovery of patches of bare soil. Their efficiency in recolonizing open areas is directly influenced by environmental conditions, such as soil temperature and water availability. In this study, our goal was to verify how seeds of three endemic species (*Bulbostylis nesiotis, Cyperus atlanticus* and *Cyperus sp.*) of Trindade Island respond to temperature fluctuations and to water deficit in bare and vegetated soil areas, and how a post-dispersion period affects the germination parameters of the seeds. Seeds were collected from dispersing individuals close to the study sites. The temperature patterns of bare and vegetated soil areas were simulated to find whether these different conditions affected germination capacity. Furthermore, we investigated the seeds responses to a water stress gradient, simulating the common conditions of water availability in the study area. Finally, both tests were repeated using stored seeds (6 and 12 months) to test their ability to stay viable at long times after dispersion. Seeds of *B. nesiotis* were able to germinate at both bare and vegetated soil temperatures, but the higher temperatures of bare soil reduced the percentage of germination and increased the mean time for germination, while the storage time had a slowly increasing trend on the mean germination time of the seeds in the bare soil treatment simulation. Both *Cyperus* species showed no germination at temperature treatments simulating bare soil conditions (20/55ºC) but showed high levels of germination at temperatures simulating vegetated soil temperature conditions (20/30ºC). A reduction in the germination percentage was observed when the seeds were stored for 12 months post-collection. Also, storage treatments resulted in an increase of the mean time for germination. We concluded that *B. nesiotis* had a strong potential to colonize the disturbed areas, which apparently make the environment more suitable for other species, being possibly followed by the *Cyperus* species in the vegetation recovery process at the study areas.

Keywords: soil seed bank, *Cyperaceae*, seed germination, temperature, water stress, seed storage

Introduction

In a global context, oceanic islands provide an extremely high contribution to the world species biodiversity, especially when proportion of their land area and endemism occurrence are compared to the same proportion on continental lands (Whittaker & Fernández-Palacios, 2007). However, many oceanic islands have been severely damaged by introduced and invasive species. Self-regeneration strategies are the main mechanism for reestablishment of faunal and plant biodiversity, particularly for islands that are far from the mainland. In tropical regions, plant species have several key strategies of regeneration, which include: (1) seeds – seed rain (recently dispersed seeds) and seed banks (persistent and dormant seeds in soil), (2) seedlings – seedling banks (established seedlings under temporarily suppressed growth) and (3) regeneration of adults – resprouting of damaged individuals (Garwood, 1989). Thus, soil seed banks play a key role on the vegetation recovery of insular environments. Its importance is linked to the geographic isolation and the limited number of endemic species that can lead to this recovery (Weerasinghe *et al*, 2008; Tanaka *et al*, 2010; Cross *et al*, 2015; Ortiz-Alcaráz *et al*, 2016).

Seeds into the soil seed bank are subjected to abiotic influences that might be positive or negative, including temperature, water and light (Baskin & Baskin, 2014). In seed banks, these abiotic factors control processes such as dormancy, maintenance of viability, promotion of germination and subsequently seedling recruitment (Parker *et al*, 1989; Ooi *et al*, 2014; Liyanage & Ooi, 2017). Understanding seed bank dynamics and their abiotic drivers, in combination with assessing seeds traits, is therefore essential for vegetation management and recovery. The knowledge of favorable conditions that promote germination from seedbanks in damaged areas is of particular interest.

Among the abiotic factors that influence seed germination, soil temperature is one of the primary factors that affects plant recruitment and distribution, affecting seeds viability, dormancy, germination and subsequently overall early stage recruitment dynamics (Parker *et al*, 1989; Ooi *et al*, 2012, Lyianage & Ooi, 2017). In disturbed areas cleared of above-ground vegetation, soils are subjected to more extreme abiotic conditions such as high air and soil temperatures. These are critical and may lead to a decrease in soil moisture level (Rodriguez-Iturbe *et al*, 1999) which may increase the difficulty of vegetation recovery or favor more tolerant species (Harte *et al*, 1995; Pierson & Wright, 1991; Breshears *et al* 1998; Flerchinger & Hardegree, 2004). Higher temperatures may also increase loss of seed viability and change processes related to germination, ultimately influencing the seed bank composition (Breshears *et al*, 1998; Royer *et al,* 2011). In contrast, areas with vegetation cover usually present soils with lower temperatures and smaller diurnal ranges and are less challenging for seedling recruitment. In addition, the availability and quality of light in the environment is also an important factor. Species that can lead to natural recovery of degraded areas (earlier successional species) often require light availability as an intrinsic aspect of their germination, meaning that seeds have to stay on the top layers just below the soil surface in order to germinate. However, for these groups of seeds, burial cannot only be considered a negative factor, since buried seeds will improve the seed bank composition, dispersing the germination of the dispersed seeds over time (Leck *et al,* 1989; Parker *et al,* 1989; Baider *et al*, 1999; Tobe *et al*, 2005).

Besides abiotic influences on vegetation regeneration from seed banks, anthropic disturbances and occupation of natural areas by non-native species, are also responsible for a huge reduction in the distribution, and even extinction, of many endemic species around the world, mostly in insular environments, where native and endemic species have lower competition strategies to handle the presence of invasives/exotics (Wilcove *et* al, 1998; Myers, 2000; Sax & Gaines, 2008; Kueffer *et al*, 2009). Hence, the several different pressures on insular biodiversity have led insular endemic species to make up one-third of all endangered plant species known on Earth (Groombridge, 1992).

On Trindade Island, a Brazilian oceanic island located in the South Atlantic Ocean, the main cause of disturbance of the native vegetation was herbivory from introduced mammals, particularly those of goats and pigs, which lead to a strong decrease in plant and animal biodiversity (Barth, 1958; Alves, 1998). The introduction of these large grazing animals has become the main cause of damage and extinction of most island floras around the world, including that of Trindade, Galapagos, St. Helena, Philip Island, Nakoudojima and Hawaii (Barth 1957; Schofield, 1989; Groombridge, 1992; Weerasinghe *et al*, 2008; Cole 2012). Since the eradication of goats and pigs on Trindade in 2005, the island showed signs of vegetation recovery, including the recovery of some endemic species that were considered to be extinct, as a result of decades of herbivory by these introduced species. The natural recovery of these endemic species was driven by seed persistence in the soil seed banks or by small relict populations in undisturbed regions (Martins & Alves, 2007; Alves *et al*, 2011; Silva *et al*, 2013).

However, after the eradication of herbivores, even though some regeneration has occurred (Sylvestre, 2007; Alves *et al*, 2011, Fisch & Port, 2017), large areas on the island continue to present bare soils patches, with little evidence for vegetation recovery. The lack of coverage may drive changes in the microclimatic patterns in these locations, influencing the dynamics of plant communities by increasing soil temperatures and reducing soil water availability (Harte *et al,* 1995; Pierson & Wright, 1991; Breshears *et al* 1998; Flerchinger & Hardegree, 2004). Some of the consequences already reported for these bare patches are a reduction of streams and depletion of soil layers, however the direct influence on seeds and on potential for vegetation recovery is still unknown.

In this study, we set out to investigate the influence of abiotic factors on seeds of native species found in the soil seed bank on Trindade Island. We hypothesized that soil temperature along with moisture availability have an influence on seed viability and on the germination of the species in the soil seed bank, leading to a decrease in both richness and abundance of seeds at extreme conditions. The determination of whether spontaneous recovery in bare patches is possible is key factor for understanding the capacity for recovery of vegetation on Trindade Island. In a broader sense, the results of this study can lead to a better understanding of the association between functional traits and environmental conditions that may influence the recovery of degraded native areas, particularly important for the recovery of endemic species on Trindade Island.

Methods

Study site

The study site is located on Trindade (20° 30' S, 29° 22' W), a volcanic Island located in the South Atlantic Ocean, 1.200 km from the Brazilian coast. The island has a land area of 10 km², and a peak altitude of 600 meters. The mean temperature is 27,7°C during the hottest month (February) and 22,9°C during the coldest month (August), with an annual average of 25°Cn (Figure 5). The annual average rainfall is 1476 mm distributed through the year, with no defined rainy and dry seasons (Figure 5), which is classified by Koppen as Tropical with no dry season - '*Af*' - (Tropical climate, with no winter season and high precipitation levels with no dry season).

The island was subjected to heavy grazing by goats for more than 300 years prior to their eradication in 2005. After this, observations of natural recovery of the vegetation were done in some areas but large areas remain as bare soil and are subject to damage processes such as erosions. Today, the largest part of the island vegetation is formed by ferns, herbaceous and grass species including *Pityrogramma calomelanos* (L.) Link var. *calomelanos, Bulbostylis nesiotis* (Hemsl.) C.B.Clarke (Cyperaceae) and *Cyperus atlanticus* Hemsl. (Cyperaceae). Tree species are basically restricted to fern forests (*Cyathea delgadii*) with some other few species on the higher peaks and introduced species such as *Terminalia catappa L.* (Barth, 1958; Alves, 1998; Silva & Alves, 2011; Carvalho-Silva *et al*, 2013).

Among the several hills on Trindade Island, two hills, Paredão and Andradas, were selected to be the focus of this study. Each were similar in vegetation composition and coverage. Though the vegetation becomes scarcer from the bottom to the top of each hill, with bare soil areas occurring at the tops, the predominant soil coverage of the hills are sedges. Both hills were subdivided into vegetated (VEG) and bare soil (BARE) locations, resulting in four plots (Par.veg, Par.bare, And.veg, And.bare). Soil samples were collected and abiotic measurements were made of each plot.

Seed germination under different simulations of soil temperatures

Seeds were collected in January of 2016, from dispersing individuals and based on the list of the seed bank species on both areas (*Bulbostylis nesiotis* (Hemsl.) C.B.Clarke (*Cyperaceae*), *Cyperus atlanticus* Hemsl. (*Cyperaceae*), and *Cyperus sp.*(*Cyperaceae)).* To assess the influence of abiotic factors, seed germination was quantified under simulated soil temperature conditions, using the same temperatures patterns and moisture levels used and described at the chapter 1. Two sets of treatments were established, comparing conditions in bare and vegetated areas.

To determinate the influence of soil temperature patterns on seed germination, two growing chambers were used, each one simulating the temperature patterns of either vegetated (VEG) or bare (BARE) plots. The VEG treatment consisted of a daily variation of temperature that started at 21°C at 6 am for one hour. From 7 am to 11 am the temperature was increased to 30°C, staying constant at the maximum temperature for one hour. From 12 pm to 6 am the next day, the temperature was decreased constantly from 30°C to 21°C, after which the cycle was repeated. For the BARE treatment, the temperature variation was the same as that of VEG in the 6 – 7am period. However, from

7 - 11am, temperature was increased to 55°C, staying constant for one hour, then decreased to 30°C for the 12pm – 6 pm interval. From 6 pm to 6 am, the temperature was decreasing constantly to 21°C, and the cycle then restarted. In each chamber, 60 seeds of each species were divided into 4 dishes lined with moistened filter paper, which was topped off by watering daily. Germination was checked daily and germinated seeds were recorded and removed from the dishes. The experiment was carried out over 2 months and at the end of this period, a tetrazolium test was used to assess whether any of the non-germinated seeds were still viable.

Influence of storage time on seed germination

To investigate the influence of storage time on germination, we repeated the same tests (temperature fluctuations of bare and vegetated soil) on seeds of the same sample after six and twelve months. This may be an indicator of the seeds ability to remain viable in the soil, compounding the seed bank and supporting the vegetation recovery. For this experiment, seeds were considered as: M1 (fresh seeds), M6 (stored through six months), and M12 (seeds stored through twelve months). Seeds were stored in paper bags under constant 23°C.

Seed germination under different levels of water stress

To assess seed germinability under conditions of water stress, seeds of the three species were subjected to a gradient of PEG 6000 solutions, according to Villela *et al* (1991). This is a very common solute used to simulate water stress conditions by being a chemically inert solute and by do not show toxicity upon the seeds. Based on broad literature, the PEG solutions used were -0.2; -0.4; -0.6; -0.8 and -1.0 MPa, indicating the capacity of seeds to preserve their viability, or even germinate, under conditions of water stress. In a chamber set to a 12 hours photoperiod and a constant temperature of 25°C, 60 seeds of each species were divided among four dishes lined with moistened filter paper, using the PEG concentrations for the treatments and distilled water as control. Germination was checked daily and germinated seeds were recorded and removed from the dishes. The experiment was carried out for 2 months and at the end of this period, a tetrazolium test was used to assess whether any of the non-germinated seeds were still viable.

For both tests (temperature and water stress), germination percentage and mean germination time were the analyzed parameters.

Analysis

We used ANOVA and a Log transformation to compare the germination percentage and mean time for germination of each species under the treatments (factors) of temperature and storage times. Comparing the germination levels under the simulation of the gradient of water stress, we used ANOVA followed by Tukey test, with the PEG solution concentration as main factor of influence over the seeds. The responses analyzed were germination percentage and mean time for germination. Significance for all statistical tests was set at $p < 0.05$. All analyses were done using the program R Studio Version 1.0.143 and the packages readxl and lsmeans (Lenth, 2016; Wickham & Bryan, 2017).

Results

Seed germination under different simulations of soil temperatures and storage times

B. nesiotis seeds were able to germinate under both temperature patterns (BARE and VEG) and all storage times (*M1*, *M6* and *M12*), with no influence of storage time on germinability ($p = 0.05$). Concerning the germination on *BARE,* this treatment showed germination percentages to be affected by each storage times, when compared to *VEG* (Figure 13). Furthermore, seeds in BARE conditions showed a significant increase in the mean time for germination at every storage times ($p < 0.01$), when compared to VEG. For seeds in *VEG* treatments, no influence of storage time was recorded for the seeds. In addition, analyses showed that when seeds on BARE reached 6 months, the time it took to germinate was longer ($p < 0.01$). However, in comparing the M12 seeds to the M1 seeds, it is possible to note that, there was no variation in the time required to germinate (Figure 14).

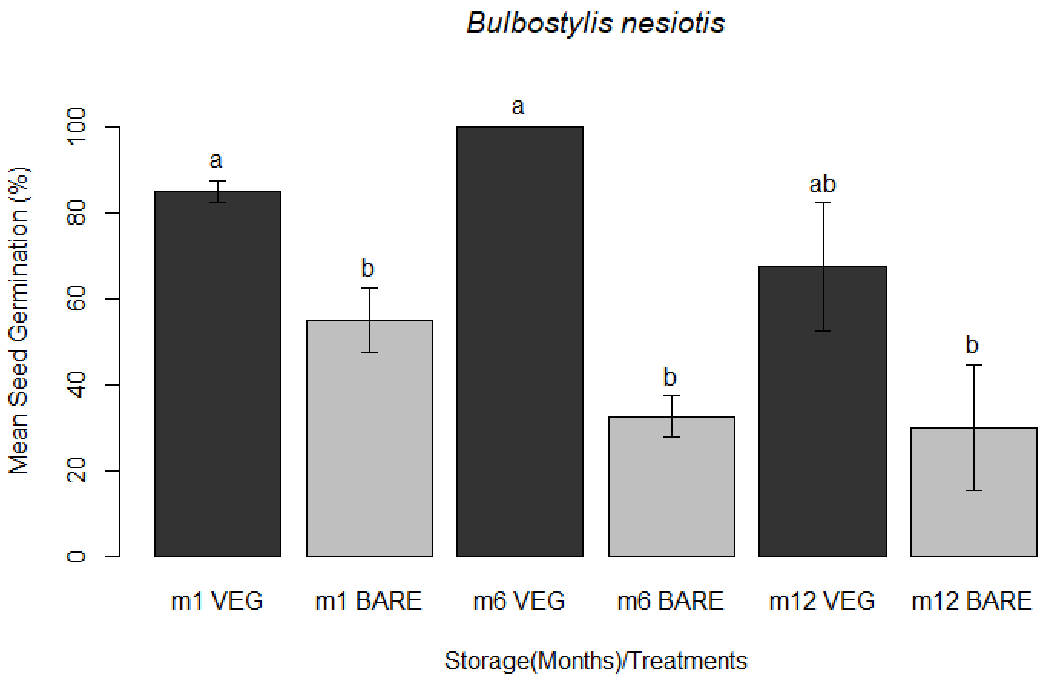

Figure 13 Mean seed germination of *Bulbostylis nesiotis* seeds under simulated temperature patterns. (■)VEG and(■) BARE indicates a 20/30°C and 20/55°C temperature range, respectively. M1, m6 and m12 indicates time post dispersion in months. Different letters indicate significant differences between treatments and species responses (p < 0.05).

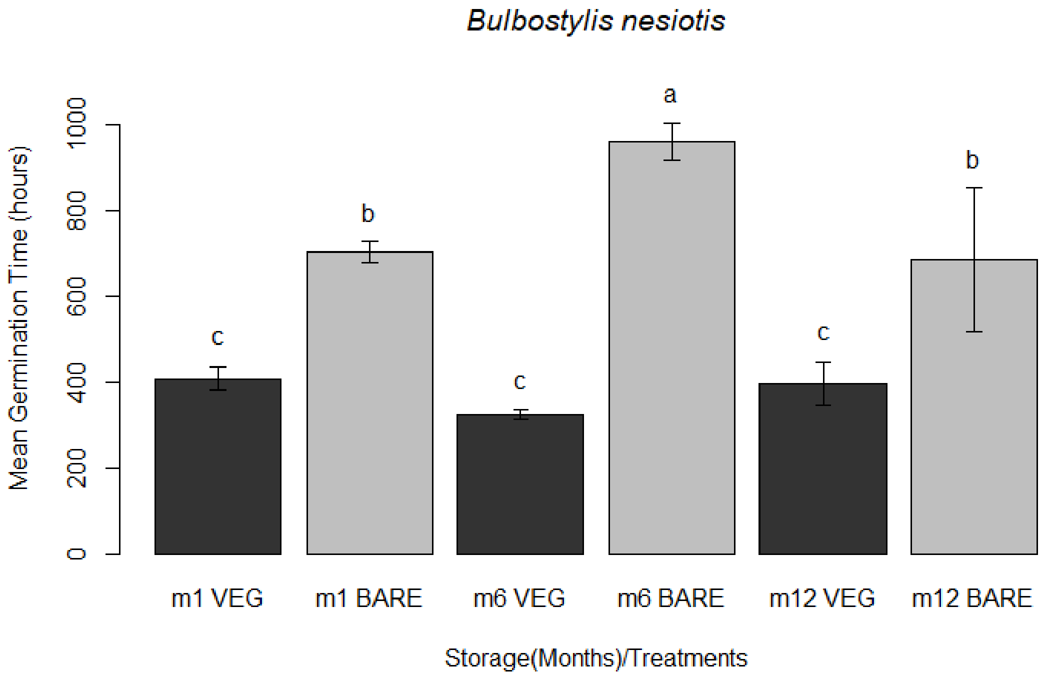

Figure 14 Mean time for germination of *Bulbostylis nesiotis* seeds under simulated temperature patterns. (■)VEG and (■) BARE indicates a 20/30°C and 20/55°C temperature range, respectively. M1, m6 and m12 indicates time post dispersion in months. Different letters indicate significant differences between treatments and species responses (p < 0.05)

The *Cyperus* species had similar results among them in every analysis, with the influence of the M12 storage affecting the seeds ($p < 0.01$), leading to decrease of germination percentage. The BARE treatment had a highly influence, causing no germination of seeds. *Cyperus atlanticus* seeds of the *VEG* treatment had no significant difference between M1 and M6 storage times in comparing germinability (Figure 15) ($p = 0.99$) and mean time for germination (Figure 16) ($p = 0.689$). Also, in the M12 treatment of BARE, a significative influence of storage time on the seeds was observed, showing a decrease of germination percentage and an increase of their mean germination time, compared to the other storage times.

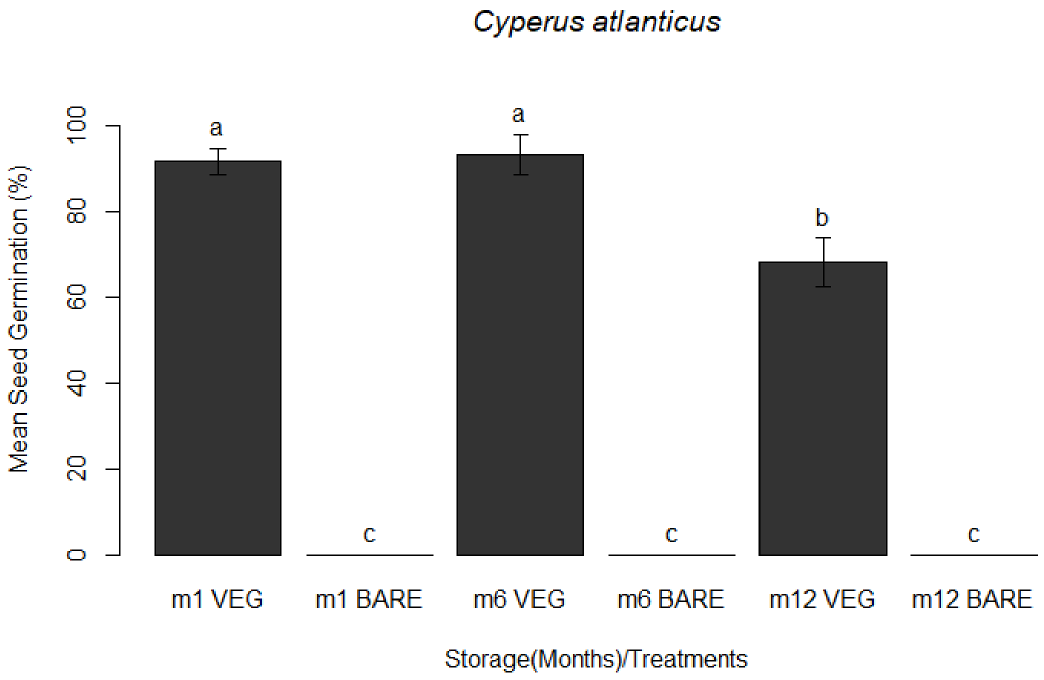

Figure 15 Mean seed germination of *Cyperus atlanticus* seeds under simulated temperature patterns. (■)VEG and(■) BARE indicates a 20/30°C and 20/55°C temperature range, respectively. M1, m6 and m12 indicates time post dispersion in months. Different letters indicate significant differences between treatments and species responses ($p < 0.05$).

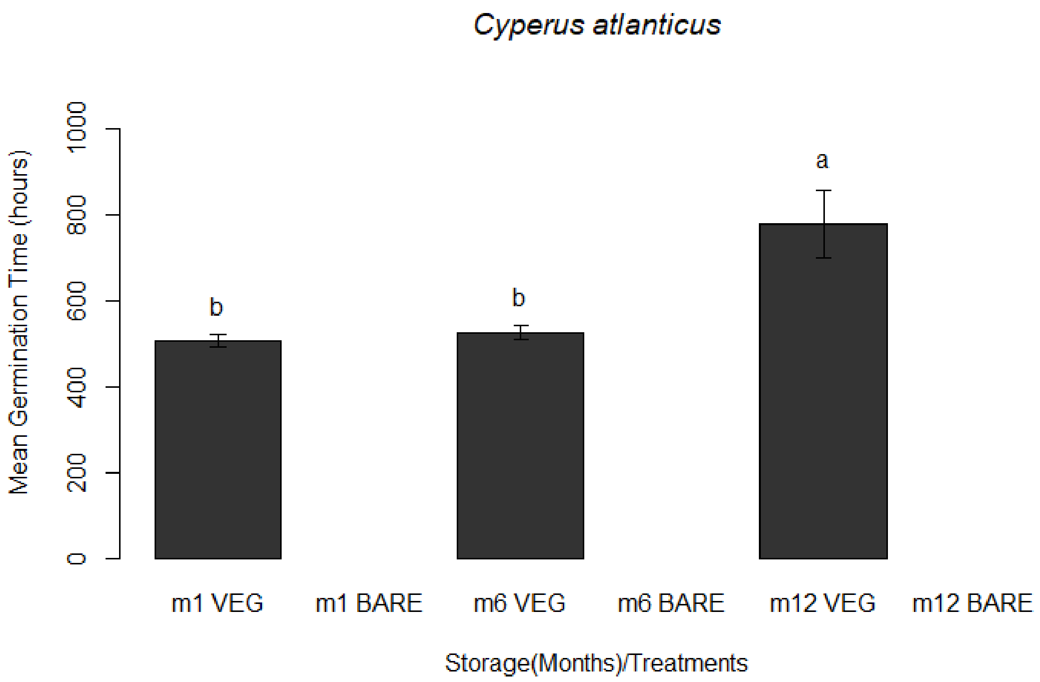

Figure 16 Mean time for germination of *Cyperus atlanticus* seeds under temperature patterns. (■)VEG and(■) BARE indicates a 20/30°C and 20/55°C temperature range, respectively. M1, m6 and m12 indicates time post dispersion in months. The absence of bars indicates no germination at the treatment. Different letters indicate significant differences between treatments and species responses (p < 0.05).

Cyperus sp. seeds showed no significant difference among M1 and M6 seeds (p = 0.738). In addition, in M12, we observed a difference from M6 (p < 0.01) and a loss of germinability (Figure 17) of *Cyperus* species over time. In this specie, the storage times did not influence the mean time for germination (Figure 18). M1 was no different from M6 (p = 0.99) nor from M12 (p = 0.129).

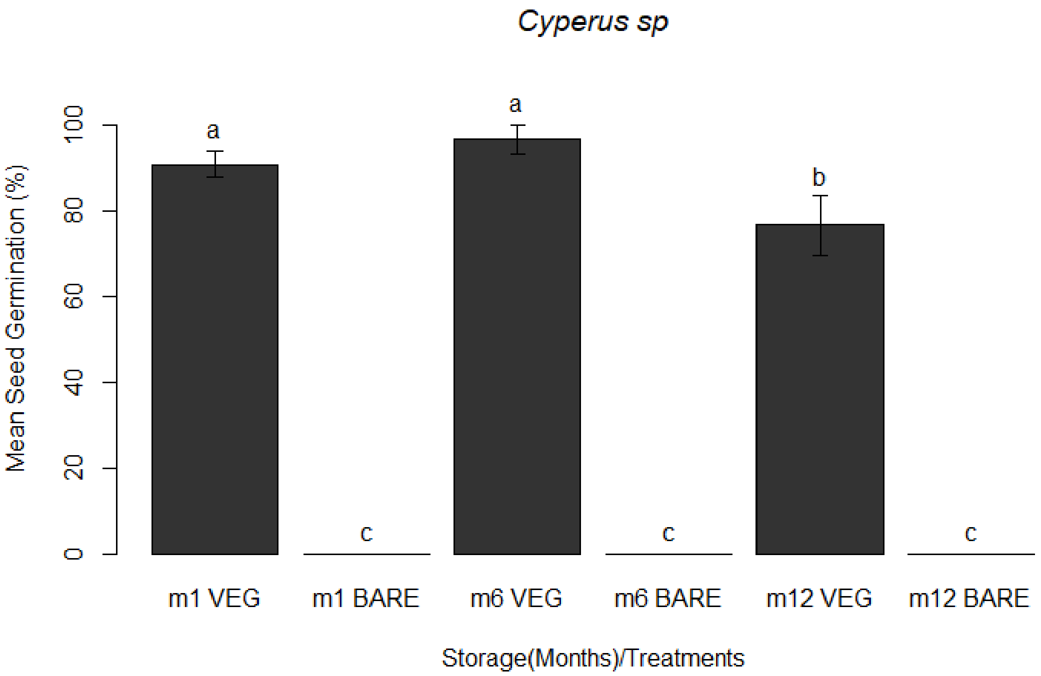

Figure 17 Mean seed germination of *Cyperus sp.* seeds under simulated temperature patterns. (■)VEG and(■) BARE indicates a 20/30°C and 20/55°C temperature range, respectively. M1, m6 and m12 indicates time post dispersion in months. Different letters indicate significant differences between treatments and species responses (p < 0.05).

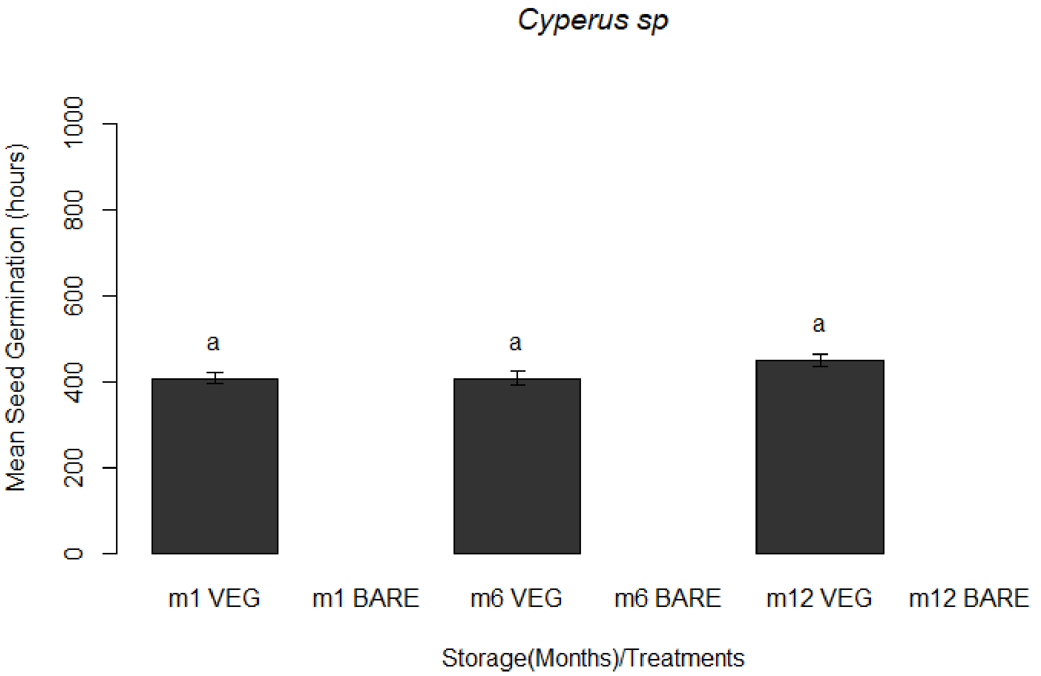

Figure 18 Mean seed germination of *Cyperus sp.* seeds under simulated temperature patterns. (■)VEG and(■) BARE indicates a 20/30°C and 20/55°C temperature range, respectively. M1, m6 and m12 indicates time post dispersion in months. The absence of bars indicates no germination at the treatment. Different letters indicate significant differences between treatments and species responses (p < 0.05).

Seed germination under different levels of water stress

The ability to germinate under simulated conditions of water stress showed a decrease in germination, proportional to the reduction of osmotic potential where they were tested. No germination was found for any species under the treatments of -0.8 and -1.0 MPa. Furthermore, we observed a trend of increase in the mean germination time of seeds under the higher levels of water stress.

B. nesiotis seeds showed a variation in their mean germination percentage from 0 to 90%, depending on the treatment (Figure 19). The -0.2 MPa was the only treatment where seeds germinated with similar values of those of the Control (p = 0.36). Besides that, seeds in -0,4 and -0,6 MPa concentrations revealed different germination percentages ($p < 0.01$), showing a gradient of influence by water stress. Seeds under treatments where we observed some germination (-0.2, -0.4 and -0.6 MPa) no difference between them ($p < 0.01$). However, when compared to the Control, these treatments showed a significant increase in the mean time for germination in response to the water stress gradient (Figure 20).

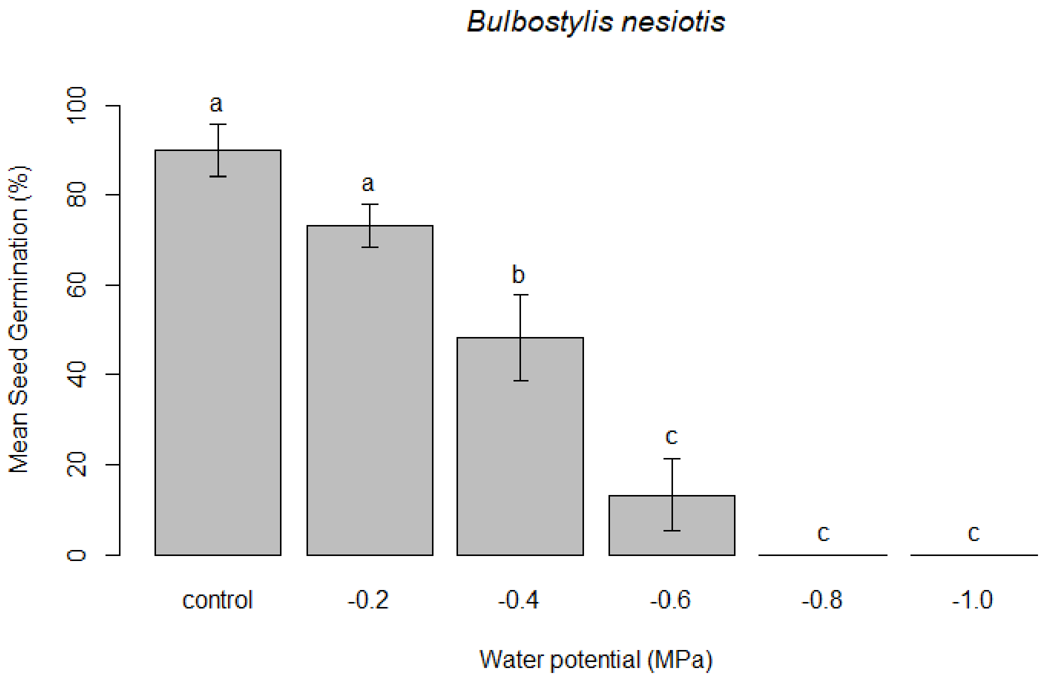

Figure 19 Mean seed germination of *Bulbostylis nesiotis* seeds under a gradient of water stress simulations. Different letters indicate significant differences between treatments and species responses (p < 0.05).

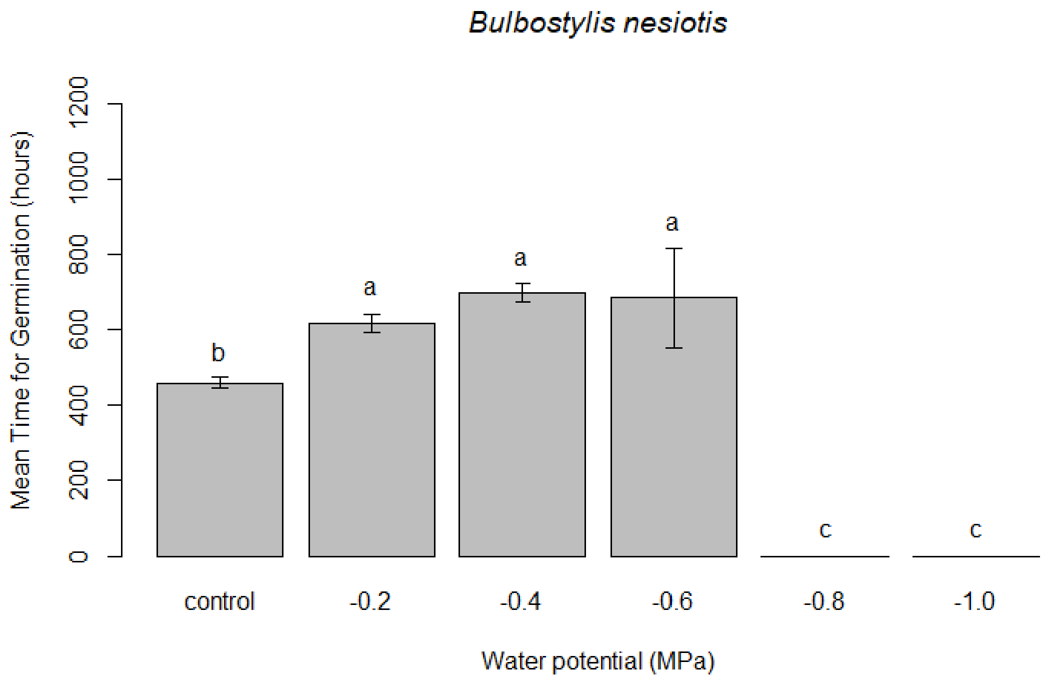

Figure 20 Mean time for germination of *Bulbostylis nesiotis* seeds under a gradient of water stress simulations. The absence of bars indicates no germination at the treatment. Different letters indicate significant differences between treatments and species responses ($p < 0.05$).

For *Cyperus atlanticus,* results showed low levels of germination for the control and for both water stress simulations where it occurred. Therefore, no significative difference was found between all of them (Figure 21). Regarding the mean time for germination, seeds were influenced by water stress (Figure 22), being significantly different ($p < 0.01$) from the Control but similar among treatments ($p = 0.99$).

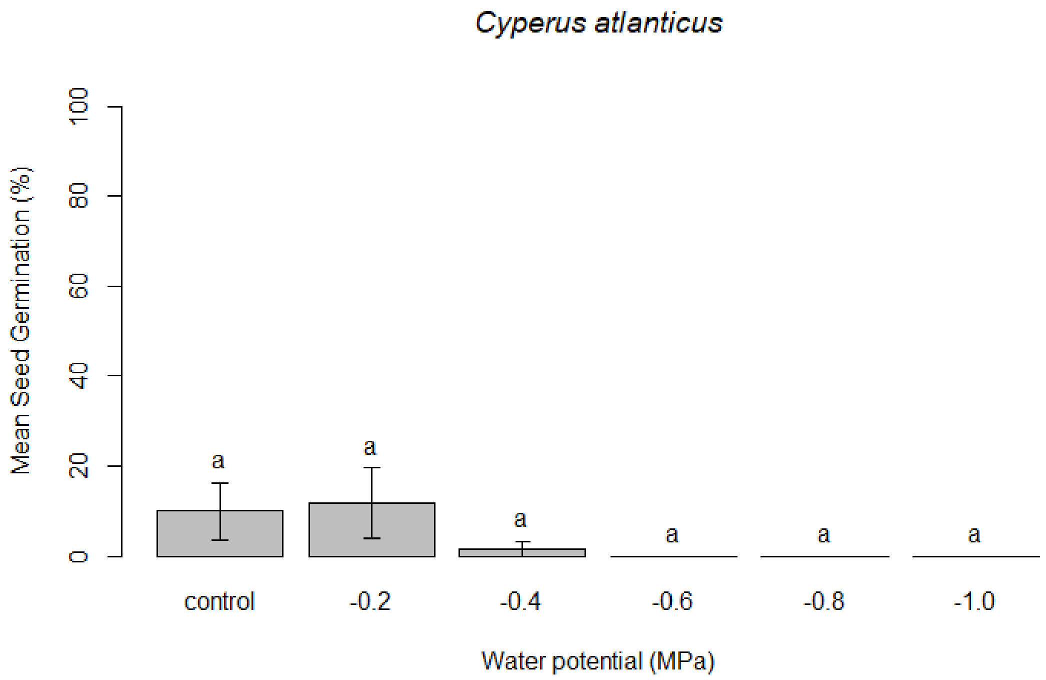

Figure 11 **Mean seed germination of *Cyperus atlanticus* seeds under a gradient of water stress simulations. Different letters indicate significant differences between treatments and species responses ($p < 0.05$).**

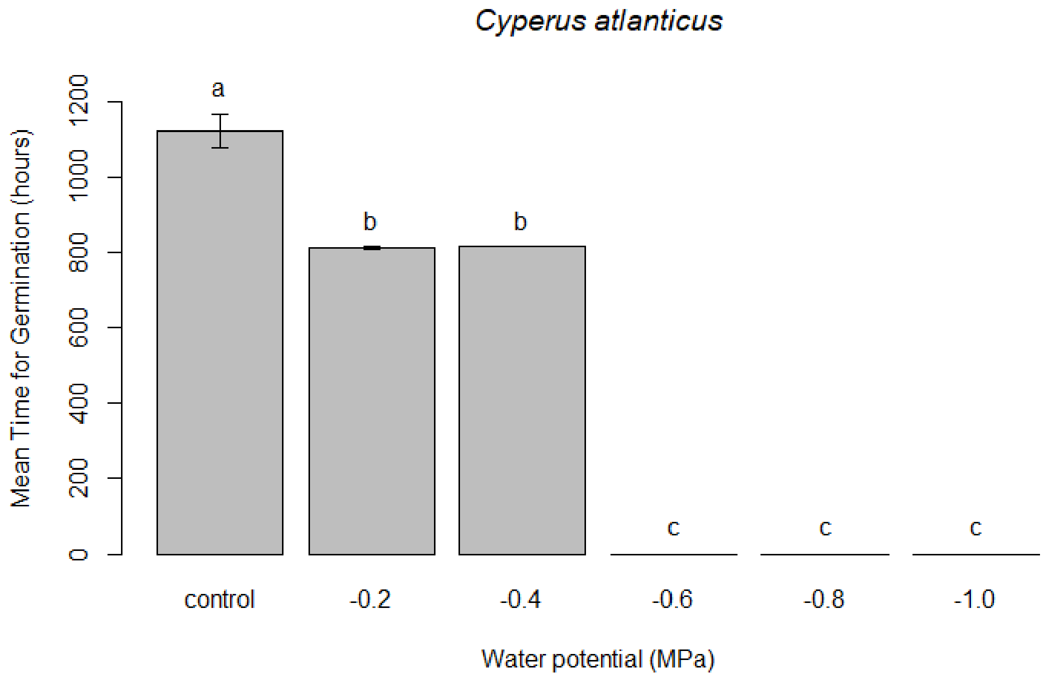

Figure 22 **Mean time for seed germination of *Cyperus atlanticus* under a gradient of water stress simulations. The absence of bars indicates no germination at the treatment. Different letters indicate significant differences between treatments and species responses ($p < 0.05$).**

For the germination percentages of *Cyperus sp.*, we found some similar responses to the water stress simulations, with a strong and gradual influence over seeds, with low rates of germination in -0.2MPa and -0.4MPa and no germination in -0.6MPa, -0.8MPa or -1.0MPa (Figure 23). The Control treatment was significantly higher when compared to the -0.2 MPa and -0.4 MPa (p < 0.01) treatments, which were similar between themselves (p = 0.84). For the mean time for germination, seeds gave similar responses at -0.2MPa and -0.4MPa, which were significantly lower than the Control seeds (Figure 24).

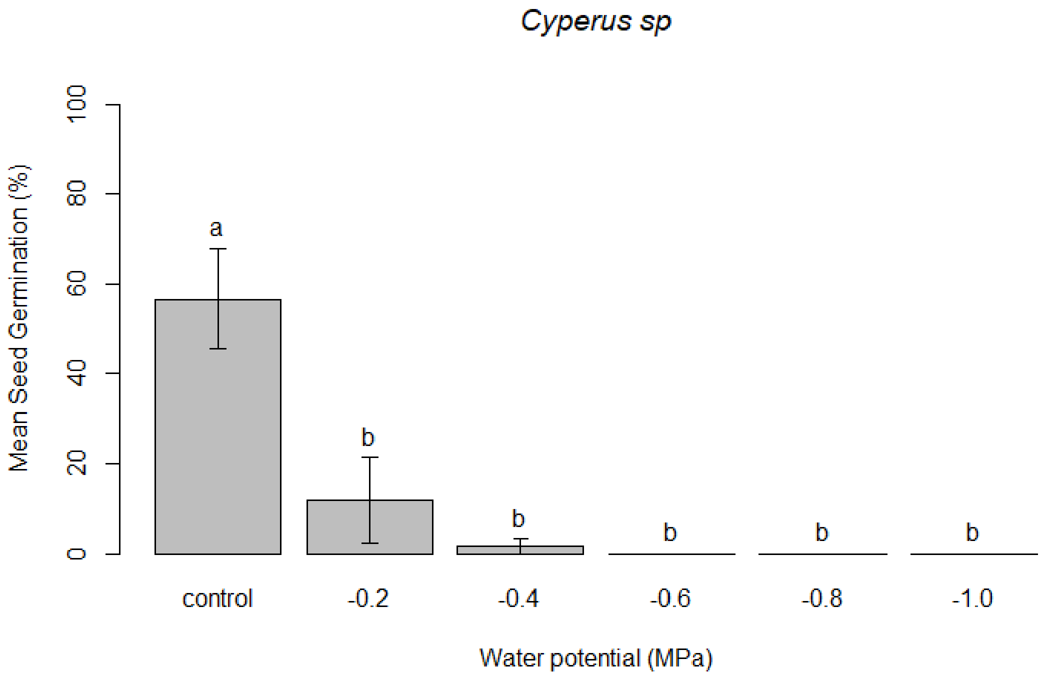

Figure 23 Mean seed germination of *Cyperus sp.* seeds under a gradient of water stress simulations. Different letters indicate significant differences between treatments and species responses (p < 0.05).

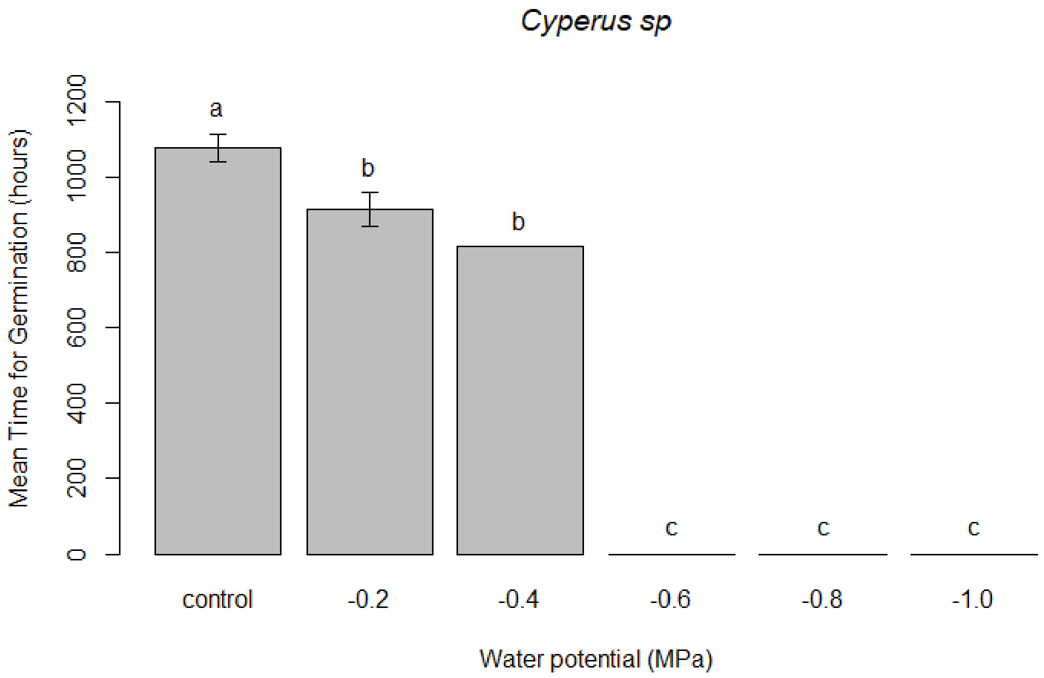

Figure 24 Mean time for seed germination of *Cyperus sp.* under a gradient of water stress simulations. The absence of bars indicates no germination at the treatment. Different letters indicate significant differences between treatments and species responses ($p < 0.05$).

Discussion

Despite low species richness and stressful abiotic conditions of temperature and water availability, our results indicates a possible way for the natural recovery of degraded areas. *Bulbostylis nesiotis* seeds could lead this process, not only due to their presence in the soil seed bank, but also because their high germination ability under bare soil conditions. Furthermore, when these seeds were exposed to extreme conditions of temperature, the increase in the mean germination time confirms another important strategy for establishment used by this species. Dispersion of germination over a longer time period, which increases the chance of recruitment for new individuals (see Leck *et al*, 1989; Parker *et al*, 1989). In contrast, both species of the *Cyperus* genus, showed a lower capacity to establish themselves in the bare areas studied. This was mainly because of an inability to tolerate the temperatures of those areas, which becomes evident when compared to *B. nesiotis*, otherwise, despite the negative influence of storage time, seeds have the ability to keep their viability over at least one year. This is a positive response, since we know that they could help compose the seed banks and favor the vegetation recovery as soon as abiotic conditions become appropriate for these species. An additional factor to favorable to *Cyperus* species is the occurrence of windows of opportunities, which are periods with pulses of required resources or free-disturbance periods during an unfavorable time that allow for germination and establishment (Balke *et al*, 2014). On Trindade, undefined rainy and dry seasons can sometimes result in rainy periods that might be long enough to provide suitable conditions for the establishment of seedlings.

In a study on Morro Vermelho, an area also disturbed by goats on Trindade, Martins and Alves (2007) found new occurrences of *B. nesiotis* and *C. atlanticus* and concluded that these species could be favoring the soil recovery as pioneers of a natural regeneration process. This study also reported the presence of the first tussocks of both species leading to more suitable conditions for the recruitment of other species, reducing soil temperature, increasing soil moisture retention and retaining dispersed seeds. In our case, Turtle Hill and Andradas Hill are located at lower altitudes (75 meters), when compared to Morro Vermelho (505 meters), presenting more extreme abiotic patterns such as higher soil and air temperatures and fewer rain occurrences (sparser and less in volume). A diverse altitude range is a strong factor that increases the diversity of habitats on Oceanic Islands by causing differences in levels of rainfall and wind incidence, which results in a variety of microclimate features (Whittaker & Fernández-Palacios, 2007; Hamann, 2011). Consequently, it is possible to note different compositions of species between habitats or degrees of intrinsic features within a single species, such as seed dormancy, vigor and germinations rates (Mendes-Rodrigues *et al*, 2010). This can be a result of maternal effects, in other words, when pressures by abiotic conditions experienced by the mother plant during seed production result in differences in the phenotypic characteristics of their seeds and, thus, of the new individuals (Roach & Wulff, 1989; Fenner, 2006; Hoyle *et al*, 2008; Ooi *et al*, 2012). Indeed, Trindade Island show a strong change in the species communities in different locations, as the habitats change (Barth, 1958; Alves, 1998). The comparison of hills characteristics, such as rainfall and temperature fluctuations, could explain why *Cyperus* species are able to support plant regeneration in the case of Morro Vermelho but their lack of germination on the Turtle and Andradas hills.

Water availability is known as one of the main factors that influences germination dynamics and the establishment of new seedlings. In environments with well-defined rainy seasons, dispersion seasons, dormancy, imbibition, and seed ability to persist in the soil will be highly synchronized with the wet and rainy season dynamics (Wolk *et al*, 1989; Thompson, 2000; Ramos *et al*, 2017).On the other hand, many different strategies were developed to prevent the loss of seeds when they face extreme or unpredictable conditions, such as mucilage production, seed dormancy, resistance to drought and germinating rapidly (Baskin, 1982; Ooi *et al*, 2007; Barbosa & Garcia, 2014). Although the island does not have a well-defined dry season, field measurements have shown that areas of bare soil conditions may become unfavorable even in short periods without rainfall. As noticed in the temperature simulations, *B. nesiotis* is also more tolerant to water stress, as compared to both *Cyperus* species. This can be observed not only by the higher levels of germination and wider range of stress treatments tolerated, but also by the ability to increase the mean time to germinate when the seeds were under stressful conditions. As indicated above, this is another important strategy for increasing the chances of recruitment in stressful environments and it can work in two main ways: (1) early germination of seeds, where they germinate as soon they find required resources, which is good if they reach a successful establishment, giving them a competitive advantage over seeds of later germination. However, it can be a high-risk strategy, since the rainy periods can be enough to germinate but not for them to stablish themselves. (2) late germination of seeds, which depends on constant and stable signals from the environment, helping seeds to avoid the influence of sporadic events such as droughts that can interfere with seedling recruitment (Labouriau, 1983; Grubb, 1977; Pujol *et al*, 2000).

Regardless of low germination rates of both *Cyperus* species in the water stress simulations, in an experiment with the same genus, Baskin & Baskin (1982) showed that *C. inflexus* seeds, after being moistened for 1 day, were able to keep their viability at least for 15 days in dry conditions, showing germination of 85% when they received water again. Though the low germination rates of the treatments in the present study, because of the presence of viable seeds of Cyperus in the bare soil seed bank we presume that the species of this genus are able to keep their viability under water stress conditions until a good season arrives.

Conclusions

The absence of plant soil coverage on the studied areas, even a long time after herbivorous eradication, shows that the negative consequences of vegetation removal by herbivory are still influencing the vegetation recovery on Trindade Island. Bare and vegetated areas generate two distinct microclimatic conditions, the vegetated areas having cooler temperatures and higher moisture levels in the upper soil layers, and higher seed densities. Despite the negative impacts of more extreme temperatures on non-vegetated areas, the surrounding standing vegetation is currently dispersing seeds in the soil seed banks. Indeed, the presence of seeds might indicate an ability for spontaneous recovery of the bare areas. However, with only *B. nesiotis* and *Cyperus* (depending on windows of opportunities) acting as agents of primary succession through the soil seed banks, the revegetation process probably will be slow, and those areas may stay vulnerable, favoring the increase of already existent erosions and the emergence of new ones. The results of the current study show how important the maintenance of plant coverage is in conservations efforts. It protects, not only the seed bank composition, which will ensure the continuity of Island recovery, but also the soil layers from erosion and their negative consequences. These results also help to explain the importance of knowing the agents involved in ecological succession, such as the areas abiotic characteristics and their main species, so that restoration can be favored.

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CONCLUSÃO GERAL

A ausência de cobertura vegetal, mesmo após 13 anos da erradicação dos herbívoros mostra que as consequências negativas da remoção da vegetação continuam influenciando na recuperação da mesma. Entretanto, as áreas perturbadas na Ilha da Trindade podem mostrar uma habilidade natural de regeneração através do banco de sementes do solo. Isto porque, apesar de limitações de algumas espécies, os três principais requisitos para a recuperação deste tipo de área foram observados neste estudo: condições abióticas que permitem a viabilidade de sementes, presença de endêmicas, e ausência de exóticas no banco de sementes. As condições ambientais se apresentaram bastante distintas em razão da presença ou ausência de vegetação, com temperaturas mais amenas e uma maior disponibilidade hídrica no solo nas áreas com cobertura vegetal. A presença de vegetação propicia um ambiente mais favorável para a manutenção de um banco de sementes abundante e o estabelecimento de novos indivíduos. Entretanto, nas áreas de solo nu, as condições extremas observadas nas camadas superficiais não reduziram a viabilidade de todas as espécies, visto que sementes de *Bulbostylis nesiotis* apresentaram germinação sob estas condições. Além disso, a presença de sementes de *Cyperus* no banco em áreas de solo nu pode estar relacionada a um mínimo grau de soterramento. Isso porque tal condição pode favorecer a manutenção da viabilidade destas sementes, uma vez que a temperatura já se mostra mais amena logo nos primeiros centímetros abaixo da superfície. Em segundo lugar, mesmo que em menor densidade quando comparado com as áreas com vegetação, foi identificada a presença de espécies endêmicas no banco de sementes, as quais poderiam atuar como colonizadoras destas áreas. Finalmente, a ausência de espécies exóticas no banco, uma vez que estas representariam uma barreira ao pleno desenvolvimento das endêmicas.

Os resultados deste trabalho colaboram com a compreensão da importância da manutenção da cobertura vegetal sobre o solo, visto que esta resulta em condições microclimáticas mais favoráveis à continuidade e expansão das populações endêmicas.

Os dados encontrados levam a crer que *Cyperus atlanticus*, apesar de ser a espécie mais representativa não só da vegetação total da ilha, como também do banco de sementes do solo das áreas testadas, dependeria de outros fatores para auxiliar na regeneração natural destes ambientes. Um destes fatores seriam as janelas de oportunidades, com períodos chuvosos que tornassem a camada superficial do solo nu mais propícia à germinação e estabelecimento de novos indivíduos, tanto em questões de temperatura do solo quanto de disponibilidade hídrica. Outro requisito seria, em um primeiro momento, o estabelecimento de indivíduos de *Bulbostylis*, visto que a espécie foi a única dentre as testadas que se mostrou hábil em germinar no solo nu sob condições normais. Logo, indivíduos já estabelecidos desta espécie gerariam um microclima capaz de favorecer a germinação e estabelecimento de novos indivíduos de *Cyperus*. Por último, o fato de *Cyperus* atualmente representar a maior cobertura vegetal da Ilha, tem levado as populações já estabelecidas a gerarem um microclima propício ao avanço de suas próprias populações sobre as áreas de solo nu através da reprodução vegetativa.

Por outro lado, com apenas *Bulbostylis nesiotis* e *Cyperus atlanticus* (dependendo de janelas de oportunidades) agindo como agentes de sucessão através do banco de sementes do solo, o processo de revegetação pode se mostrar bastante lento, e estas áreas continuariam vulneráveis à novas ocorrências de novas erosões, bem como ao agravamento daquelas já existentes.

Os resultados deste trabalho colaboram com a compreensão da importância da manutenção da cobertura vegetal sobre o solo de áreas protegidas, uma vez que sem elas a reestruturação da comunidade vegetal se torna mais difícil, devido às limitações que certas espécies têm em se estabelecer em áreas desprovidas de vegetação. Também ajudam a entender a importância de se conhecer os agentes envolvidos na sucessão ecológica, como padrões abióticos locais, tanto macro, quanto microclimáticos, e as principais espécies aptas a favorecer o processo.

Seria necessário um plano de restauração que acelerasse este processo de regeneração, visando prioritariamente a contensão dos atuais processos erosivos, através do estabelecimento de novos indivíduos, respeitando a composição de espécies nativas e evitando os efeitos das pressões abióticas desfavoráveis. Novos estudos seriam então necessários para enriquecer a lista de espécies, entre nativas e endêmicas, que seriam adequadas para uso em um projeto de recuperação das áreas ainda prejudicadas.

MATERIAL SUPLEMENTAR

Tabela 4. Resultados da análise de variância (ANOVA), no nível de significância de 5%, das medições de temperatura do solo sob diferentes coberturas e profundidades do solo.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Depth	2	9146	4573	191.40	<2e-16***
veg/bare	1	55789	55789	2334.92	<2e-16***
depth:veg/bare	2	3824	1912	80.03	<2e-16***
residuals	3954	94474	24		

Tabela 5. Resultados da análise de variância (ANOVA), no nível de significância de 5%, das medições de disponibilidade hídrica do solo sob diferentes coberturas (plot) e profundidades do solo (dep).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Plot	1	87398	87398	273.3	<2e-16***
Dep	2	145140	72570	226.9	<2e-16***
Plot:dep	2	124638	62319	194.9	<2e-16***
residuals	1074	343458	320		

Tabela 6. Resultados da análise de variância (ANOVA), no nível de significância de 5%, da porcentagem de perda da viabilidade de sementes das espécies estudadas (spp) - *Bulbostylis nesiotis, Cyperus atlanticus e Cyperus sp.* - sob simulações de temperaturas de solo (temp) – solo descoberto (BARE) e vegetado (VEG).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Temp	1	48240	48240	892.231	<2e-16***
Spp	2	1687	844	15.604	8.26e-05***
Temp:spp	2	824	412	7.622	0.0035**
residuals	20	1081	54		

Tabela 7. Análise de variância (ANOVA), no nível de significância de 5%, da porcentagem de perda da viabilidade de sementes de *Bulbostylis nesiotis* sob gradiente de déficit hídrico (wp) – concentrações de PEG 6000.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Wp	5	1883	376.7	1.832	0.157
residuals	18	3700	205.6		

Tabela 8. Análise de variância (ANOVA), no nível de significância de 5%, da porcentagem de perda da viabilidade de sementes de *Cyperus atlanticus* sob gradiente de déficit hídrico (wp) – concentrações de PEG 6000.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Wp	5	947	189.3	0.953	0.471
residuals	18	3575	198.6		

Tabela 9. Análise de variância (ANOVA), no nível de significância de 5%, da porcentagem de perda da viabilidade de sementes de *Cyperus sp.* sob gradiente de déficit hídrico (wp) – concentrações de PEG 6000.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Wp	5	1187	237.4	1.313	0.303
residuals	18	3256	180.9		

Tabela 10. Resultados da análise de variância (ANOVA), no nível de significância de 5%, da porcentagem de germinação de sementes de *Bulbostylis nesiotis* sob simulações de temperaturas de solo (temp) – solo descoberto (BARE) e vegetado (VEG) e armazenamento (strg).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Temp	1	13612	13612	38.679	1.4e-06***
Strg	2	2475	1237	3.516	0.0445*
Temp:strg	2	1913	956	2.717	0.0848 .
residuals	26	9150	352		

Tabela 11. Resultados da análise de variância (ANOVA), no nível de significância de 5%, da porcentagem de germinação de sementes de *Cyperus atlanticus* sob simulações de temperaturas de solo (temp) – solo descoberto (BARE) e vegetado (VEG) e armazenamento (strg).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Temp	1	59512	59512	1326.33	<2e-16***
Strg	2	860	430	9.58	0.00076***
Temp:strg	2	860	430	9.58	0.00076***
residuals	26	1167	45		

Tabela 12. Resultados da análise de variância (ANOVA), no nível de significância de 5%, da porcentagem de germinação de sementes de *Cyperus sp.* sob simulações de temperaturas de solo (temp) – solo descoberto (BARE) e vegetado (VEG) e armazenamento (strg).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Temp	1	63012	63012	1322.468	<2e-16***
Strg	2	435	217	4.562	0.02*
Temp:strg	2	435	217	4.562	0.02*
residuals	26	1239	48		

Tabela 13. Análise de variância (ANOVA), no nível de significância de 5%, do tempo médio de germinação de sementes de *Bulbostylis nesiotis* sob simulações de temperaturas de solo (temp) – solo descoberto (BARE) e vegetado (VEG) – e de período de armazenamento das sementes (strg).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Temp	1	1143724	1143724	96.511	4.58e-10***
Strg	2	46815	23407	1.975	.0160
Temp:strg	2	175291	87645	7.396	0.003**
residuals	25	296268	11851		

Tabela 14. Análise de variância (ANOVA), no nível de significância de 5%, do tempo médio de germinação de sementes de *Cyperus atlanticus* sob diferentes períodos de armazenamento das sementes (strg). As comparações entre temperatura não foram consideradas no modelo pela ausência de germinação no tratamento *BARE*.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Strg	2	214466	107233	15.51	0.00036***
residuals	13	89890	6915		

Tabela 15. Análise de variância (ANOVA), no nível de significância de 5%, do tempo médio de germinação de sementes de *Cyperus sp* sob diferentes períodos de armazenamento das sementes (strg). As comparações entre temperatura não foram consideradas no modelo pela ausência de germinação no tratamento *BARE*.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Strg	2	0.03037	0.015186	2.087	0.164
residuals	13	89890	6915		

Tabela 16. Análise de variância (ANOVA), no nível de significância de 5%, da porcentagem de germinação de sementes de *Bulbostylis nesiotis* sob gradiente de déficit hídrico (wp) – concentrações de PEG 6000.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Wp	5	30217	6043	42.38	2.44e-09***
residuals	18	2567	143		

Tabela 17. Análise de variância (ANOVA), no nível de significância de 5%, da porcentagem de germinação de sementes de *Cyperus atlanticus* sob gradiente de déficit hídrico (wp) – concentrações de PEG 6000.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Wp	5	592.6	118.52	1.684	0.189
residuals	18	1266.7	70.37		

Tabela 18. Análise de variância (ANOVA), no nível de significância de 5%, da porcentagem de germinação de sementes de *Cyperus sp.* sob gradiente de déficit hídrico (wp) – concentrações de PEG 6000.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Wp	5	10133	2026.7	14.03	1.12e-05***
residuals	18	2600	144.4		

Tabela 19. Análise de variância (ANOVA), no nível de significância de 5%, do tempo médio de germinação de sementes de *Bulbostylis nesiotis* sob gradiente de déficit hídrico (wp) – concentrações de PEG 6000.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Wp	5	1995238	399048	124.5	3.19e-12***
residuals	16	51298	3206		

Tabela 20. Análise de variância (ANOVA), no nível de significância de 5%, do tempo médio de germinação de sementes de *Cyperus atlanticus* sob gradiente de déficit hídrico (wp) – concentrações de PEG 6000.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Wp	2	0.12156	0.06078	36.99	0.0263*
residuals	2	0.00329	0.00164		

Tabela 21. Análise de variância (ANOVA), no nível de significância de 5%, do tempo médio de germinação de sementes de *Cyperus sp.* sob gradiente de déficit hídrico (wp) – concentrações de PEG 6000.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Wp	2	71126	35563	7.145	0.0478*
residuals	4	19908	4977		