



CRISTINA BALDAUF

**“ECOLOGY, CONSERVATION AND SUSTAINABLE
MANAGEMENT OF JANAGUBA (HIMANTHUS DRASTICUS;
APOCYNACEAE) IN THE BRAZILIAN SAVANNA”**

***“ECOLOGIA, CONSERVAÇÃO E MANEJO SUSTENTÁVEL DE
JANAGUBA (HIMANTHUS DRASTICUS; APOCYNACEAE) NO
CERRADO BRASILEIRO”***

**CAMPINAS
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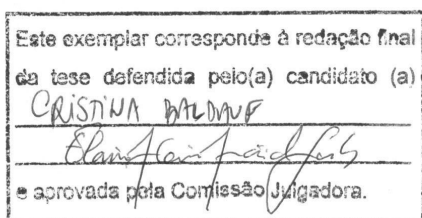


UNIVERSIDADE ESTADUAL DE CAMPINAS
INSTITUTO DE BIOLOGIA

CRISTINA BALDAUF

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OF JANAGUBA (*HIMATANTHUS DRASTICUS*; APOCYNACEAE)
IN THE BRAZILIAN SAVANNA**

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JANAGUBA (*HIMATANTHUS DRASTICUS*; APOCYNACEAE)
NO CERRADO BRASILEIRO***



Dissertation presented to the Institute of Biology in partial fulfillment of the requirements for the degree of Doctor in Plant Biology

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Orientador: Prof. Dr. Flavio Antonio Maës dos Santos
Coorientadora: Profa. Dra. Anete Pereira de Souza

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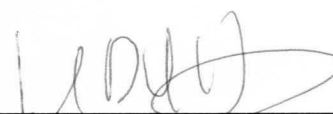
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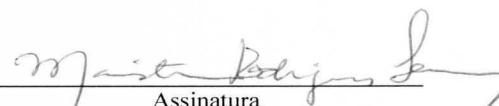
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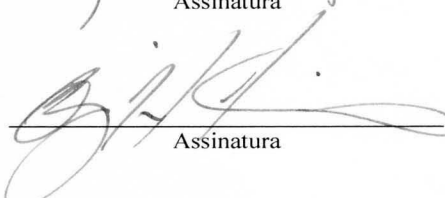
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ABSTRACT

Himatanthus drasticus (Apocynaceae), popularly known as *janaguba*, is a tree species commonly harvested in the Brazilian savanna (Cerrado). It has been exploited since the 1970's due to the medicinal value of its latex for the treatment of various forms of cancer. We evaluated the ecological impacts of harvesting on natural populations of *janaguba* using different scales and complementary approaches in the Cariri region, Ceará State, Brazil. An ethnoecological survey assessing the harvesting activity and traditional ecological knowledge was undertaken using semi-structured interviews and participant observation. Three management systems were identified, which may have different ecological impacts on *janaguba* populations, depending on the amount of bark removed and on the time interval between harvestings. To assess the effect of harvesting at individual level we carried out an experiment in which different percentages of the bark were removed, simulating the two main management systems employed. We found no differences in the bark regeneration indexes between the treatments. Reproductive phenology of harvested and non-harvested individuals was also compared. Flower and fruit production were higher in individuals with 100% of debarking when compared with partially stripped or non-harvested individuals. At the population level we sampled populations subjected to contrasting rates of harvesting and situated in different ecosystems (open savanna - *cerrado* and savanna woodland - *cerradão*). Matrix models were employed to describe population dynamics. Microsatellite markers were developed to compare the genetic diversity of managed populations in two groups (adults and seedlings) and management systems. The population growth rates were equal to one in all populations sampled in the first interval population, while in the second interval we observed a reduction in population growth rate of overexploited populations in the area of *cerradão*. Genetic diversity was high in all populations and generations. We observed a decrease in allelic richness in the seedling group of the high-harvest populations. However, the majority of the genetic diversity is maintained. We verified that the bark regrowth is slower in the *cerradão* than in the *cerrado*. Thus greater intervals between harvesting events are needed in the former. Overall, our results suggest that there are no significant impacts caused by harvesting at the genetic, individual, or population levels. However, populations located in savanna woodland are more vulnerable to harvesting and, therefore, should be less exploited. In conclusion, *janaguba* harvesting remains ecologically sustainable even after 40 years of exploitation and despite the increasing market demand for its latex. A workshop was organized in order to present the results of the thesis to a wide range of stakeholders. During the workshop, guidelines for the sustainable harvesting were developed through a deliberative and participatory approach. The decisions taken in the event constitute the core of the "Sustainable Management Plan" for the harvesting of *janaguba*, which aims to guarantee the conservation of natural populations and the livelihood improvement of local communities in the Cariri region.

RESUMO

Himatanthus drasticus (Apocynaceae), popularmente conhecida como janaguba, é uma espécie arbórea bastante explorada no Cerrado brasileiro. Ela vem sendo alvo de extrativismo desde a década de 1970, em função do valor medicinal de seu látex para o tratamento de vários tipos de câncer. Nós avaliamos os impactos ecológicos do extrativismo sobre as populações naturais de janaguba usando diferentes escalas e abordagens complementares na região do Cariri, Estado do Ceará, Brasil. Um levantamento etnobotânico foi realizado para avaliar o conhecimento ecológico tradicional e a atividade extrativista através de entrevistas semi-estruturadas e observação participante. Três sistemas de manejo foram identificados, os quais podem ter impactos ecológicos distintos, em função da quantidade de casca removida e dos intervalos entre coletas. Para quantificar o efeito do extrativismo em nível individual, nós realizamos um experimento no qual diferentes percentuais de casca foram removidos, simulando os principais sistemas de manejo empregados. Não foram detectadas diferenças na regeneração das cascas entre os tratamentos. A fenologia reprodutiva de indivíduos explorados e não explorados também foi comparada. A produção de flores e frutos foi maior nos indivíduos com 100% de remoção de casca quando comparados com indivíduos que sofreram remoção parcial ou com indivíduos não-explorados. No nível populacional, nós amostramos populações sujeitas a diferentes intensidades de manejo e localizadas em ecossistemas distintos (cerrado e cerradão). Modelos matriciais foram empregados para descrever a dinâmica populacional, enquanto marcadores microssatélites foram desenvolvidos para comparar a diversidade genética em dois grupos (adultos e plântulas) e sistemas de manejo. As taxas de crescimento populacional foram iguais a um em todas as populações amostradas no primeiro intervalo demográfico, enquanto no segundo intervalo demográfico observamos uma redução na taxa de crescimento das populações muito exploradas na área de cerradão. Nós registramos uma redução da riqueza alélica das plântulas das populações muito exploradas. Entretanto, a maior parte da diversidade genética foi mantida. Verificamos que a regeneração das cascas é mais lenta no cerradão que no cerrado. Portanto, intervalos maiores entre coletas são necessários no primeiro caso. Globalmente, nossos resultados sugerem que não existem impactos significativos causados pelo extrativismo nos níveis genético, individual e populacional. Entretanto, as populações situadas em áreas de cerradão são mais vulneráveis ao extrativismo e, portanto, devem ser menos exploradas. Em conclusão, o extrativismo de janaguba continua ecologicamente sustentável mesmo após 40 anos de exploração e apesar da demanda crescente por seu látex. Um workshop foi organizado a fim de apresentar os resultados da tese para uma ampla gama de interessados. Durante o workshop, diretrizes para o manejo sustentável foram desenvolvidas através de uma abordagem deliberativa e participativa. As decisões tomadas no evento constituem o “Plano de Manejo Sustentável” para a espécie, o qual tem como objetivo garantir a conservação das populações de janaguba e a melhoria dos meios de vida das comunidades de extratores da região do Cariri.

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DEDICATÓRIA

“Aos esfarrapados do mundo e aos que neles se descobrem e, assim descobrindo-se, com eles sofrem, mas, sobretudo, com eles lutam.”

Paulo Freire

“TO BE HOPEFUL in bad times is not just foolishly romantic. It is based on the fact that human history is a history not only of cruelty, but also of compassion, sacrifice, courage, kindness. What we choose to emphasize in this complex history will determine our lives. If we see only the worst, it destroys our capacity to do something. If we remember those times and places—and there are so many—where people have behaved magnificently, this gives us the energy to act, and at least the possibility of sending this spinning top of a world in a different direction.”

Howard Zinn

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*“Berat sama dipikul, ringan sama dijinjing”*¹

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¹ Provérbio indonésio cuja tradução literal é: “Pesado carregamos juntos, leve carregamos juntos”. É interpretado localmente como “um problema compartilhado é um problema pela metade, enquanto uma alegria compartilhada é uma alegria dobrada”.

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INTRODUÇÃO GERAL

“Que difícil é ser breve na batalha das ideias”

Fidel Castro Ruz

1. Produtos florestais não-madeireiros na América Latina

A pré-história americana é objeto de grandes debates gerados por descobertas controversas acerca da data da chegada dos ameríndios no continente e de suas respectivas rotas migratórias (Bonatto e Salzano 1997; O'Rourke 2011; Pitblado 2011; Gonçalves *et al.* 2013). Entretanto, independente da data precisa que a espécie humana chegou à América, é inegável o fato de que as populações humanas têm utilizado e manipulado uma grande amplitude de ecossistemas e recursos genéticos vegetais por milhares de anos neste continente.

O sítio arqueológico de Monte Verde no Chile abriga restos de vários produtos florestais não-madeireiros (PFNM)¹ empregados pelos povos pleistocênicos. Folhas de boldo (*Pneumus boldus*) estão entre as muitas espécies de plantas medicinais registradas nas proximidades de um lugar que parece ter sido uma "casa de cura" neste sítio arqueológico. Também foram encontradas 45 espécies de plantas comestíveis no mesmo local (Dillehay *et al.* 1986, Dillehay *et al.* 2008), uma forte evidência de que o histórico de manipulações humanas resultou na transformação das paisagens originais em ambientes ricos em recursos úteis para populações tradicionais (Wiersum 1997).

Na região amazônica, Roosevelt *et al.* (1996) encontraram depósitos que continham uma grande quantidade de frutas e fragmentos carbonizados de madeira de espécies comuns tropicais como a castanha-do-pará (*Bertholetia excelsa*), pitomba (*Talisia esculenta*), tarumã (*Vitex cf. cimos*a) e várias espécies de palmeiras. Os autores estimaram que o local foi ocupado pela primeira vez entre 11.200 a 10.500 anos antes do presente.

Na época do contato europeu, os povos amazônicos promoviam, manejavam ou cultivavam 138 espécies em diferentes graus de domesticação (Clement 1999). Entretanto,

¹ O termo “produtos florestais não-madeireiros” (PFNM ou NTFP, do inglês “Non-timber Forest Products”) vêm sendo utilizado desde a década de 1980 para designar “*todos os materiais biológicos, exceto madeira, que são extraídos das florestas para uso humano*” (De Beer e McDermott 1989). No entanto, não existe uma definição única do que seja PFNM. Neste trabalho assumimos que *Himatanthus drasticus* é um PFNM, ainda que seja obtido principalmente em áreas de savana (Cerrado) e não em florestas *stricto sensu*. Para uma revisão sobre as definições de PFNM consulte Shackleton *et al.* (2011) Non-timber Forest Products: Concept and Definitions. In: Shackleton, S.; Shackleton, C. and Shanley, P. (orgs). Non-timber Forest Products in the Global Context. Springer, Heidelberg. Pp 3-22.

inicialmente os conquistadores usaram a floresta apenas como fonte de madeira e terras florestais foram consideradas improdutivas (Gabay *et al.* 2012). Assim, as paisagens amazônicas modificadas pelos seres humanos foram abandonadas após o contato europeu e posterior redução das populações indígenas. Conseqüentemente, as populações de plantas domesticadas que ocorriam nestas paisagens desapareceram ou retornaram ao seu estado selvagem (Clement 1999). Por outro lado, alguns PFNM tornaram-se *commodities* durante os períodos iniciais de colonização (Sills *et al.* 2011), um caso bastante emblemático é o da quina ou chinchona (*Cinchona officinalis*), a qual figura na bandeira da República do Peru em função de sua importância na época da independência do país na década de 1820. Sua casca foi utilizada mundialmente contra a malária até a Segunda Guerra Mundial (Martínez Alier 2002).

A colonização marcou também uma ruptura de mecanismos de reprodução social dos povos nativos, seguida pela perda da propriedade comunal e mercantilização dos recursos naturais (Hobsbawm 1962; Gabay *et al.* 2012). Mais recentemente, com a evolução do capitalismo, ocorreu a erosão de tradições culturais adaptativas e perda de parte do conhecimento tradicional, concomitantemente com o aumento da degradação ambiental (Esquit-Choy 2012; García Latorre e García Latorre 2012). Entretanto, os PFNM permaneceram como elementos fundamentais dos meios de vida das populações tradicionais e rurais (Laird 2011). Estima-se que 1,5 bilhões de pessoas no mundo dependam de produtos florestais, tanto para autoconsumo quanto para venda no comércio local e regional (FAO 2001).

Em termos de comércio internacional, os PFNM têm tido importância variável ao longo da história. Representaram importantes *commodities* durante os períodos coloniais, conforme já mencionado. Após a Segunda Guerra Mundial ocorreu um significativo declínio no comércio de produtos florestais e PFNM tais como gomas, resinas, fibras e plantas medicinais foram largamente substituídas por derivados sintéticos mais baratos (Sills *et al.* 2011).

No final dos anos 80, em um contexto de uma crescente crise ambiental onde a indústria madeireira era considerada uma das grandes vilãs, os PFNM voltaram a chamar a atenção, em virtude de seu potencial de conciliar os objetivos de geração de renda e conservação da biodiversidade (Nepstad e Schwartzman 1992; Boot e Gullison 1995).

Entretanto, atualmente considera-se que o uso de PFNM tem uma ampla gama de impactos sobre a conservação da biodiversidade (Laird *et al.* 2011) e, em muitos casos, as pressões de mercado conduziram a sobre-exploração das espécies (Ruiz-Perez *et al.* 2004; Sunderland *et al.* 2011). Por outro lado, uma abrangente revisão dos estudos sobre extrativismo de PFNM em países em desenvolvimento apresentou resultados mais otimistas, especialmente para a América Latina (Stanley *et al.* 2012). Os autores verificaram que, em aproximadamente dois terços dos casos, que envolviam estudos realizados no período compreendido entre 2000 e 2010, foi demonstrada a sustentabilidade da coleta, ao passo que apenas 17,8% dos estudos registraram a coleta insustentável de PFNM (Stanley *et al.* 2012).

Do ponto de vista da pesquisa florestal, o manejo de PFNM levou a uma mudança paradigmática que vem forçando os profissionais dessa área a buscar métodos silviculturais apropriados e tecnologias para manejar simultaneamente madeira e PFNM (Gautam e Watanabe 2002). No entanto, os dados sobre taxas de crescimento e produtividade são abundantes para espécies madeireiras, ao passo que a ecologia e biologia de PFNM permanecem em grande parte desconhecida. Até mesmo no caso de espécies amplamente comercializadas existem lacunas consideráveis no conhecimento ecológico, sobretudo em relação às espécies fornecedoras de cascas e exudatos (Shanley *et al.* 2002; Ticktin and Shackleton 2011).

Por outro lado, as comunidades que utilizam determinado recurso vegetal já possuem inúmeros saberes acerca dos ciclos da planta em questão, bem como dos aspectos que beneficiam ou desfavorecem a ocorrência de populações da espécie. Portanto, a união de estudos e experimentos científicos ao conhecimento tradicional pode representar o caminho mais indicado para o desenvolvimento de técnicas de manejo que contribuam para a conservação das espécies vegetais, dos ecossistemas em que ocorrem e, conseqüentemente, da atividade extrativista (Redford e Padoch 1992; Shanley e Stockdale 2008; Fortmann e Ballard 2011).

2. Extrativismo de PFNM no Bioma Cerrado

O Bioma Cerrado² é considerado um dos ‘hotspots’ para a conservação da biodiversidade mundial, visto que possui a mais rica flora dentre as savanas do mundo e apresenta alto nível de endemismo (Mittermeier 2005; Ribeiro e Walter 2008). A riqueza de espécies de aves, peixes, répteis, anfíbios e insetos é igualmente grande, embora a riqueza de mamíferos seja relativamente pequena (Klink e Machado 2005).

A despeito da biodiversidade do Cerrado, as taxas de desmatamento neste bioma têm sido historicamente superiores às da Floresta Amazônica (Klink e Machado 2005). De acordo com Machado *et al.* (2004) a ocupação do Cerrado ocorreu em diferentes momentos e velocidades, mas muito provavelmente a abertura de áreas de pastagem para a criação de gado de corte foi a principal causa de desmatamento do bioma. Mais recentemente, a monocultura intensiva de grãos, especialmente a soja, vem se constituindo em uma das grandes ameaças à biodiversidade do Cerrado. Ainda que os esforços do governo brasileiro para reduzir o desmatamento tenham obtido algum sucesso (La Rovere *et al.* 2013), a aprovação do novo código florestal constitui uma nova ameaça à conservação dos biomas brasileiros, incluindo o Cerrado (Garcia 2012).

Por outro lado, diversas iniciativas têm incentivado o aproveitamento econômico de espécies do Cerrado, sobretudo PFNM, como uma forma de geração de emprego e renda, manutenção de comunidades locais em áreas rurais e conservação da biodiversidade (Schmidt 2005). A coleta de PFNM ocorre há pelo menos nove mil anos neste bioma (Klink e Moreira 2002) e dentre as espécies manejadas, algumas têm considerável valor econômico, tais como: barbatimão (medicinal e tanante), buriti (alimentícia e artesanato), faveira (medicinal), mangaba (frutífera e medicinal), piaçava (têxtil) e pequi (óleo e fruto) (Borges-Filho e Felfili 2003). No entanto, a exploração predatória de algumas dessas espécies vem acarretando reduções drásticas em suas populações naturais, visto que alguns destes produtos têm um volume de comercialização bastante elevado (Felfili e Silva Junior 1988; Borges-Filho e Felfili 2003; Zardo e Henriques 2011).

² Optamos por referir-nos ao Cerrado como um bioma com diferentes fisionomias, conforme Ribeiro e Walter (2008) e MMA (2011). Entretanto, a definição de bioma é controversa. Para outros autores, um bioma seria delimitado por uma única fisionomia vegetacional e, desta forma, o Cerrado seria constituído de um complexo de biomas ou, ainda, um domínio morfoclimático (Coutinho 2006; Batalha 2011).

Devido ao cenário recém-exposto, é fundamental a implementação de políticas que permitam aliar as demandas de exploração dos PFTM com a conservação dos remanescentes de Cerrado e, para tanto, informações ecológicas básicas sobre as espécies exploradas, bem como sobre os impactos do manejo sobre as populações são imprescindíveis. Neste contexto, destacam-se as pesquisas realizadas com o capim-dourado (*Syngonanthus nitens*) (Schmidt 2011) e o buriti (*Mauritia flexuosa*) (Sampaio 2012), as quais forneceram importantes subsídios para o manejo sustentável destes recursos. Todavia, considerando a riqueza biológica encontrada neste bioma, pode-se afirmar que existem informações disponíveis acerca da autoecologia e efeitos do manejo nas populações de um número bastante reduzido de espécies.

O extrativismo de plantas medicinais é parte integrante da economia regional em áreas de Cerrado no nordeste do Brasil, constituindo-se em fonte de renda principal ou complementar para comunidades locais. No Estado do Ceará, particularmente na região da Chapada do Araripe, destaca-se o extrativismo do “leite de janaguba” (*Himatanthus drasticus* (Mart.) Plumel), espécie explorada desde a década de 1970, em função de seu uso popular na cura de doenças do sistema digestivo, inflamações, mas, sobretudo, no tratamento do câncer (Baldauf e Santos, 2013).

Os resultados de estudos farmacológicos vêm demonstrando a eficácia do uso medicinal de *H. drasticus* como analgésico, antitumoral e imunoestimulante (Colares *et al.* 2008; Lucetti *et al.* 2010; Sousa *et al.* 2010, Mousinho *et al.* 2011). O sucesso dos tratamentos com o “leite de janaguba” vem causando um aumento da demanda pelo produto, o que poderia representar uma maior geração de renda para as comunidades extrativistas, mas, por outro lado, ameaçar a viabilidade ecológica do extrativismo da espécie. Neste contexto, a falta de dados sobre a autoecologia da espécie, assim como a ausência de estudos avaliando o impacto ecológico dos sistemas de manejo empregados para a coleta do látex de janaguba foram as principais motivações para o desenvolvimento desta tese de doutorado.

3. Objetivos da tese

"Aprendió la lengua de los vencedores y en ella habló y escribió. Nunca escribió sobre los vencidos, sino desde ellos."

Eduardo Galeano

O principal objetivo desta tese foi avaliar a sustentabilidade do extrativismo de janaguba (*Himatanthus drasticus* (Mart.) Plumel) em áreas de Cerrado no nordeste Brasileiro. Para tanto, utilizamos várias abordagens complementares. Inicialmente caracterizamos o conhecimento tradicional sobre a espécie no que se refere à autoecologia e sistemas de manejo empregados para coleta do látex (Capítulo 1). A finalidade de tal caracterização foi o reconhecimento dos principais sistemas de manejo empregados pelos extratores, para posterior avaliação de seus respectivos impactos ecológicos.

A seguir, desenvolvemos marcadores moleculares específicos (Capítulo 2) para identificar possíveis efeitos dos principais sistemas de manejo sobre a diversidade genética das populações (Capítulo 3), bem como caracterizamos a estrutura genética espacial e os padrões de fluxo gênico da espécie, visando o estabelecimento de estratégias de conservação genética *in situ* e *ex situ* (Capítulo 4). Adicionalmente, avaliamos a influência dos sistemas de manejo sobre a fenologia reprodutiva da espécie (Capítulo 5), para verificar possíveis *trade-off* entre a exploração do látex e a reprodução da espécie. Com o intuito de sugerir um intervalo adequado para a coleta sustentável, caracterizamos os padrões e taxas de reposição de cascas de plantas de janaguba submetidas a diferentes sistemas de manejo em ecossistemas distintos (Capítulo 6). Finalmente, estimamos os parâmetros demográficos das populações de janaguba a fim de verificar se estes variam em função da intensidade de manejo empregada ou do ecossistema considerado (Capítulo 7).

Os principais resultados obtidos deram origem a um plano de manejo sustentável para a espécie, o qual foi submetido ao Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) para aprovação. O referido plano de manejo foi desenvolvido de forma participativa (Capítulo 8) e publicado na forma de literatura de cordel (anexos), a fim de retornar os resultados da pesquisa para as comunidades extrativistas de forma acessível, bem como fomentar processos de divulgação científica na área de estudo.

A seguir serão explicitados os principais aspectos relacionados à espécie estudada e à área de estudo. Posteriormente, serão apresentados os capítulos da tese, os quais foram,

em sua maioria, redigidos na forma de artigos e de acordo com as normas dos periódicos nos quais foram publicados ou submetidos.

4. A espécie *Himatanthus drasticus* (Mart.) Plumel

*“Da família Apocinácea,
A mesma da mangabeira,
A Janaguba é um tesouro,
Uma planta de primeira;
Desde muito admirada,
Conhecida e utilizada
Pela gente brasileira”*

Francisco Willian Brito

A família Apocynaceae destaca-se pela frequente ocorrência de plantas ricas em compostos bioativos (Raffauf 1964; Moura e Agra 1989; Cunningham *et al.* 2008). Muitos gêneros de Apocynaceae vêm sendo utilizados medicinalmente pelas populações tradicionais em todo o mundo (Arseculeratne *et al.* 1981; Bussman *et al.* 2006). Na América do Sul, o gênero *Himatanthus* é empregado popularmente para tratamento de várias enfermidades, sobretudo o câncer (Lima 2005; Agra *et al.* 2007; Colares *et al.* 2008). No Brasil, a importância deste gênero é demonstrada através de sua presença na primeira farmacopeia brasileira (Silva 1929). Hoje em dia, provavelmente a espécie mais comercializada no país é *Himatanthus drasticus* (Mart.) Plumel, conhecida popularmente como janaguba, joanaguba ou tiborna (Figura 1).

Himatanthus drasticus é uma árvore com altura aproximada entre 1-7m, ramos jovens castanho-escuros com manchas castanho-claras e ramos mais velhos castanho-claros. Apresenta folhas oblanceoladas, elípticas, às vezes obovadas, ápice obtuso ou arredondado, às vezes obtuso-acuminado, base das folhas jovens agudas, decurrentes e folhas adultas obtusas, decurrentes e coriáceas (Spina 2004). As flores são pequenas e o fruto é polispérmico com germinação faneroepígea (Amaro 2006).

Apresenta uma distribuição restrita ao Brasil, onde recebeu diversos nomes vernaculares, entre eles: “janaguba”, “tiborna”, “joanaguba”, “janaúba” e “pau de leite” (Spina 2004; Spina 2013). Sua distribuição abrange as áreas de transição entre Cerrado e Caatinga, Cerrado, Campo Rupestre, Carrasco, Caatinga, Mata de Galeria e em Campo com afloramento granítico (Spina 2004). No Estado do Ceará, essa espécie ocorre com maior frequência na Chapada do Araripe, situada no extremo sul do estado (Amaro 2006).



Figura 1: A espécie *Himatanthus drasticus* (janaguba). Detalhe de um ramo com flor (A), exsudação do látex após a remoção da casca (B), extrativista coletando o látex da espécie (C) e produto pronto para a comercialização (D).

5. Área de estudo

*“Chapadão do Araripe,
Beleza sem outra igual,
Mataria exuberante,
Clima ameno, especial,
Altitude avantajada,
Média de chuva dobrada
Em face ao Sertão Central.”*

Francisco Willian Brito

A área de estudo está situada na região do Cariri, sul do Estado do Ceará (Figura 2). Nesta região encontra-se a Chapada do Araripe, formada por uma superfície tabular estrutural, com o topo conservado em um nível de 800 a 1000m de altitude que, além de bom aquífero, possui solos profundos e bem drenados (BRASIL, 1981). A Chapada do Araripe é reconhecida como uma das áreas prioritárias para a conservação do bioma Cerrado no Brasil (Cavalcanti e Joly 2002).

A área dos cerrados nordestinos está localizada sob influência de outros biomas ou domínios fitogeográficos como da Caatinga a leste, da Amazônia a oeste - noroeste, da Mata Atlântica a sudeste e do cerrado central ao sul - sudoeste (Vieira 2012). Outrora caracterizados como ambientes de baixa diversidade, os cerrados nordestinos atualmente são considerados muito diversos, tendo sido contabilizadas 936 espécies de plantas lenhosas, pertencentes a 84 famílias botânicas, em um levantamento recente (Vieira 2012).

O cerrado da chapada do Araripe é uma disjunção situada a altitude de 800 a 900m, encravado no domínio semi-árido da caatinga e é geralmente caracterizado como uma “ilha de habitat”, devido às temperaturas mais amenas e aos maiores níveis de precipitação que os das áreas circundantes (Costa *et al.* 2004). A Chapada do Araripe constitui um dos cinco centros de endemismos locais do cerrado nordestino (Vieira 2012).

Em termos históricos, as terras localizadas na Chapada do Araripe eram habitadas pelos índios Kariri, antes da chegada dos portugueses no interior brasileiro durante o século XVII. Atualmente, por processo de auto-reconhecimento, foi identificada apenas uma comunidade de remanescentes de índios Kariri, composta por 50 famílias e localizada próxima ao município de Crato, Ceará (MDA 2010). Hoje em dia, a região tem como eixo central o aglomerado urbano formado pelas cidades vicinais de Juazeiro do Norte, Crato e Barbalha, que ficam a uma distância aproximada de 700 km das capitais nordestinas.

A região caracteriza-se pela economia de base agrária, pelas atividades de extração (produtos madeireiros e não madeireiros) e indústrias de transformação, principalmente de gesso (MDA 2010). Destacam-se ainda como importante fonte de renda as atividades comerciais relacionadas à vida cultural e religiosa, sobretudo em função da memória do padre Cícero Romão Batista, líder religioso que fundou o município de Juazeiro do Norte, um dos maiores centros de peregrinação religiosa do país.

A primeira Unidade de Conservação criada na região foi a Floresta Nacional do Araripe (FLONA Araripe), localizada entre as latitudes 07°11'42" Sul e 07°28'38" Sul e longitudes 39°13'28" W e 39°36'33" W. Com uma área de 38626,32 hectares esta unidade de conservação abrange parte dos Municípios de Santana do Cariri, Jardim, Crato e Barbalha (Ibama 2004). O solo predominante na FLONA Araripe é o do tipo latossolo distrófico vermelho-amarelo (Cavalcanti e Lopes 1994) e o clima é classificado como tropical úmido e seco ou savânico (Aw), conforme o sistema de classificação de Köppen.

Em termos florísticos, foram registradas 188 espécies e 55 famílias de Angiospermas na FLONA Araripe (Ribeiro-Silva *et al.* 2012). Dentre as famílias com maior número de espécies encontram-se Fabaceae, Rubiaceae e Asteraceae, seguidas de Bignoniaceae e Myrtaceae (Ribeiro-Silva *et al.* 2012). As áreas amostradas na tese pertencem à FLONA Araripe e estão localizadas em duas fitofisionomias do Bioma Cerrado, o “cerrado *sensu stricto*”, definido como “*vegetação dominada por arbustos e árvores de 3-8 metros de altura, com menos de 30% de cobertura do dossel e ainda com uma quantidade considerável de vegetação herbácea*” e o “cerradão”, o qual é descrito como “*uma floresta quase fechada, com cobertura de dossel entre 50 e 90%, composta por árvores de 8-12 metros de altura*” (Oliveira e Marquis 2002).

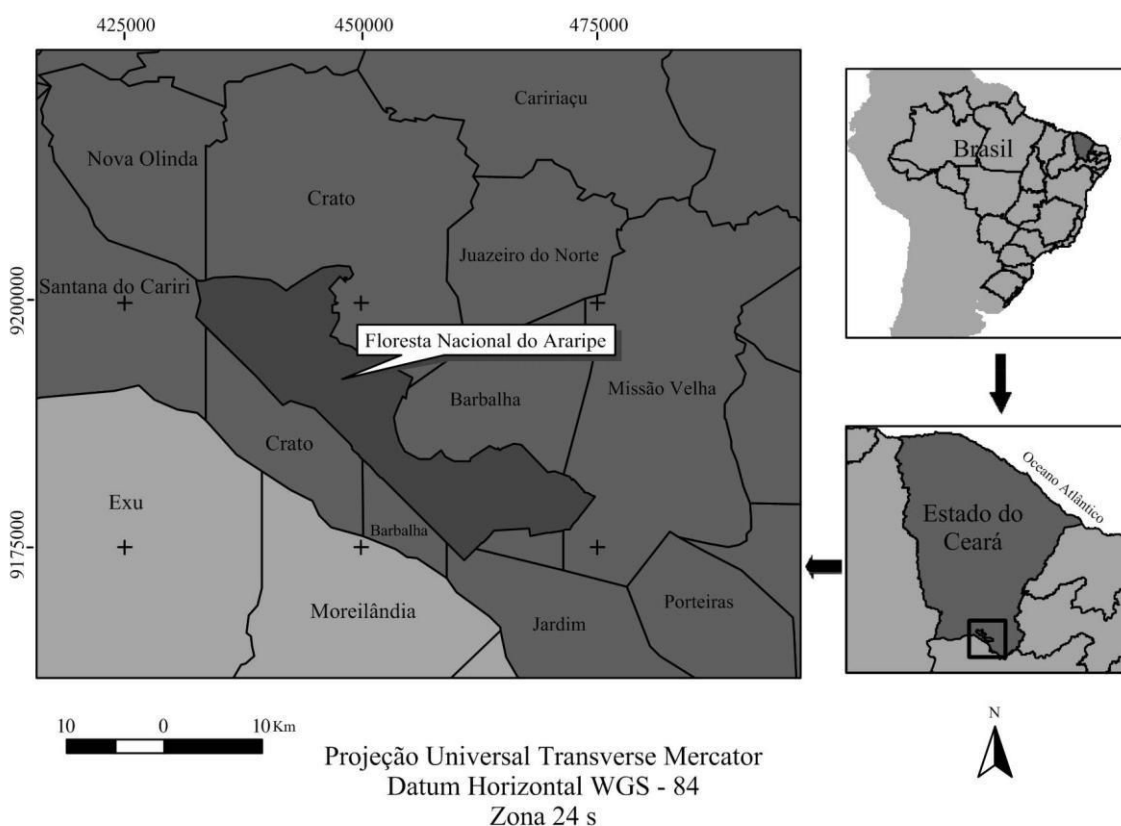


Figura 2: Localização da Floresta Nacional do Araripe, Ceará, Brasil.

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CAPÍTULO 1

Ethnobotany, traditional knowledge and diachronic changes in non-timber forest products management: the case study of *Himatanthus drasticus* in the Brazilian savanna³

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ABSTRACT

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The analysis of factors and processes that affect the traditional knowledge and the management practices deriving from it are essential for devising conservation strategies for non-timber forest products. The purpose of this study is to assess the traditional knowledge and analyze diachronic changes in management systems for non-timber forest products in a case study on an intensely exploited species from the Brazilian savanna, the *Himatanthus drasticus*, commonly known as "janaguba". The janaguba produces a latex of commercial value, widely used in popular medicine in Brazil. Recent pharmacological evidence of its medicinal properties has increased harvesting pressure on this resource. For this reason, we carried out an ethnobotanical characterization of the management systems used to harvest the janaguba latex and of the traditional ecological knowledge associated with such practices. Three management systems were identified in the harvesting of the janaguba latex, which may have different ecological impacts on janaguba populations, depending on the amount of bark removed and on the time interval between harvestings. Among the factors that can influence changes in the management systems over time are: market pressure and growing demand for the product, loss of traditional knowledge, the system of land tenure, and the biological characteristics of the species, especially its high biomass regeneration capacity.

Keywords: Medicinal plants, janaguba, ethnoecology, conservation, Cerrado, harvesting

RESUMO

A análise dos fatores e processos que afetam o conhecimento tradicional, bem como as práticas de manejo derivadas dele, é fundamental para a elaboração de estratégias de conservação de produtos florestais não-madeireiros. O objetivo desse trabalho é acessar o conhecimento tradicional e avaliar as mudanças diacrônicas nos sistemas de manejo de produtos-florestais não-madeireiros, a partir de um estudo de caso com uma espécie altamente explorada na savana brasileira. O látex de janaguba (*Himatanthus drasticus*) é comercializado devido à sua ampla utilização na medicina popular no Brasil. A recente comprovação farmacológica das propriedades medicinais da espécie vem aumentando a pressão de coleta deste recurso. Foi realizada uma caracterização etnobotânica dos sistemas de manejo de látex de janaguba, bem como do conhecimento ecológico tradicional associado a estas práticas. Foi possível identificar três sistemas de manejo empregados para coleta do látex de janaguba, os quais podem ter impactos ecológicos distintos sobre as populações exploradas, uma vez que se diferenciam em função da quantidade de casca retirada e intervalos entre explorações. Entre os fatores que podem influenciar a mudança dos sistemas de manejo ao longo do tempo encontram-se a influência do mercado e crescente demanda pelo produto, a perda do conhecimento tradicional, a posse da terra e as características biológicas da espécie, especialmente sua alta capacidade de regeneração de biomassa.

Palavras-chave: plantas medicinais, janaguba, etnoecologia, conservação, Cerrado, extrativismo

INTRODUCTION

The dialectical relationship between ethnobiological knowledge and the local management practices of natural resources shapes ecosystems and affects plant populations (Ghimire et al. 2004). In a highly complex scenario of the interactions between cultural, socioeconomic and biological factors, it is important to understand the motivations and contexts that lead to certain practices in the management of plant genetic resources.

Ruiz-Perez et al. (2004) compared the harvesting of 61 species of non-timber forest products (NTFPs) and concluded that in most cases, the strategies adopted by harvesters were market-oriented, reinforcing the idea that an increase in demand might lead to overexploitation and/or domestication. In addition to these economic aspects, other factors, such as land tenure, the biological characteristics of a species and its cultural significance, also influence the management intensity of plant resources (González-Insuasti et al. 2008). Although a number of the factors that tend to intensify plant management have been identified, how these factors or their mutual influence and relationship with other variables operate is unknown (González-Insuasti et al. 2008).

In this context, the ethnobotanical characterization of traditional ecological knowledge and local management techniques provides precise information on the practices that interact with the population ecology of the species in question and on the relationship between knowledge, management practices and institutional relations. Moreover, understanding the heterogeneity of knowledge and practices within a specific area is crucial for conducting research to suggest the best management systems for the species under study. However, research exhibiting this focus is rare, even considering species that are of economic importance, such as medicinal plants (Ghimire et al 2004). Therefore, more case studies are necessary to assess the traditional knowledge of species from the different botanical families that are subject to harvesting.

The Apocynaceae family is known for its medicinal compounds (Raffauf 1964, Moura and Agra 1989, Botsaris 2007). In South America, the genus *Himatanthus* is used in popular medicine to treat several diseases, especially cancer (Lima 2005, Agra *et al* 2007, Colares *et al.* 2008). Within this genus, one of the species that is most widely used and economically important is the *Himatanthus drasticus* (Mart.) Plumel-Apocynaceae, an endemic Brazilian medicinal tree, commonly known as janaguba.

Since the 1970s, the harvesting of janaguba latex has become a source of income for many communities in the Araripe Plateau, northeastern Brazil. This practice led the now defunct Brazilian Institute for Forestry Development (IBDF) to prohibit the harvesting of janaguba to avoid overharvesting of the species, which resulted in the establishment of an illegal market (Bezerra 2004).

The Araripe National Forest (FLONA Araripe), located in the State of Ceará, is a site of intense janaguba harvesting. In 1989, the office of IBAMA (Brazilian Institute for Environment and Natural Renewable Resources, now ICMBio- Chico Mendes Institute for Biodiversity Conservation) in the Araripe region decided to legalize the harvesting of janaguba milk in the FLONA Araripe, where the species occurs in high densities and has potential for sustainable management. A set of rules was defined, including the registration of the harvesters, the definition of collection sites and the introduction of a fee that harvesters should pay to the FLONA Araripe, equivalent to 10% of the market value of one liter of janaguba latex (Francisco Willian Brito, FLONA Araripe, April 7, 2009).

The pharmacological studies of the leaves, barks and latex of *H. drasticus* have confirmed its analgesic, antitumor and immunostimulant properties (Lucetti et al. 2010; Colares et al. 2008; Sousa et al. 2010, Mousinho et al. 2011). According to the harvesters and traders, both the trade and the number of people involved in the activity are increasing, most likely because of the scientific recognition of the medicinal properties of *H. drasticus*. In this context, understanding the management systems changes over time (diachronic approach) is crucial, since the increase in janaguba harvesting may affect the natural *H. drasticus* population, depending on the management practices adopted by the harvesters.

The objectives of this study are as follows: (1) to perform an ethnobotanical characterization of the traditional ecological knowledge of *H. drasticus*, (2) to identify the management systems used to extract latex from *H. drasticus* and discuss their potential ecological impacts, and (3) to conduct a diachronic analysis of the management systems found in the study site to identify and associate the factors that influenced the adoption of each of these management systems.

METHODS

Study site

The sub-region of Araripe is located in the center of Northeast Brazil, on the border of the Ceará, Pernambuco and Piauí states. The Chapada do Araripe, a preserved plateau at a height of 800-1000 m, with a good aquifer and deep and well-drained soils, is located in this region (BRASIL 1981). The vegetation physiognomies in the sub-region of Araripe consist of *cerrado* and *caatinga*. The *cerrado* in the Araripe plateau is a disjunction at a height of 800-900 m in the semi-arid *caatinga* (Costa et al. 2004). The climate is dry-hot tropical, with a rainy season. The temperature ranges from 15 °C to 25 °C, and the average rainfall is 1100 mm/year (Bezerra 2004).

Surrounding Chapada do Araripe is the Cariri region of Ceará. The economic characteristics of the region include harvesting (timber and non-timber products), agriculture, cattle raising, manufacturing industries, crafts, commerce, services, tourism and cultural and religious activities (pilgrimages) (Bezerra 2004).

The first protected area established in the region was the Araripe National Forest (FLONA Araripe), located between 07°11'42" and 07°28'38" latitude and 39°13'28" and 39°36'33" longitude. This protected area covers 38,626.32 ha, including part of the municipalities of Santana do Cariri, Missão Velha, Jardim, Crato, and Barbalha. In the FLONA Araripe, many non-timber forest products (NTFPs) are harvested, and these products represent an important source of income for the local communities as well as food security for the families of the harvesters (Bezerra 2004).

Data collection and analysis

A data set provided by the FLONA Araripe was used to select harvesters living in the municipalities of Crato and Barbalha, which are areas that demonstrate the highest rates of latex harvesting in the FLONA Araripe and surrounding areas (Bezerra 2004). It is estimated that approximately 124 harvesters are involved in latex harvesting in these municipalities (Franca 2006); however, only 31 harvesters are officially registered in the FLONA Araripe. Additionally, we used the snowball technique (Patton 2001) to identify non-registered harvesters in the study area.

The data were collected during 42 semi-structured interviews (31 registered and 11 non-registered harvesters) conducted to identify aspects of the management practices of the

janaguba population, with the aim of recording the ecological knowledge associated with these practices. No inclusion criterion, such as age, gender, or level of experience, was considered. The interviews were conducted individually.

The variables used in this study were as follows: the height (HEI, in cm) and width (WID, in cm) of the removed bark; the number of sides harvested in the plant (SID, values between one and four, which are equivalent to the debarking of approximately 25%, 50%, 75%, and 100% of the tree circumference); the time interval between harvestings for each plant (INT, in months); the length of time of the activity (ACT, in years); and the traditional ecological knowledge (TEK). The TEK was defined based on the answers to eight questions concerning the species autoecology, focusing on the following themes: habitat, demography and natural regeneration, phenology, seed dispersal, pollination and herbivory. The knowledge of each respondent was assessed by attributing zero (0) to the responses that did not agree with the data from the literature and one (1) to the responses that agreed with the data from the literature. This approach is similar to the “*index of ecological knowledge*” described by Reyes-Garcia et al. (2006). To evaluate the answers inter-correlation, the Cronbach’s α (Cronbach 1951) was calculated using the software SPSS 14.0 (SPSS Inc 2006). As the result was relatively high (Cronbach’s $\alpha = 0.71$), the questions were considered equally and the scores for each question were summed to obtain the variable “traditional ecological knowledge”.

The presence of outliers in the data set was analyzed using the software PC-ORD version 4.25 (McCune and Mefford 1999). This analysis was based on Euclidean distance, and the objects were considered outliers when their values were higher than two standard deviations. To identify the main management systems, a principal component analysis (PCA) based on the described variables was carried out. The data were standardized and the Euclidean distance and the correlation matrix were employed. The PCA was performed using the software MVSP version 3.2 (Kovach 2010).

RESULTS AND DISCUSSION

Characterization of traditional ecological knowledge

In general, the local ecological knowledge of the janaguba matches the information found in the literature, as will be discussed below. Amaro (2002) observed that the

janaguba has the characteristics of a pioneer species in which germination usually depends on light. In agreement with the literature, most of the respondents (73.8%) reported that the germination of this species is successful in open areas. However, Amaro et al. (2006a) confirmed the neutral photoblastism of the janaguba seeds, therefore, the successful survival of the janaguba in more open environments may not be related to increased seed germination but to the ease of establishment of seedlings in environments with lower litter depth, as described by 45.2% of the harvesters.

Concerning the leaf phenology of the janaguba, Modesto (1997) observed that, in the same study area, the leaves fall between May and October; however, only 28.5% of the harvesters cited that period for leaf fall. It should be emphasized that 40.4% of the respondents stated that the species is evergreen, which is a perception that may be derived from the fact that the leaves fall from only some of the individuals of the population and that leaf abscission from individual trees may be partial (pers. obs.).

Regarding flowering time, 64.2% of the responses matched the period cited in the literature, with a flowering peak in November and December. The question regarding fruiting produced the most diverse answers; October, November, December, and January were mentioned most often (42.5% of the respondents). The data in the literature suggest that the trees fruit throughout the year, which could explain the lack of agreement among the responses to this question (Spina 2004). However, the fruiting peak observed at our study site matches exactly the period mentioned by most of the harvesters (pers.obs.).

The only answer to the questions about the species ecology that was unanimous referred to seed dispersal. The harvesters who answered this question (83.3% of the total) stated that the seeds disperse by the wind, as cited in the literature (Amaro et al. 2006b).

There are no data in the literature regarding flower visitors and potential pollinators of *H. drasticus*, and the respondents could not relay any information about this topic. Even without the data for the species, moths of the family Sphingidae are considered the main pollinators of the genus *Himatanthus*, visiting the scented white tubular flowers (Linhares 2010).

A study conducted by Schlindwein et al. (2004) on the reproductive biology of other species of the genus (*Himatanthus phagedaenicus*), a pioneer arboreal species found in the Atlantic forest and in the Tabuleiro Nordestino (a region of savanna-like vegetation typical

of coastal ecosystems), confirmed that this species is pollinated by deceit because it does not produce nectar. The authors found no flower visitors after seven months of observation, which demonstrates how difficult it is to detect the pollinators of the genus. Therefore, it is understandable that the traditional ecological knowledge exhibits the same gap in information, especially because the pollination of the genus is nocturnal, when latex is not harvested.

To characterize the traditional ecological knowledge (TEK) of each respondent, we summed the values for each question related to aspects of the species ecology. These values ranged from one to seven, and the mean was 3.9 ($s = 2.3$), demonstrating that the respondent group is reasonably informed about the topic (48.7% of correct answers). However, the high standard deviation obtained demonstrates an unequal distribution of knowledge among the respondents. The older harvesters score higher values for TEK, close to the maximum, which is reinforced by the positive correlation between age and TEK ($r_s = 0.612$; $p < 0.01$). This result is not surprising because in general, TEK develops from experience accumulated over generations, and therefore, it is usually associated with older people in a given community (Davis and Wagner 2003). Similarly, the TEK associated with the janaguba comes from diachronic observation and experience accumulated by harvesters over forty years of activity in the region.

We also observed the existence of different forms of knowledge transmission associated with the janaguba. In this case, in addition to the vertical transmission (from parent to child), there is a horizontal transmission (between two individuals of the same generation) (Cavalli-Sforza and Feldman 1981). Zarger (2002) demonstrated the importance of the horizontal transmission for the ecological knowledge of the use and management of this plant species. For the janaguba, we observed complementary forms of inherited knowledge, which according to Cavalli-Sforza and Feldman (1981) prevents the erosion of knowledge.

Ethnobotanical characterization of harvesting

The time it takes to reach the harvesting areas depends on the means of transportation available. In general, when the harvesters go on foot or by bicycle, their activity begins at approximately 3 AM; when riding a motorbike, they leave home a little later, but they always start working before 5 AM. This time is considered the best time of

the day for harvesting because it corresponds to the period during which the janaguba “releases” more “milk”. The harvesters usually stop working at approximately 10 AM because they believe that the heat prevents the trees from exuding latex. Following the same reasoning, they identified the period from December to May as optimal for harvesting because this is the rainy season and the temperatures are milder.

The sites are selected considering the proximity of the harvester’s houses as well as the density of the target species. After arriving at their harvesting sites, the harvesters select trees that are at least 7-cm thick (they call this measurement “*grossura de litro*”, i.e., the diameter of a one-liter bottle of a soft drink), which is a size they consider “safe” for harvesting given that thinner plants require special skills to avoid damage to the tree. The harvesters do not relate the productivity in terms of latex volume with the diameter or the age of the trees; they exploit the trees randomly from those that measure 7 cm in diameter at breast height (D.B.H) to the oldest trees, which can reach approximately 40 cm D.B.H. Taking this criterion into account, the number of exploitable trees varies from 28.5 to 33.3 individuals per hectare, which can be considered very high when compared with the densities of other NTFPs exploited in the same region (Bezerra 2004); thus, in terms of resource availability, the janaguba shows a potential for sustainable management.

Vertical cuts are made in the selected trees using a machete or a scythe to remove the bark from 5-10 individual plants. Next, the harvesters go back to the first trees and scrape the bark with a spoon to remove the exuding latex. The latex removed is poured into a vessel or a bottle containing a small amount of water, and the harvesting cycle starts over.

This description is an overall representation of the harvesting procedure; however, there are differences in many aspects of the harvesting process. An important variation is the percentage of bark removed from each tree, which harvesters call the “number of sides” exploited. Exploiting one side refers to the removal of approximately 25% of the bark; two sides, 50% of the bark; three sides, 75% of the bark; and four sides, 100% of the bark. The bark removed is the “outer bark”, or the rhytidome, a complex of tissues consisting of phellogen, epidermis, cortex, and primary and secondary phloems. All tissues external to the phellogen are dead in a mature tree (Romero 2006).

Few harvesters (11.9%) do not remove the bark to harvest the latex; instead they cut the bark using a system similar to that used to tap latex from rubber trees. Of the

respondents that remove the bark to extract the janaguba latex, 7.1% remove it from one side, 33.8% remove it from two sides, 16.2% remove it from three sides, and 31.0% remove all of the bark.

Concerning the frequency of harvesting, time intervals of up to six months were considered sufficient for the recovery of an individual tree and the beginning of a new harvesting by 16.7% of the respondents. For 23.8% of the harvesters, 7 to 12 months was considered the ideal interval between harvests. Most respondents (40.5%) indicated that a time interval between 13 and 17 months was necessary before starting a new harvesting period, and finally, 19.0% thought that an interval of more than 18 months was essential before harvesting the same individual again. A correlation between the amount of bark removed and the interval required for a new harvesting was observed, i.e., a larger amount of bark removed indicated a longer interval that the harvesters allowed for an individual tree to recover from intense harvesting ($r_s = 0.561$; $p < 0.05$). Nevertheless, the ecological studies on bark regeneration showed no differences in the regeneration speed of trees subjected to different degrees of debarking (pers.obs.).

The length of the cuts did not differ greatly because generally, the cuts were at “arm’s reach”, which corresponds to approximately two meters of bark removed. The width of the cuts varied from 5 to 20 cm, according to the diameter of the individual tree and the most frequent width of bark removed was 10 cm (reported by 59.5% of the respondents).

Aspects related to the species biology may govern the management practices used, especially with regard to the regeneration capacity of biomass. In the study site, it is notable that in a large number of individual trees with barks almost completely removed do not show signs of morbidity several years after debarking. This trend has been confirmed by biomass experimental studies in which the trees that were almost 100% debarked did not die or exhibit signs of morbidity four years after debarking (Baldauf, pers. obs).

In general, it is expected that individual harvested trees would die shortly after the harvesting, most likely because of the interrupted flow of water and/or nutrients (Borges-Filho and Felfili 2003, Delvaux et al. 2007; Geldenhuys et al. 2007; Guedje et al. 2007). It is possible that in the case of the janaguba, most of the harvesters remove mainly dead bark during their harvesting activity, which would not hinder the flow of water or photosynthates. Moreover, plants belonging to families such as Apocynaceae, Canellaceae

and Moraceae exhibit high resilience after debarking partly because the wound is protected by exudates after the bark is removed (Cunningham 2001). On the other hand, there was no evidence of attacks by fungi or other pathogens in the debarked janaguba trees, most likely due to the presence of secondary metabolites in the latex; the genus *Himatanthus* is known for its antimicrobial compounds (Souza et al. 2004). Moreover, some species harvested for bark and exudates presented a reduction in the number or viability of the seeds in overharvested trees when compared with unharvested trees (Gaoue and Ticktin 2008; Stewart 2009). This information is not available for the *H. drasticus* and could be addressed in further studies.

The management systems

To interpret the ordination graph, we considered the first two axes, which explained more than 75% of the data variance.

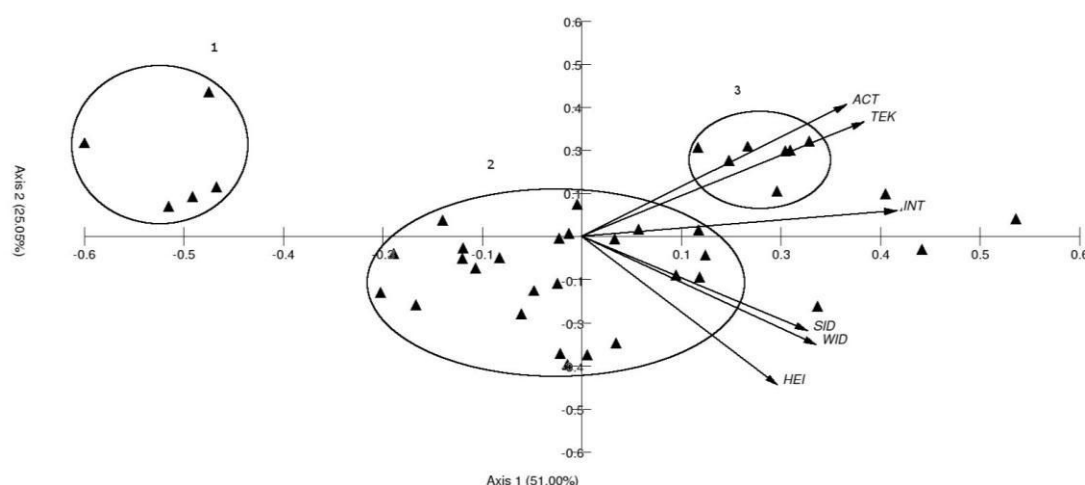


Figure 1: Ordination graph of janaguba (*Himatanthus drasticus*) harvesters: axes 1 and 2 of the principal component analysis (PCA).

Considering the ordination produced along axes 1 and 2, it is possible to observe the formation of three groups representing the three distinct management systems observed.

Management system 1 (MS 1) consists of a small group of five harvesters, located on the negative side of the first axis. MS 1 is used by harvesters with poor to medium local ecological knowledge and few years of activity. These harvesters differ from the others by not removing the barks when extracting the latex; they make several cuts in the bark, similar to the process used to tap latex from rubber trees. This system is used by 11.9% of

the respondents, however, they recognize that the productivity is low because of the small amount of latex exuded.

Management system 2 (MS 2) consists of the group located in the center of the graph, formed by the majority of the respondents. This group represents a more recent management system used by harvesters with a short to medium length of time in the activity and a poor local ecological knowledge of the species. The harvesters who use this MS remove the largest amounts of bark and allow for shorter time intervals between harvestings. This system is used by 61.9% of the respondents.

Management system 3 (MS 3) consists of the group located on the positive side of the two axes, formed by seven harvesters. The harvesters with a longer length of time in the activity and a deep local ecological knowledge use this management system. This MS is characterized by the removal of smaller amounts of bark from each tree and by longer intervals between harvestings. MS 3 is used by 16.7% of the respondents.

It should be emphasized that four harvesters were not included in any of the management systems identified because they use different management characteristics that do not constitute a management system *per se*.

Considering the potential impacts of other management systems, we observed that MS 3, which is used by harvesters with deeper traditional ecological knowledge and longer lengths of time in the activity, is more cautious concerning the amount and frequency of bark removal compared to MS 2, which is used by harvesters with shorter lengths of time in the activity. As a result, we hypothesize that a diachronic change occurred in the management systems regarding the management practices for the janaguba. The context that might have triggered this change will be discussed below.

Among the factors that exert strong pressure on the intensity of plant resource management, reported by González-Insuasti et al. (2008), the system of land tenure and the species biology might influence the management practices in the harvesting of the janaguba latex. Linhares (2010) observed that in small plantations in the state of Maranhão, young janaguba trees are preserved to protect the species and to guarantee future harvesting. These tolerant practices are considered evidence of incipient management or domestication (Clement 1999, González-Insuasti and Caballero 2007). Similar practices were not found in our study site, most likely because in this case, most respondents harvest the latex in the

FLONA Araripe. Because the harvester does not own the land, neither significant changes in the landscape nor investment in techniques to increase productivity are expected.

This aspect of land tenure also influences the management systems in another way; the national forests in Brazil are conservation areas that allow for the sustainable management of forest species (Brasil 2000). For this reason, the managers of the FLONA Araripe developed a system to register harvesters, and in an attempt to establish the communal management of resources, a participatory mapping that defines the harvesting areas for each family.

However, the harvesters feel they are being deceived by this mutual agreement because a number of harvesters do not comply with the zoning rules and harvest latex in areas designated to other families, either because access is easier or because plant density is higher. Moreover, there are cases of broken rules and evidence of “theft”, i.e., unauthorized harvesting of *janaguba* latex in areas of the FLONA Araripe by harvesters from the same community or from neighboring communities. The “theft” of latex is the most likely explanation for the differences between the FLONA Araripe statistics on the volume of traded latex (approximately 1,000 liters of latex per year) and the numbers obtained in another study of the same study area (Franca 2006), which estimated that approximately 8,700 liters of latex are harvested annually.

The breaking of the rules and “theft” are related to political and socioeconomic decollectivization processes and may have serious implications for the livelihood of the harvesters, as well as for the management and conservation of the species and ecosystems in question (Fernandez-Gimenez 2000, Duchelle et al 2011). The heavy demand for the *janaguba* species in the regional and national markets has increased the number of individuals involved in harvesting, which motivates some of the old harvesters to intensify their activity because they believe that “if we don’t extract it, others will come and do it”. Thus, the market pressure has stimulated competition among social actors and is possibly causing the adoption of more intensive management systems, as in MS 2, and the abandonment of management systems based on the traditional knowledge, as in MS 3, a situation that resembles the “tragedy of the commons” (Hardin 1968). Nevertheless, in order to discuss strategies to improve livelihoods and reduce the anthropogenic pressure on

the janaguba, a workshop with a wide range of stakeholders recently took place in the study area.

One of the outcomes of this workshop was a guideline for the sustainable harvesting of the species formulated using a deliberative and participatory approach (Baldauf 2012). However, for this strategy to be successful, it is necessary to follow a series of principles for community-based management, such as resource monitoring and sanctions for the appropriators who violate operational rules as discussed by Ostrom (1990) and Cox et al. (2010).

Another important aspect is the lack of social organization of the harvesters. There are no harvester associations or organized groups; the majority of the harvesters depend on the village traders to commercialize the latex. The improved organization of the harvesters is vital for increasing their bargaining power and equity within the supply chain. If the harvesters could sell the product directly to consumers instead of to traders, their contribution margin would increase considerably, improving their incomes. Another option is to introduce a certification process to enhance the generation of income and to promote the sustainable harvesting of *H. drasticus*. However, the certification of janaguba latex must be investigated carefully because businesses and consumers are responding slowly to certification messages, and markets for certified products remain narrow (Shanley et al 2005).

CONCLUSION

The level of ecological knowledge of the respondents as a whole is considered intermediate and is in line with the studies available in the literature to date. The deepest ecological knowledge observed is restricted to older individuals and/or to those individuals with a longer history in the activity.

The diachronic analysis of the management systems used to harvest the janaguba latex suggests that the market affects the management practices. In addition, factors such as the loss of traditional knowledge, species resilience to high levels of exploitation and the regimes of access to land may influence the harvesting practices. Nevertheless, these changes demonstrate the dynamism of the ecological knowledge and of the practices deriving from this knowledge, which according to Parlee and Berkes. (2006), are not permanent and continue to evolve every season based on observation and interpretation.

Finally, we emphasize that the different management systems identified in this study may have distinct ecological impacts on *H. drasticus* populations. Thus, the characterization of the ethnobotanical knowledge related to management systems represents a fundamental step for devising sustainable management strategies for any plant species.

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CAPÍTULO 2

Characterization of microsatellite loci in *Himatanthus drasticus* (Apocynaceae), a medicinal plant from the Brazilian Savanna⁴

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ABSTRACT

- Premise of the study: We developed a new set of microsatellite markers for studying the genome of the janaguba tree, *Himatanthus drasticus*, which is employed in folk medicine in northeastern Brazil. These novel markers are being used to evaluate the effect of harvesting on the genetic structure and diversity of natural populations of this species.
- Methods and Results: Microsatellite loci were isolated from an enriched *H. drasticus* genomic library. Nine primer pairs successfully amplified polymorphic microsatellite regions, with an average of 8.5 alleles per locus. The average values of observed and expected heterozygosity were 0.456 and 0.601, respectively.
- Conclusions: The microsatellite markers described here are valuable tools for population genetic studies of *H. drasticus*. The majority of the primers also amplified sequences in the genome of another species of the same genus. This new set of markers may be useful in designing a genetic conservation strategy and a sustainable management plan for the species.

Keywords: conservation; harvesting; medicinal plants; molecular markers; sustainable management

INTRODUCTION

Himatanthus drasticus, commonly known as janaguba, is an important medicinal tree species of the Brazilian Savanna that has been used by traditional populations of northeastern Brazil since the 1970s in the treatment and healing of a great number of diseases. Recently, the medicinal value of the *Himatanthus* genus was confirmed through pharmacological studies (Colares et al., 2008; Ferreira et al., 2009). This may increase the level of harvesting of bark and latex and lead to an overexploitation of natural populations. Harvesting can affect not only the individual physiology and metabolism, but also the genetic diversity and structure of managed populations (Ticktin, 2004).

The characterization of genetic diversity, associated with demographic studies, has been applied to the establishment of exploitation criteria for some plant species (Reis et al., 2000; Mariot et al., 2002). However, our present knowledge of the genetic diversity and structure of harvested species is still very incipient, especially regarding savanna species. To evaluate the impact of bark and latex harvesting on the genetic diversity of *H. drasticus* populations, we developed a set of highly polymorphic microsatellites, which can be used to determine the genetic diversity and structure, spatial genetic structure and gene flow of *H. drasticus* populations and to perform paternity analysis. The genetic information obtained with these novel markers will be applied in a sustainable management plan for the species and may also be employed in conservation and genetic improvement programs.

METHODS AND RESULTS

Genomic DNA was isolated from dried leaves of an individual *H. drasticus* tree, which were sampled in northeastern Brazil (07°23'51''S; 39°20'02''W) using a DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA). A microsatellite-enriched library was constructed according to the protocol described by Billotte et al. (1999). Genomic DNA samples were digested with the *RsaI* restriction enzyme, and the resulting DNA fragments were linked to *RsaI* adapters. A library was enriched for dinucleotide sequences using (CT)₈ and (GT)₈ biotin-labeled probes bound to Streptavidin MagneSphere Paramagnetic Particles (Promega, Fitchburg, WI, USA). Selected DNA fragments were PCR-amplified using primer sequences complementary to the adapters, and the amplicons were cloned into a pGEM-T Easy (Promega) vector. Plasmids were transformed into *Escherichia coli* XL1-

Blue competent cells, and positive clones were selected using the β -galactosidase gene and grown overnight in an HM/FM medium with ampicillin. A total of 96 positive clones were bidirectionally sequenced in an automated ABI 377 sequencer (Applied Biosystems) using T7 and SP6 primers and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

The sequences were assembled and edited using SeqMan software (DNASTar). MICROSAT software (A.M. Risterucci, CIRAD, personal communication) was employed to eliminate adapters and restriction sites from the sequences. The repetitive regions were identified using the Simple Sequence Repeat Identification Tool (Temnykh et al., 2001), and primer pairs were designed using the Primer3Plus software (Untergasser et al., 2007). After evaluation of the patterns of amplified fragments, nine of the fifteen developed markers were selected (Table 1) and the forward primer from each pair was labeled with various fluorochromes (6-FAM, HEX and TET). The remaining loci were discarded, due to amplification failures, nonspecific amplification patterns or unexpected product sizes.

Polymerase chain reaction (PCR) amplifications were performed in 15 μ L total volume containing 5.0 ng of template DNA, 0.8 mM of each forward and reverse primers, 20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM MgCl, 0.15 mM of each dNTP and 1 U of Taq DNA Polymerase. A PTC-100 thermal cycler (MJ Research, Waltham, MA, USA) was utilized for PCR with the following program: 96 °C for 1 min followed by 30 cycles of denaturation at 94 °C for 1 min, 1 min at a specific annealing temperature (T_a) and a final extension of 72 °C for 5 min. Amplification products were verified by electrophoresis on 3% agarose gels containing 0.1 mg ethidium bromide per mL in 1x TBE buffer (89 mM Tris-borate, 2 mM EDTA, pH 8.0) and were genotyped on 6% denaturing polyacrylamide gels stained with silver nitrate. Amplicons were electrophoretically separated in an ABI 377 automated sequencer (Applied Biosystems) using the GS500 TAMRA marker as the size standard (Applied Biosystems). Fragment size and allele identification were determined using Genescan v. 3.1.2 and Genotyper v. 2.5.2 software (Applied Biosystems). Two populations were sampled for genetic diversity analysis: Population 1: 07°18'40''S/39°27'48''W; n=51, and Population 2: 7°23'58''S/39°20'00''W; n=56. Descriptive statistics and tests of Hardy-Weinberg Equilibrium were performed using

Arlequin 3.0 analysis (Excoffier et al., 2005). Cross-species amplification was evaluated on *Himatanthus obovatus* (Müll Arg) Woodson and *Plumeria rubra* L.

Table 1. Characteristics of nine microsatellite primers developed for *Himatanthus drasticus*. Depicted for each primer pair are the forward and reverse sequences, repeat motifs, sizes of the original fragments (bp), annealing temperatures when analyzed individually (*Ta*) and the GenBank accession numbers.

Primer	Sequence	Repeat	Size	<i>Ta</i>	GenBank
HD1	F: TTGCTTTTTGTGCTGAGAGG R: CACCAATCCTTGATTCCAGA	(TG) ₄ CAG(TG) ₄	155	55	HQ423414
HD3	F: TGGTCTTCCCAGCAGTTGT R: GCGAAAAGTGTAGCACTGGA	(GA) ₁₃	253	55	HQ423416
HD7	F: CTTGTAGGTAACCGTCTTCACG R: CAAAGTGGCTGACGTTTGG	(CT) ₉	142	57	HQ423419
HD9	F: CTGCTTTGAGTCCATAACCTG R: GGTCCAGCCGATATGATTGT	(GT) ₆ (TA) ₄	167	57	HQ423420
HD11	F: CCGCCAATTTCTCTTCCTCT R: ACCACCACCATCCACTTCAT	(CT) ₈ T(TC) ₁₂	274	53	HQ423421
HD14	F: AGGGAGGGTGGAGAGAGAAG R: TTCGGCACGTCCTATATCCT	(AG) ₁₀	255	57	HQ423424
HD18	F: CAAGCTTGAATTAAACAACCAA R: GCATTGCCATTCTAGCA	(AAT) ₄	250	57	HQ423426
HD19	F: TGGCAATACACTGTCTCAAGTC R: TCTGGGAAAAGCAGCAGAAG	(TG) ₉ (GC) ₄	233	60	HQ423427
HD21	F: GCACCTAATTTCCCTACCTC R: CATCCTATCTACCGTCAGCA	(AC) ₉ AT ₄	285	57	HQ423428

All of the loci were polymorphic and presented a varied degree of diversity (Table 2). The average number of alleles was 8.5, ranging from two to 14 alleles per locus. Observed and expected heterozygosities ranged from 0.018 to 0.774 (0.456 on average) and from 0.018 to 0.868 (0.601 on average), respectively. The fixation index presented values

between -0.020 and 0.491 (0.215 on average). Six loci in Population 1 and eight in Population 2 significantly departed from the Hardy-Weinberg equilibrium ($P < 0.05$). The excess of homozygotes detected in this finding may be related to a probable mixed mating system, as well as partial self-incompatibility and mating among relatives.

Table 2: Results of initial primer screening in populations of *Himatanthus drasticus*. Shown for each primer pair are the number of alleles (A), number of effective alleles (A_e), values of observed (H_o) and expected (H_e) heterozygosity, fixation index (F) and P-values for Hardy-Weinberg equilibrium tests (HW) using the Markov chain method with 100000 dememorization steps. The sample size for each population is exhibited in parentheses.

Population	Primer	A	A_e	H_o	H_e	F	HW
Pop 1 (n=51)	HD01	2	1.040	0.039	0.038	-0.020	1.000
	HD03	12	5.255	0.412	0.810	0.491	0.000
	HD07	4	2.088	0.333	0.521	0.360	0.000
	HD09	2	1.963	0.510	0.491	-0.039	1.000
	HD11	12	6.208	0.529	0.839	0.369	0.000
	HD14	13	5.513	0.468	0.819	0.427	0.005
	HD18	5	2.021	0.510	0.505	-0.009	0.053
	HD19	8	3.217	0.686	0.689	0.004	0.024
	HD21	5	3.102	0.451	0.678	0.334	0.000
	HD01	12	1.018	0.018	0.018	-0.009	1.000
Pop 2 (n=56)	HD03	5	6.892	0.679	0.855	0.206	0.018
	HD07	8	1.585	0.214	0.369	0.420	0.000
	HD09	5	2.116	0.393	0.527	0.255	0.001
	HD11	13	7.593	0.571	0.868	0.342	0.000
	HD14	14	5.381	0.774	0.814	0.005	0.005
	HD18	9	3.640	0.679	0.725	0.064	0.001
	HD19	13	3.325	0.407	0.699	0.417	0.000
	HD21	11	3.502	0.536	0.714	0.250	0.000

Five of the nine loci were positively amplified in *H. obovatus* (55.5%). Some of the primer pairs that demonstrated specificity to *H. drasticus* (HD1, HD18, HD19 and HD21) may also be useful in differentiating these two species, which can coexist in some regions. For *P. rubra*, cross-amplification failed for the majority of loci, and locus HD1 alone was amplified in this species. These results were expected, as the species belongs to another genus of the Apocynaceae family.

CONCLUSIONS

The microsatellites isolated and characterized in this work are potentially useful tools for genetic studies of *H. drasticus* populations. These microsatellites can be used to analyze genetic diversity and structure, mating systems, gene flow and fine-scale spatial genetic structure as well as to perform paternity analysis. All of this information can be applied to a sustainable management plan for the species. Because cross-tests were positive at certain sequences, the primers might be broadly applicable for genetic studies in *H. obovatus*. These results also indicate a high level of genetic diversity in the studied populations, suggesting that conservation in situ may be an important strategy for the species.

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Appendix 1. Taxa used in this study. Specimens were deposited in UEC the Herbarium (Universidade Estadual de Campinas herbarium).

Taxon – Specimen Voucher, Collector

Himatanthus drasticus – UEC 1217, Barros et al.

Himatanthus obovatus – UEC 7537, Shepherd et al.

Plumeria rubra – UEC 496, Spina

CAPÍTULO 3

Tapping latex and alleles? The impacts of bark and latex harvesting on the genetic diversity of *Himatanthus drasticus* (Apocynaceae)⁵

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Abstract

The importance of genetic variation for maintaining biological diversity and evolutionary processes is widely accepted; however, limited action has been taken to protect genetic diversity in natural populations. Until recently, it was assumed that the exploitation of non-timber forest products, such as fruits, barks and exudates, had low environmental impact, but recognition that the harvesting of these plants can affect several biological levels, including genetic diversity and structure, has been growing. Nevertheless, the effect of harvesting non-timber forest products on the genetic diversity of the harvested species is poorly understood. The objective of this work is to assess the impact of harvesting on the genetic diversity of *Himatanthus drasticus* (Apocynaceae), a tree of the Brazilian Savanna (Cerrado) that is highly exploited for its medicinal latex. We tested the hypothesis that harvesting can cause a trade-off between survival and reproduction in *H. drasticus*, reducing the effective population size as well as the genetic diversity in highly exploited populations. The study was conducted in the Araripe National Forest, a preserved Cerrado area in Ceará State, Brazil. We sampled six populations that included two different levels of harvesting (high-harvest and low-harvest) and characterized the genetic diversity and structure in two groups (adults and seedlings) using microsatellite markers. We verified high levels of polymorphism in *H. drasticus*; however, significant levels of inbreeding in all studied populations were also observed. We found a higher degree of genetic structure in the seedling group when compared with the adult group, a difference that was most likely due to seed rain deposited in nearby locations. No differences were detected in adult genetic diversity levels between populations under different harvesting intensities. However, seedling allelic richness was significantly lower in the high-harvested populations, indicating allelic losses that may be associated with the exploitation of bark and latex. Evidence of genetic loss due to overharvesting was supported by significant bottleneck signatures observed in the seedling group of two out of three high-harvest populations. The establishment of a conservation strategy is imperative to avoid future genetic losses and ensure the long-term viability of *H. drasticus* populations. The information obtained in this study has been integrated with ecological information about the effects of latex tapping on phenology, demography and biomass recovery of exploited populations, with the goal of proposing a sustainable management plan for the species.

Keywords: sustainable management, janaguba, non-timber forest products, Brazilian Savanna, Cerrado

Introduction

The importance of genetic variation for maintaining biological diversity and evolutionary processes is widely recognized (Frankel and Soulé, 1981; Lande, 1988; Frankham et al., 2004; Laikre et al., 2009). Despite some studies that showed no association between genetic diversity and fitness (Savolainen and Hedrick, 1995; He and Lamont, 2010), high levels of genetic diversity are generally assumed to act as a basis for adaptation and to provide resilience to environmental stress and change (Li and Wu, 1996; Frankham, 2005; Schaberg, 2008; Richter-Boix et al., 2011). Modern genomic approaches can identify the adaptive genetic variation that is related to traits that are key for response to climate change, such as phenology or drought tolerance. The identification of adaptive genetic variation allows environmental management to focus on maintaining adaptive genetic potential (Allendorf et al., 2010). Monitoring adaptive change has already provided important results concerning organismal adaptations to human-altered environments (Hansen et al., 2012).

A decline in genetic diversity may have an adverse effect on the ability of a species to survive, with possible ramifications for other components of the forest ecosystem (FAO, 2002; Crutsinger et al., 2006). Because of the ecological and economic consequences of genetic losses, the conservation of genetic diversity is an explicit objective of the Convention on Biological Diversity (CBD). The relevance of genetic diversity conservation was also highlighted in the Global Strategy for Plant Conservation 2011-2020 (SCBD, 2011). One of the goals of this strategy is the improvement of the “*status of biodiversity by safeguarding ecosystems, species and genetic diversity*,” including the almost utopian target of “*at least 75% of the most important areas for plant diversity of each ecological region protected with effective management in place for conserving plants and their genetic diversity*”.

Some authors have discussed the viability of genetic assessment and monitoring because genetic monitoring is laborious and expensive and therefore impractical for use in management and routine operational purposes (Jennings et al., 2001; Sheil, 2001).

Nevertheless, there are several conservation programs for animal and plant species based on the successful use of genetic indicators (Rieseberg and Swensen, 1996; Reis et al. 2000; Maudet et al., 2002; Frankham et al., 2004; Bergl and Vigilant, 2007; Vinceti et al. 2013). Moreover, with the development of methods for multiplexing PCR, the costs of genotyping have been decreasing over the last years (Guichoux et al. 2011).

Although the establishment of criteria for selecting species for genetic diversity assessment and monitoring is controversial, there is a consensus that species threatened in the wild and species that are intensively exploited should be prioritized. Another recommendation is to prioritize tree species for which genetic markers are already available (Konnert et al., 2011). Given these criteria, the tree species *Himatanthus drasticus* (Apocynaceae) is of particular interest because it has been subjected to high levels of exploitation in a Brazilian biodiversity hotspot (Cerrado) and microsatellite markers are already available for its genetic characterization (Baldauf et al., 2011).

Himatanthus drasticus, commonly known as *janaguba*, has been intensively harvested for its medicinal latex since the 1970s. Its distribution comprises the entire northeastern region of Brazil (Spina, 2004), where the latex of the species is commonly mixed with water (“leite de janaguba”) and used to treat gastritis, hemorrhoids, anemia, inflammations, worms and several types of cancer (Lucetti et al., 2010). Preliminary results of pharmacological studies with leaves, bark and latex from this species have supported its popular use as an analgesic, antitumor and immunostimulant (Colares et al., 2008; Lucetti et al., 2010; Sousa et al., 2010; Mousinho et al., 2011). Validation of the medicinal properties of *H. drasticus* caused an increase in trade that may have negatively impacted natural populations. The most common technique employed for tapping the latex of *H. drasticus* involves removing large quantities of bark and latex. Because of the potential ecological and genetic impact of latex harvesting, the objective of this work is to assess the effect of harvesting on the genetic diversity of *H. drasticus* populations with the goal of defining strategies for its sustainable management. We tested the hypothesis that harvesting can cause a trade-off between survival and reproduction in non-timber forest products, reducing the effective population size as well as the genetic diversity in highly exploited populations.

2. Materials and Methods

2.1. Study area and experimental design

The study area was a plateau named Chapada do Araripe that is situated in the Cariri region, Ceará State, of northeastern Brazil (Figure 1) and is recognized as a priority area for conservation of Brazil's Cerrado biome (Cavalcanti and Joly, 2002). The cerrado of the Chapada do Araripe is a disjunction located at an altitude of 800 to 900 m, characterized as a habitat island due to higher rainfall and lower temperature when compared to the surrounding Caatinga biome (semi-arid forest) (Costa, 2004).

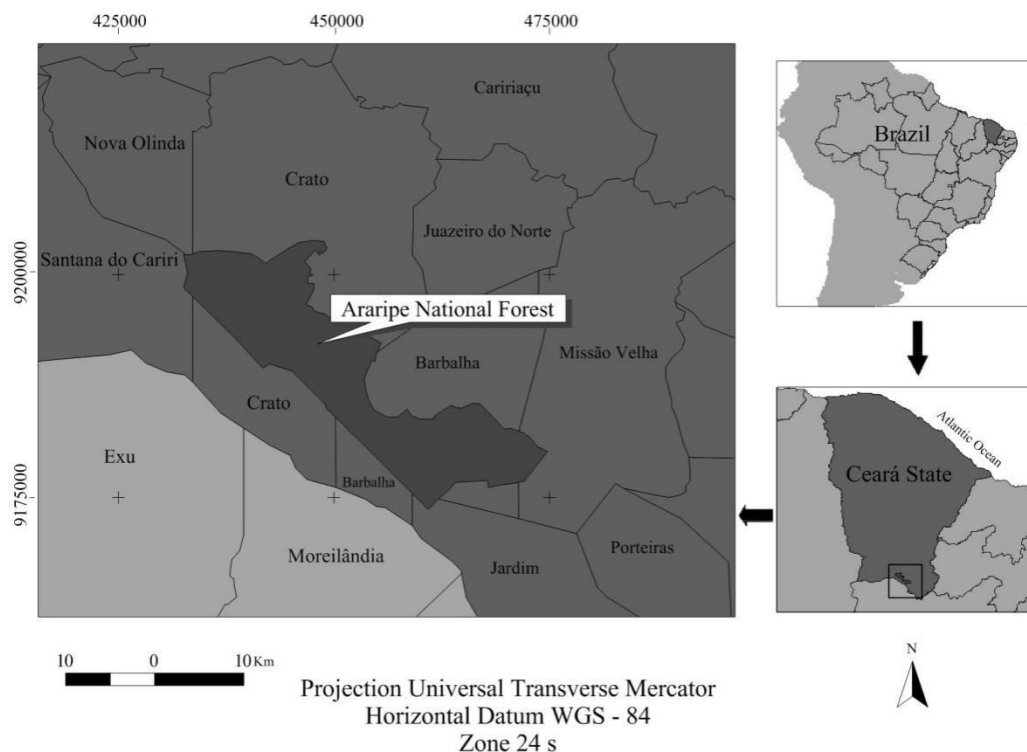


Figure 1: Location of the Araripe National Forest (FLONA- Araripe), Ceará State, Northeastern Brazil.

The survey was carried out in one of the protected areas of Chapada do Araripe, the Araripe National Forest (07°11' S, 39°13' W, 938 m elevation), which is considered the only pristine Cerrado area in Ceará State (Figure 1). The area is covered by the “cerrado *sensu stricto*”, which is defined as “vegetation dominated by 3 to 8 m tall trees and shrubs with more than 30% crown cover but with still a fair amount of herbaceous vegetation

between them” (Oliveira and Marquis, 2002). The soil is a red-yellow *dystrophic latosoil* (Cavalcanti and Lopes, 1994), and the climate is Tropical wet and dry or Savanna climate (Aw) according to the Köppen classification system (Köppen, 1948). The rainfall in this area is concentrated between November and May.

To characterize genetic diversity, we sampled six populations with two different harvesting levels in a completely randomized design. All populations were sampled in an area where non-timber forest products harvesting is permitted, but logging, grazing and agriculture are not. The climate, water availability and soil type are the same in the six populations; therefore, the main difference between the areas is the harvesting intensity. Three populations were subject to high rates of bark harvesting (high-harvest populations- HH), and three populations had low rates of harvest (low-harvest populations- LH). In high-harvest populations, more than 50% of adult trees were debarked, and individuals generally had more than 50% of the bark circumference removed. In low-harvest populations, less than 20% of adult trees were debarked, and individuals had, at maximum, 50% of the bark circumference removed. We assessed the levels of genetic diversity in two different groups (seedlings and adult). In each population, samples were collected from all adult trees within a 1 ha plot. The number of adult plants in the sampled populations ranged from 43 to 70 individuals. A minimum number of 50 seedlings were randomly collected in 100 subplots of 2 x 2 m located inside each 1 ha plot, totaling 654 genotyped individuals.

2.2 DNA extraction and microsatellite genotyping

The nine microsatellite markers used in this study were previously developed by Baldauf et al. (2011). Genomic DNA was extracted from approximately 25 mg of fresh leaves grinded with liquid nitrogen using the DNeasy kit (Qiagen, Valencia, California, USA). DNA quantification was conducted by comparison of standard DNA (DNA λ) of known concentration in a 0.8% agarose gel stained with ethidium bromide. Microsatellite loci were amplified through PCR as follows: 15 μ L total volume containing 5.0 ng of template DNA, 0.8 mM of each forward and reverse primers, 20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM MgCl, 0.15 mM of each dNTP, and 1 U of Taq DNA polymerase, 96° C for 1 min followed by 30 cycles of denaturation at 94° C for 1 min, 1 min at a specific annealing temperature (Ta), 1 min at 72° C, and a final extension of 72° C for 5 min.

Amplification products were checked using 3% agarose gel electrophoresis stained with ethidium bromide.

Fluorescent amplicons were genotyped on 6% denaturing polyacrylamide gels in an ABI 377 automated sequencer (Applied Biosystems) using the GS500 TAMRA marker as the size standard (Applied Biosystems). Fragment size and allele identification were determined using Genescan v. 3.1.2 and Genotyper v. 2.5.2 software (Applied Biosystems) and then exported to Excel.

2.3 Data analysis

After defining the allele frequencies of each population and group, diversity indices were estimated as follows: percentage of polymorphic loci ($P_{95\%}$), number of alleles per locus (A), effective number of alleles (A_e), observed heterozygosity (H_o), expected heterozygosity (H_e) and fixation index (F). The significance of the F values was calculated using permutations (1,000) and a sequential Bonferroni correction for multiple comparisons (95%, $\alpha=0.05$). Genetic indexes and F-statistics were estimated using GENALEX 6.5 software (Peakall and Smouse, 2006).

The software BOTTLENECK (Piry et al. 1999) was used to detect recent population bottlenecks in the studied populations and groups. This analysis was based on the Stepwise Mutation Model (SMM or strict single step mutations) using two different tests: the ‘sign test’ and the ‘Wilcoxon signed-ranks test’. Bottleneck tests assume that populations that have experienced a recent reduction in effective population size exhibit a more rapid reduction of allelic diversity than heterozygosity at polymorphic loci (Maudet et al., 2002).

To compare the average values of A , H_o , H_e and F between adults, groups and harvesting intensities, a 95% confidence interval for each parameter was calculated using a Jackknife procedure over loci using FSTAT software, version 2.9.3.2 (Goudet 1995). An extra analysis of gene frequency using the two-class classification (common frequencies $\geq 5\%$ and rare frequencies $< 5\%$) was performed by resampling methods (10,000 bootstraps) and comparison of the confidence intervals obtained for each generation and harvesting level.

3. Results

3.1 Genetic diversity and structure

The nine loci studied showed high levels of polymorphism. The total number of alleles was 148, ranging from 6 (locus HD1) to 24 (locus HD14), with a mean of 7.52 alleles per locus. The effective number of alleles ranged from 1.0 to 7.34 (average of 3.20). The average of effective number of alleles was inferior to the average number of alleles per locus, which indicates a large amount of low-frequency alleles (Table 1). The percentage of polymorphic loci reached 100% in all but one population. Only one population (population 2— low-harvested adults) presented one monomorphic locus (HD3). The observed heterozygosity varied from zero (the only monomorphic locus) to 0.847 (average of 0.447), and the expected heterozygosity ranged between zero and 0.864 (average of 0.614). Deviations from Hardy-Weinberg equilibrium and significant values of intra-population fixation index (F) were detected in all studied populations.

Table 1: Indices of genetic diversity obtained in adults and seedlings groups of *H. drasticus* subject to different levels of harvesting. The following parameters are presented: size of each sample (N), number of alleles per locus (A), percentage of polymorphic loci ($P_{95\%}$), observed heterozygosity (H_o), expected heterozygosity (H_e) and Fixation Index (F). LH= low-harvest; HH= high-harvest. Values in parenthesis are the standard error.

	n	P	A	H_o	H_e	F
Adults						
LH1	70	100.0	8.22 (0.293)	0.379 (0.022)	0.577 (0.021)	0.341(0.029)
LH2	59	88.9	7.33 (0.458)	0.497 (0.029)	0.589 (0.028)	0.136 (0.027)
LH3	43	88.9	9.78 (0.457)	0.468 (0.027)	0.621(0.030)	0.230 (0.018)
HH1	51	88.9	6.78 (0.461)	0.431 (0.019)	0.598 (0.028)	0.220 (0.025)
HH2	51	100.0	7.56 (0.389)	0.462 (0.029)	0.583 (0.023)	0.274 (0.042)
HH3	54	100.0	7.67 (0.342)	0.467 (0.015)	0.596 (0.021)	0.185 (0.021)
Mean						
Seedlings						
LH1s	55	100.0	8.67 (0.455)	0.372 (0.023)	0.624 (0.019)	0.398 (0.038)
LH2s	53	100.0	8.67 (0.347)	0.421 (0.027)	0.642 (0.018)	0.381(0.032)
LH3s	53	100.0	7.44 (0.033)	0.496 (0.026)	0.665 (0.017)	0.291 (0.034)
HH1s	55	88.9	5.33 (0.272)	0.446 (0.022)	0.522 (0.026)	0.173 (0.027)
HH2s	50	100.0	5.89 (0.263)	0.428 (0.023)	0.546 (0.020)	0.203 (0.033)
HH3s	59	100.0	6.89 (0.340)	0.476 (0.030)	0.609 (0.017)	0.274 (0.040)

The F-statistics indicated high and significant levels of inbreeding in both the population average (F_{IS}) and in the set of populations (F_{IT}) in adult and seedling populations (Table 2). The genetic divergence (F_{ST}) was significantly higher in the seedlings than that observed in the adult group. The F_{ST} value indicates that only 5.6% of the genetic diversity was found among populations, whereas 94.4% of it was within populations in the adult group. With regard to seedlings, 9.7% of the genetic diversity was found among populations, whereas 90.3% was within these populations.

Table 2: F-statistics from different groups of janaguba (*H. drasticus*).

	HD 01	HD 03	HD 07	HD 09	HD 11	HD 14	HD 18	HD 19	HD 21	Mean
$F_{IS(A)}$	0.335	0.268	0.453	0.021	0.392	0.208	0.018	0.375	0.201	0.248*
$F_{IS(S)}$	0.761	0.218	0.505	0.075	0.362	0.119	0.119	0.431	0.138	(0.151 - 0.330)
$F_{IT(A)}$	0.373	0.304	0.479	0.054	0.405	0.282	0.013	0.460	0.225	0.303*
$F_{IT(S)}$	0.867	0.257	0.521	0.092	0.378	0.176	0.143	0.525	0.180	(0.171 - 0.427)
$F_{ST(A)}$	0.058	0.050	0.048	0.034	0.023	0.093	0.030	0.136	0.030	0.288*
$F_{ST(S)}$	0.443	0.050	0.033	0.019	0.025	0.064	0.027	0.165	0.049	(0.179 - 0.371)
										0.349*
										(0.206 - 0.486)
										0.056*
										(0.036 - 0.077)
										0.097*
										(0.035 - 0.177)

F_{IS} = inbreeding in each population, F_{IT} = inbreeding in the set of populations, F_{ST} genetic divergence among populations, A= adults, S= seedlings * $p < 0.05$. Values in parenthesis are the interval confidences obtained after 10,000 bootstrap.

3.2 Effects of harvesting on genetic diversity

Among adult plants, no differences in genetic diversity were detected between populations with different harvesting intensities (Table 3). However, among seedlings, allelic richness was significantly lower in the high-harvested populations, indicating allelic losses that may be associated with heavy harvesting (Table 3). Conversely, the fixation index (F) of seedlings was higher in low-harvest populations compared to high-harvest populations.

Table 3: Indices of genetic diversity obtained in *H. drasticus* pooled according to the level of harvesting. The following parameters are presented: number of alleles per locus (A), observed heterozygosity (H_o), expected heterozygosity (H_e) and Fixation Index (F). LH= low-harvest; HH= high-harvest. Different letters refer to significant differences based on the p -values obtained after 10,000 permutations.

	LH adults	HH adults	LH seedlings	HH seedlings
A	7.801 ^a	7.004 ^a	7.964 ^a	5.790 ^b
H_o	0.444 ^a	0.454 ^a	0.425 ^a	0.452 ^a
H_e	0.600 ^a	0.600 ^a	0.651 ^a	0.567 ^a
F	0.261 ^a	0.243 ^a	0.347 ^b	0.203 ^a

The analysis of gene frequency using the two-class classification (common frequencies $\geq 5\%$ and rare frequencies $< 5\%$, Table 4, Figure 2) indicated that the allelic losses identified in the seedlings involved low-frequency alleles. Differences were not found in the number of high-frequency alleles between populations and generations. A greater number of private alleles in the seedlings of the less exploited populations was also detected (Figure 2).

Table 4: Average number of common alleles (frequencies $\geq 5\%$) and rare alleles (frequencies $< 5\%$) in *H. drasticus* populations pooled according to the level of harvesting. LH= low-harvest; HH= high-harvest. Values in parenthesis are the confidence intervals (95%).

	LH adults	HH adults	LH seedlings	HH seedlings
<i>Common</i>	3.519 (3.221 - 3.518)	3.297 (3.111 - 3.407)	3.629 (3.334 - 3.371)	3.260 (3.111 - 3.370)
<i>Rare</i>	4.925 (4.111 - 5.445)	4.036 (3.333 - 4.406)	4.631 (3.667 - 5.185)	2.778 (2.227 - 3.185)

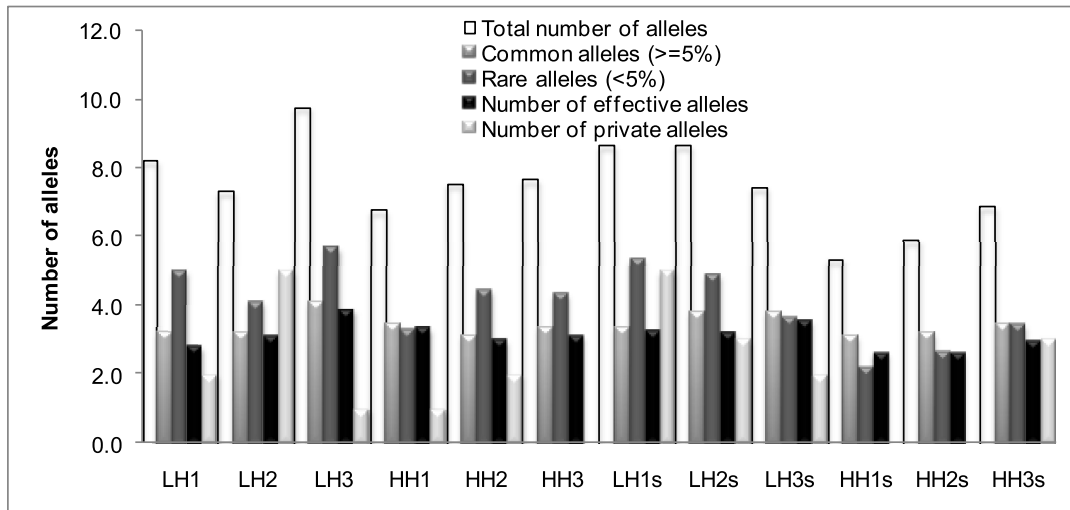


Figure 2: Allelic patterns across cohorts of *H. drasticus* under different levels of harvesting (LH= low harvest; HH= high harvest, s= seedling).

Bottleneck signatures were found in four out of six populations when the sign test was used. However, when the Wilcoxon test was employed, evidence of bottleneck was only found in two populations (Table 5). Nevertheless, the two approaches converged on the same results, with populations HH1s (high-harvest 1– seedlings) and HH2s (high-harvest 2– seedlings) presenting bottleneck signatures in both tests. The other populations that suffered bottlenecks according to the sign test were LH2 (low-harvest 2– adults) and LH3 (low-harvest 3– adults).

Table 5: Results of bottleneck detection tests on populations of *Himatanthus drasticus*

	Sign test	Wilcoxon ranks test
LH 1	–	–
LH 2	+	–
LH 3	++	–
HH 1	–	–
HH 2	–	–
HH 3	–	–
LH 1s	–	–
LH 2s	–	–
LH 3s	–	–
HH 1s	++	++
HH 2s	++	++
HH 3s	–	–

p-Value for bottleneck detection: – $P > 0.05$, + $P < 0.05$, ++ $P < 0.01$. (LH= low harvest; HH= high harvest, s= seedling)

4. Discussion

4.1 Genetic diversity and structure

The present study detected high levels of genetic diversity in the nine SSR loci that were evaluated. The detected levels of genetic diversity were similar to the values reported for other Cerrado species studied using SSR markers. An average of 7.2 alleles was estimated for the populations in the present study. Moura et al. (2009) assessed the genetic diversity of two species from the genus *Solanum* and found allelic averages of 4.6 and 6.2 for *S. lycocarpum* and *S. crinitum*, respectively. Zucchi et al. (2003) reported an average number of 10.4 alleles per locus, with a range from 3 to 22 alleles. Compatible values of alleles per locus were also observed for *Caryocar brasiliense* (Collevatti et al. 2001) and *Hymenaea stigonocarpa* (Moraes et al. 2007). Regarding observed heterozygosity, this study found an average value of 0.447, whereas Tarazi (2009) found a value of 0.342 for *Dipterix alata*. Higher H_e values were found in *Copaifera langsdorfii* ($H_o = 0.720$) (Tarazi et al., 2009) and *Caryocar brasiliense* ($H_o = 0.856$) (Collevatti et al., 2001). Again, the results obtained from *H. drasticus* ($H_o = 0.447$) are within the range found in species of the Cerrado biome.

It was observed high and significant levels of inbreeding in the population average (F_{IS}). The high rate of inbreeding is most likely due to self-pollination and/or mating among relatives. Significant levels of genetic divergence were also observed in *H. drasticus*. In the adult group, an $F_{ST} = 0.056$ was found, whereas in the seedlings group a higher value was registered ($F_{ST} = 0.097$). However, no significant differences between the genetic structure of seedlings and adults were detected (Table 2). The genetic structure of the studied populations can be considered relatively weak when compared to values obtained for other Cerrado species. Gomes and Moura (2010) compiled information about the genetic structure in plant populations from the Cerrado and found values of genetic divergence ranging from 0.053 in *Solanum lycocarpum* to 0.273 in *Tibouchina papyrus*.

4.2 Effects of harvesting on genetic diversity of *H. drasticus*

Several studies have highlighted the demographic and genetic unsustainability of timber logging, even for species under reduced impact logging (Finkeldey and Ziehe, 2004; Lacerda et al., 2008; Sebbenn et al., 2008; Wernsdörfer et al., 2011; Zimmerman and

Kormos, 2012). However, the effect of NTFP harvesting on the genetic diversity of the target species is poorly understood. Until recently, it was generally assumed that exploitation of NTFP had low environmental impact, but recognition is growing that harvesting these plants can affect biological processes on several levels, including genetic diversity and structure (Ticktin, 2004; Ticktin and Shackleton, 2011).

In the present study, neither levels of heterozygosity among janaguba populations nor the number of alleles in the adult group showed any differences. Among seedlings, the number of alleles was significantly lower in high-harvest populations, denoting allelic losses associated with harvesting that are most likely due to a reduction in effective population size. A reduction of effective population size results in the decline of both the number of alleles and the heterozygosities, but allele diversity declines more rapidly due to the loss of rare alleles. These losses have only a weak influence on heterozygosity (Nei, 1987; Gonzales and Hamrick, 2005). Rapid allele loss seems to explain the situation in the studied janaguba populations, where allelic losses involved only rare alleles and did not affect heterozygosity levels. Evidence of genetic loss due to overharvesting in janaguba populations was supported by statistically significant signs of bottleneck in the seedling group of two out of three high-harvest populations.

The genetic diversity of NTFP has been widely examined (Collevatti et al. 2001; Muchugi et al., 2006; Kyndt et al., 2009; Omondi et al., 2010; Muchugi et al. 2012), but few studies have addressed questions related to harvesting or sustainable management of NTFP. Cruse-Sanders and Hamrick (2004) studied populations of American ginseng, *Panax quinquefolius* L. (Araliaceae), and observed that the average expected heterozygosity was significantly greater within protected populations than within populations in which harvesting was allowed. Padmini et al. (2001) and Shaankar et al. (2001) assessed the genetic diversity of three NTFP species harvested for fruits in a wildlife sanctuary in India and found no clear pattern genetic diversity loss related to different disturbance regimes. However, Ramesha et al. (2007) described the population structure and genetic diversity of an economically important rattan, *Calamus thwaitesii*, in the core, buffer and peripheral regions of three protected areas in the central Western Ghats, southern India. Their results indicated that in all protected areas, the core and buffer regions presented higher genetic diversity than the peripheral regions of the protected area.

Domestication can also reduce the genetic diversity of NTFP species. Otero-Arnaiz et al. (2005) assessed management effects on the levels and distribution of genetic variation in wild, managed and cultivated sympatric populations of *Polaskia chichipe*, a columnar cactus with edible fruits under artificial selection in central Mexico. They observed that when populations were pooled by management type, the highest expected heterozygosity and the lowest fixation index were observed in wild populations. However, the reduction of genetic diversity in high-harvest populations of janaguba does not seem to be connected with domestication because no signs of artificial selection were detected in past and current management systems (Baldauf and Santos, 2013).

Because timber logging reduces tree density in the reproductive population, the effects of logging on genetic structure are widely discussed in the literature (Cloutier et al., 2006; Conte et al., 2006; Sebbenn et al., 2008, Lacerda et al., 2008; Fageria and Rajora, 2013). On the other hand, there is no information available on the possible impacts of NTFP harvesting on the genetic structure of the target species.

In this study, we found a higher fixation index (F) in the seedlings of the low-harvest populations compared to the seedlings of the high-harvest populations. One possible explanation is that human disturbance, especially by trampling, in the areas subjected to high levels of harvesting has caused an increase in the mortality of seedlings. Trampling is recognized as one of the factors that may affect recruitment (Harper, 1977). Since most of the seeds of *H. drasticus* are dispersed at short distances (Baldauf et al., unpublished data), harvesters will likely step on clustered and genetically-related seedlings when exploiting latex, causing their death and, therefore, decreasing the fixation index in high-harvest populations.

Bottleneck signatures were found in four populations when the sign test was used. However, when the Wilcoxon test was employed, evidence for bottleneck was found in only two populations (Table 4). The other populations that suffered bottlenecks, according to the sign test, were LH2 (low-harvest 2– adults) and LH3 (low-harvest 3– adults). Nevertheless, the two approaches converged on two populations, with HH1s (high-harvest 1– seedlings) and HH2s (high-harvest 2– seedlings) presenting bottleneck signatures in both tests.

Usually, the detection of recent bottlenecks in natural populations is related to the process of fragmentation, which can cause large and negative effects on genetic diversity (for review see Aguilar et al. 2008), but factors that do not radically change the landscape, such as forest management, may also affect the genetic diversity and structure of plant populations (Kanashiro et al., 2002; Sork and Smouse, 2006).

In the case of NTFP, if non-reproductive parts are harvested, only indirect selection effects on the viability or the fecundity of affected individuals might be genetically significant (Namkoong et al., 1996). There is no evidence of the influence of harvesting on the viability of *H. drasticus*; however, latex tapping increases reproductive activity in the exploited trees (Baldauf et al., unpublished data). The fact that exploited trees produce more flowers than non-exploited trees can alter pollinator behavior because pollinator movement is primarily governed by foraging economy (Levin and Kerster, 1974). If the density of flowers on a tree is high, pollen vectors can visit several flowers in the same tree or in a limited area, increasing selfing or mating among closely related plants and therefore reducing gene flow in high-harvest populations.

4.3. Implications of this study for sustainable management and conservation

Very limited action has been taken to protect genetic diversity on a global scale, and important findings from conservation genetic research have not been translated into concrete conservation action (Laikre, 2010). To achieve conservation targets, geneticists worldwide need to become more actively involved in policy work and in their countries' implementation of the CBD (Laikre, 2010). Using the need for conservation planning as a context, we assessed the genetic diversity of a highly exploited species in a biodiversity hotspot, aiming toward the development of a sustainable management plan that addresses the CBD's broad targets of conservation and sustainable use. The results obtained in this study contribute to the accomplishment of strategic goals B (reduce the direct pressures on biodiversity and promote sustainable use) and C (improve the status of biodiversity by safeguarding ecosystems, species and genetic diversity) under the 2020 Aichi Biodiversity Target section (SCBD 2011).

The growing demand for *H. drasticus* in regional and national markets has increased the number of people involved in its harvesting and has caused the adoption of more

intensive management systems (Baldauf and Santos, 2013). For this reason, the establishment of a conservation plan for this species is imperative to avoid future genetic losses and to ensure the long-term viability of its populations.

H. drasticus presented high levels of genetic diversity in natural populations, indicating the possibility of conserving these populations *in situ*. However, in high-harvest populations, we verified the loss of rare alleles, which have been shown to be important for ensuring the survival of a range of biological populations by enabling the development of resistance following exposure to a variety of selective forces (Schaberg et al., 2008). Accordingly, a complementary strategy of *ex situ* conservation, such as the establishment of a living gene bank, as well as participatory breeding programmes, is recommended to avoid future genetic losses. The SSR analysis methods have improved considerably over the last years, decreasing genotyping costs (Guichoux et al, 2011). For this reason, and in order to preserve the species' genetic diversity, sampling for *ex situ* conservation should be guided by genetic monitoring.

Finally, we suggest that seed collection for *ex situ* conservation must prioritize populations with low-level harvesting because they have a greater number of rare alleles. A study on the spatial genetic structure and gene flow of *H. drasticus* revealed that coancestry values were significant in the intervals 0 to ~15 and 18-35 m. These results suggest that near-neighbour individuals are relatives (Baldauf et al., unpublished data). However, the same study revealed that, despite the fact that most of the pollen and seeds are dispersed at short distances, they could reach maximum dispersal distances of approximately 200m. Therefore, considering the levels of inbreeding and the relatedness associated with geographic distance (SGS) reported for this species (Baldauf et al., unpublished data), the seed collection must be conducted from trees spaced 150-200 m apart to increase genetic variability and avoid the overlapping of gene pools.

5. Conclusion

We obtained information on the genetic diversity and the effects of harvesting on the genetic variability of *H. drasticus* populations. We verified that *H. drasticus* populations have a high genetic diversity and a lower number of rare alleles in areas with intense harvesting.

The long-term conservation of plant genetic resources requires an interdisciplinary approach that integrates socioeconomic, ecological, and genetic information (Bawa, 1997). Other studies about the effects of latex tapping on phenology, demography, and biomass recovery in exploited populations have demonstrated the traditional management systems' potential for sustainable harvesting (Baldauf et al., unpublished data; Baldauf and Santos, *in press*; Baldauf and Santos, 2013). On the other hand, whenever more intense management systems are employed, negative impacts such as the allelic reduction observed in this study may occur. Consequently, this genetic information has been integrated with the ecological data available to form a sustainable management plan for the species, which might help to ensure its adaptive potential over time.

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CAPÍTULO 4

Genetic diversity, spatial genetic structure and realised seed and pollen dispersal of
Himatanthus drasticus (Apocynaceae) in the Brazilian savanna⁶

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Running title: SGS and gene flow in *Himatanthus drasticus*

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Abstract

Studies on gene flow can be very useful in developing conservation strategies for harvested plant species. We assessed the pollen and seed dispersal patterns, genetic diversity, inbreeding and spatial genetic structure of *Himatanthus drasticus* (Apocynaceae), a tree native to the Brazilian Savanna (Cerrado) that is heavily exploited for its medicinal latex. The study was conducted in the Araripe National Forest, Ceará State, Brazil. Within a one hectare plot, samples were collected from all adult trees, adult trees located in the immediate vicinity of the plot, and seedlings. All sampled individuals were mapped and genotyped using microsatellite markers. High levels of polymorphism and significant levels of inbreeding were found, which indicates that self-fertilisation and mating among relatives occurs in this population. Both the adults and seedlings had significant spatial genetic structure up to approximately 40 m and our results confirmed the occurrence of isolation by distance. Pollen and seeds were dispersed over short distances and immigration of pollen and seeds into the plot was estimated to be 13% and 9%, respectively. Due to the increasing exploitation of the species in the region, a complementary *ex situ* conservation strategy is recommended for *H. drasticus*. Taking into consideration the degree of inbreeding, relatedness, intrapopulation spatial genetic structure and pollen dispersal distance, we recommend collecting seeds from a large number of trees spaced at least 150 m apart to avoid collecting seeds from related individuals and to avoid an overlapping pollen pools among seed trees.

Introduction

Gene flow is a general term that describes any movement of genes between populations (Slatkin 1985). It is considered a fundamental factor in the evolutionary biology of plants that affects the dynamics of populations, communities and ecosystems (Connell and Slatyer 1977; Hardy et al. 2006). More specifically, factors such as the type of pollination, diversity of the breeding systems, seasonality of pollination at the community level, and longevity of flowers may influence gene flow patterns in tropical tree populations (Bawa 1990).

Anthropogenic pressure can also alter gene flow patterns in plant populations. Deforestation, urbanisation and intensive agriculture can affect pollination and seed dispersal and these activities have reduced, and continue to reduce, the size of natural habitats (Bennett et al. 2006; Lander et al. 2010). The fragmentation of habitats divides existing populations into smaller, isolated subpopulations which can lead to an increase in inbreeding and genetic drift, as well as a reduction in gene flow (Hall et al. 1996; Aldrich et al. 1998; Jump and Peñuelas 2006; Rosas et al. 2011). Forest logging can also affect gene flow and spatial genetic structure in a population (Kanashiro et al. 2002); logging can increase the distance between co-specifics and thereby reduce the density of reproductive trees in a population (Lacerda et al. 2008). In cases where non-timber forest products (NTFP) are exploited, even when the individual tree is not removed, the overharvesting of plant structures, such as bark and exudates, could reduce the quantity and viability of seeds (Rijkers et al. 2006; Stewart 2009), which could have a negative impact on gene flow.

Restricted gene flow frequently results in intrapopulation spatial genetic structure (SGS), which is defined as the non-random spatial distribution of genotypes (Vekemans and Hardy 2004). In plant populations, SGS depends on seed and pollen dispersal distances and as such it is primarily the result of the diaspore that has the most restricted dispersal. If neither seeds nor pollen are widely dispersed a high degree of SGS is expected because of the aggregation of parental alleles in clusters around trees from both parents (Sebbenn et al. 2011). Sebbenn et al. (2011) studying *Copaifera langdorffii*, a Neotropical species that has seeds dispersed by gravity, is insect (bee) pollinated and occurs in savanna habitats, found significant SGS in both adult and seedling cohorts. But the parentage analysis showed that the SGS was mainly determined by maternal alleles due shorter seed dispersal distances

than pollen dispersal distances. Similar results were also found for other tropical tree species, including the wind-dispersed and bee-pollinated *Myracrodruon urunduva*, which also occurs in savanna habitats (Gaino et al. 2010).

Neutral genetic markers are commonly used to ascertain gene dispersal patterns and this approach is well established in the literature (Hardy et al. 2006). The use of microsatellite markers in combination with powerful analytical tools, such as likelihood-based parentage analysis, provides a method for determining pollen donor patterns and the level of gene flow based on pollination and seed dispersal (Sork and Smouse 2006; Kalinowski et al. 2007). The results obtained from fine-scale SGS and contemporary gene flow studies are being used to reveal patterns of gene flow and support strategies for the *in situ* and *ex situ* conservation of timber and non-timber forest products (Rathmacher et al. 2010; Manoel et al. 2011; Gaino et al. 2010; Sant'Anna et al. 2013).

In the Brazilian savanna (Cerrado), the demand for and exploitation of marketable medicinal plants has caused a decline in the natural populations of several species (Felfili and Silva-Junior 1988; Zardo and Henriques 2011). In this context, studies on the gene flow of species that have been overharvested can be very helpful in order to guide future management and genetic conservation planning. *Himatanthus drasticus* (Apocynaceae), commonly known as *janaguba*, is one of the most heavily harvested tree species in the Cerrado biome. The medicinal importance of the tree's latex in cancer treatments has been recently recognised in pharmacological studies (Sousa et al. 2010; Mousinho et al. 2011; Rebouças et al. 2011). This attention has caused an increase in trade, which could have negative impacts on natural populations of the species. The traditional management system used to harvest the latex is characterized by the removal of small amounts of bark from each tree and long intervals between harvesting events. However, an increasing number of harvesters are employing different management techniques, including the removal of a large amount of bark which often causes the death of the exploited tree (Baldauf and Santos 2013).

The aim of this study was to assess the genetic diversity, SGS, and pollen and seed dispersal patterns of *H. drasticus* populations so that strategies for its sustainable management could be developed. We addressed the following three questions: *i)* Are there differences in the levels of genetic diversity and inbreeding between adults and seedlings?

ii) Is there SGS among adults and seedlings? iii) Is there seed and pollen immigration into the sampled plot?

Materials and methods

The species *Himatanthus drasticus* (Mart.) Plumel

Himatanthus drasticus (Apocynaceae) is a tree species found in the Brazilian savanna that has a mean height below 7 m and dense foliage on the terminal ends of branches. The leaves are obovate, shiny and glabrous and the plant has terminal inflorescences with sympodial growth. The insect-pollinated flowers are small, white and fragrant and they are arranged in a thyrsoid inflorescence, typical of the Apocynaceae family (Liede and Weberling 1995). The fruit are horn-shaped follicles measuring 15 to 20 cm in length and 2.5 cm wide that contain many concentric winged-seeds. The bark is rough and exudes a white latex when removed (Plumel 1991; Lorenzi and Matos 2002). The latex of *H. drasticus* mixed with water (“leite de janaguba”) is commonly used to treat several diseases, such as gastritis, anaemia, inflammation, worms and many types of cancer (Lucetti et al. 2010).

Study area and experimental design

The study area is located on a plateau situated in the Cariri region, Ceará State (Chapada do Araripe), northeastern Brazil. This area is recognised as a priority for conserving the Cerrado biome in Brazil (Cavalcanti and Joly 2002). The Cerrado of the Chapada do Araripe is a species range disjunction located at an altitude of 800 to 900 m and is characterised as an island habitat because it experiences higher levels of rainfall and lower temperature than the surrounding Caatinga biome (dry forest) (Costa et al. 2004). The study was conducted in the Araripe National Forest (07°11' S, 39°13' W, altitude of 938 m), a protected area of Chapada do Araripe, which is considered the only preserved Cerrado area in Ceará State (Oliveira and Marquis 2002). The area is covered by the “cerrado *sensu stricto*”, defined as “a vegetation dominated by 3 to 8 m-tall trees and shrubs with more than 30% crown cover but with still a fair amount of herbaceous vegetation between them” (Oliveira and Marquis 2002). The soil is a red-yellow dystrophic latosol (Cavalcanti and Lopes 1994) and the climate is tropical wet and dry, or savanna

(Aw) according to the Köppen classification system (Köppen 1948). The majority of the rainfall in this area occurs between November and May. The most species-rich families of the National Forest are Fabaceae (31), Rubiaceae (17), Asteraceae (14), Bignoniaceae (10), Myrtaceae (9), Euphorbiaceae (7), Apocynaceae and Malpighiaceae (7) and Annonaceae and Solanaceae (5) (Ribeiro-Silva et al. 2012).

In the study area there are no unexploited populations of *H. drasticus*; therefore, we selected one natural population that has been subjected to low levels of harvesting (less than 20% of adult trees were debarked) to assess the genetic diversity, inbreeding, SGS and seed and pollen dispersal patterns of the species. In order to have a sample that is consistent with the demographic and spatial patterns of *H. drasticus*, we considered Milfont's (2011) study on the population and spatial structure of the species across 12 areas. Based on this study, we selected a population that represents the most common distribution pattern of the species. In the selected population, we established a 1 ha (200 x 50 m) rectangular plot divided into 100 subplots of 10 x 10 m (Fig. 1). Despite the fact that the 1 ha plot is a sample of a much larger population, the density of adult plants and seedlings in the chosen area is suitable for the purpose of this study (183 adult plant individuals and approximately 10,325 seedlings) (Milfont 2011). Samples were collected from all of the adult trees within the plot (183 individuals) and from several trees in the area immediately surrounding the plot (26 individuals) that could be releasing seeds into the plot. The limit of the sampling around the plot was arbitrarily defined based on the assumption that the trees located closest to the plot (within approximately 10 meters) are most likely to be dispersing their seeds into the plot. The definition of "adult trees" was based on the presence of reproductive structures obtained from demographic and phenological surveys conducted in the area (Baldauf et al., unpublished data). The diameter at breast height (dbh) of the sampled adults ranged from 6.2 to 24.5 cm (mean \pm SD: 10.69 ± 2.65 cm, median of 10.5 cm). Due to the high density of seedlings in the study area, we created sub-samples using 2 x 2 m plots located within each 10 x 10 m subplot. Seedling samples were taken from throughout the 1-hectare plot; however, due to the uneven distribution of the species (Milfont 2011), some of the 2 x 2 m subplots have no seedlings while in others several seedlings were sampled. Additionally, we also sampled all seedlings within a 10 x 10 m subplot (bottom left corner, Fig. 1) aiming to detect SGS at a reduced spatial scale. The

total number of sampled seedlings was 149 individuals. All 358 sampled plants were mapped with a laser distance meter and the coordinates for each individual were obtained using MAPA software (Santos 2001).

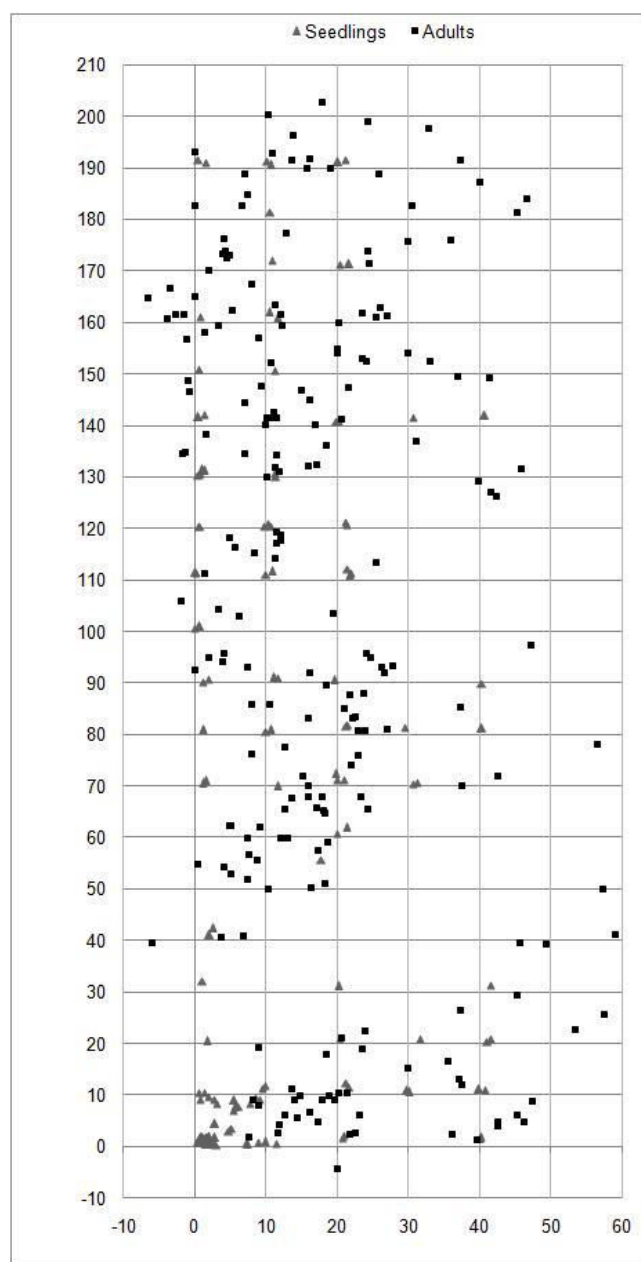


Fig 1 Map of *Himatanthus drasticus* adults and seedlings in the studied plot.

DNA extraction and microsatellite genotyping

We collected two leaves per individual and collected samples were stored at -80° C until DNA extraction. For each sample, the DNA was isolated from approximately 25 ng of fresh leaves ground with liquid nitrogen using the DNeasy kit (Qiagen, Valencia,

California, USA). DNA quantification was accomplished by comparing samples with a standard DNA (DNA λ) of known concentration in a 0.8 % agarose gel stained with ethidium bromide. Samples were then diluted to obtain a solution of 5.0 ng/mL. The microsatellite markers used in this study were previously developed by Baldauf et al. (2011) and included the following markers: HD1, HD3, HD7, HD9, HD11, HD18, HD19 and HD21. Two loci, however, had a high frequency of null alleles and were therefore excluded from SGS and parentage analysis. All loci used contain dinucleotide repeat motifs except for HD18, which is a trinucleotide. Microsatellite loci were amplified by PCR in a 15 μ L total volume containing 5.0 ng of template DNA, 0.8 mM each of forward and reverse primers, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl, and 0.15 mM of each dNTP and 1 U of Taq DNA polymerase. PCR was performed using a PTC-100 thermal cycler (MJ Research, Waltham, Massachusetts, USA) with the following sequence: 96° C for 1 min, followed by 30 cycles of denaturation at 94° C for 1 min, 1 min at a specific annealing temperature (T_a), 1 min at 72° C and a final extension of 72° C for 5 min. Amplified PCR products were verified by electrophoresis on 3% agarose gels containing 0.1 mg ethidium bromide per mL in 1 x TBE buffer (89 mM Tris-borate, 2 mM EDTA, pH 8.0). Fluorescent amplicons were genotyped on 6% denaturing polyacrylamide gels in an ABI 377 automated sequencer (Applied Biosystems, Foster City, CA) using the GS500 TAMRA marker as the standard size (Applied Biosystems). Fragment size and allele identification were determined using Genescan v. 3.1.2 and Genotyper v. 2.5.2 software (Applied Biosystems) and then exported to Excel.

Analysis of genetic diversity and fixation index

The genotypic disequilibrium test was carried out for adults and seedlings using the FSTAT program (Goudet 1995). In order to avoid false positives, we used a Bonferroni correction for 95% probability ($\alpha = 0.05$). The genetic diversity of adults, seedlings and the total sample (adults + seedlings) was estimated by the number of alleles (A), observed heterozygosity (H_o) and expected heterozygosity at Hardy-Weinberg equilibrium (H_e) for each locus and for the average across all loci. Because the sample size for the adults and seedlings was different, we also calculated the allelic richness (R) using a rarefaction method (El Mousadik and Petit 1996). The levels of inbreeding for the adult and seedling

populations were estimated using the fixation index (F). In seedlings, the intra-individual fixation index was calculated using reference allele frequencies from the adult trees with the SPAGEDI 1.3 program (Hardy and Vekemans 2002). The significance of the F values was calculated using the permutations of the alleles among individuals (1,000 repetitions) and a sequential Bonferroni correction for multiple comparisons (95%, $\alpha = 0.05$). Except for the intra-individual fixation indexes in seedlings, all other analyses were also run using the FSTAT program, version 2.9.3.2 (Goudet 1995). To investigate if the average values of A , R , H_o , H_e and F were significantly different between the adult trees and seedlings, a paired t -test (Sokal and Rohlf 1995) was used.

Analysis of the intrapopulation spatial genetic structure

The SGS was determined using an estimation of the average coancestry coefficient (θ_{xy}) between pairs of adult trees, pairs of seedlings and pairs from the total sample (adults + seedlings). These analyses were conducted using the SPAGEDI 1.3 program (Hardy and Vekemans 2002). For the analysis of the seedlings, we used the reference allele frequencies calculated for the adult cohort (Sebbenn et al. 2011). For the analysis of the adult trees and the total sample, we assumed that the reference population had the same allele frequencies found in the current adult generation. To visualise the SGS, θ_{xy} values were averaged over a set of ten 10 m distance classes (0-10 m, 10-20 m, 20-30 m, 30-40 m, 40-50 m, 50-60 m, 60-70 m, 70-80 m, 80-90 m and 90-100 m) and then plotted against the distances. The number of pairs of individuals averaged within these distance classes ranged from 858 to 1972 for adult trees and from 432 to 1320 for seedlings. To test whether there was significant deviation from a random structure, the 95% CI was calculated for each observed value and each distance class from 1,000 permutations of individuals within the distance class. To compare the SGS between the adults and seedlings, the statistic Sp (Vekemans and Hardy 2004) was calculated as $Sp = -b_k / (1 - \theta_1)$, where θ_1 is the average coancestry coefficient calculated between all pairwise individuals within the first distance class (0-10 m), and b_k is the slope of the regression of coancestry coefficient on the logarithm of spatial distance (0-100 m). To test for SGS, the spatial position of each individual was

permuted (1,000 times) to obtain the frequency distribution of b_k under the null hypothesis that θ_l and $\ln(d_{xy})$ are not correlated.

Analysis of the historical gene dispersal from SGS

The historical gene dispersal for the adults, seedlings and the total sample (adults + seedlings) was estimated from the SGS with the assumption that the observed SGS was in isolation-by-distance equilibrium (Hardy et al. 2006). The historical gene dispersal in terms of neighbourhood size (Nb) was estimated as $Nb = -(1 - \theta_l) / b_k$ (Vekemans and Hardy 2004), where b_k is the regression slope within the distance class of $\sigma_g < d_{ij} < 20\sigma_g$. This estimation of Nb is dependent on the assumed value for effective density, D_e (Hardy et al. 2006). Thus, D_e was estimated as: $D_e = D(N_e / N)$, where the effective density is the ratio of the effective population size to the census population size (Vekemans and Hardy, 2004). Based on other plant studies (Hardy et al. 2006), we used $D/10$ and $D/2$ as a minimum and maximum estimate of D_e , respectively. With a fixed D_e , the lower and upper boundaries for the 95% confidence interval (CI) of Nb were estimated as $Nb_{(lower)} = (\theta_l - 1) / (b_k - 2SE_b)$ and $Nb_{(upper)} = (\theta_l - 1) / (b_k + 2SE_b)$, where SE_b is the standard error of b_k calculated by jackknifing data over each loci (Hardy et al. 2006). The 95% CI of σ_g was estimated as $\sigma_g = \sqrt{Nb / 4\pi D_e}$ using the lower and upper Nb boundaries (Hardy et al. 2006). When $b_k < SE_b$, the upper boundary was reported as infinite, ∞ (Hardy et al. 2006).

Parentage analysis

The combined non-exclusion probability of the parent pair and combined non-exclusion probability of genetic identity were calculated using the CERVUS 3.0 program (Marshall et al. 1998; Kalinowski et al. 2007). Parentage analysis was conducted by maximum-likelihood maternity and paternity assignment (Meagher 1986) based on the multilocus genotypes of the 149 seedlings and all 209 adult trees from within and outside the plot. The most likely parents and parent pairs were determined by the Δ statistic (Marshall et al. 1998) using the reference allele frequencies calculated for the adult population (Meagher

and Thompson 1987). For this analysis, we considered all 209 adult trees to be parent candidates when assigning the mother and father of the seedling. Significance for Δ was determined using maternity and paternity tests simulated by the software (critical Δ), which used a confidence level of 80%, genotyping error ratio of 0.01, mean proportion of typed individuals of 0.93, and 10,000 repetitions. The calculation of critical Δ values was based on the assumption that 80% of the candidates sampled were located within the plot. If a mother or father candidate or parent pair had a Δ value higher than the critical Δ value calculated by simulations, it was considered to be the true parent or true parent pair. If the same individual was found to be both the maternal and paternal parent, the seedling was considered selfed. Thus, the estimate of the realised selfing rate (s) was calculated as the number of selfed seedlings (n_{selfed}) divided by the total number of seedlings:

$s = n_{selfed} / n_{total_seeds}$. If a single parent was identified, it was assumed to be the maternal parent. If two parents were identified inside the plot, we used two approaches to determine parentage: a) the near-neighbour parent was assumed to be the seed parent; and b) the more distant parent was assumed to be the seed parent. These assumptions were used to determine the likely seed and pollen donor because seeds of *H. drasticus* are dispersed by wind and pollen is most likely dispersed by hawkmoths (Sphingidae). The cryptic gene flow, or the probability of assigning a candidate mother or father inside the population when the true father is outside of the population, was calculated as described in Dow and Ashley (1996). The seed and pollen immigration rate (m) was calculated as the proportion of seedlings that had either no parents ($n_{immigrant (seed)}$) or only one parent ($n_{immigrant (pollen)}$) inside the population area relative to the total number of sampled seedlings

$m = n_{immigrant} / n_{total}$ (Burczyk et al. 1996). As all sampled individuals had a known spatial position, the realised seed dispersal distance was calculated based on the position of the seedlings relative to their likely mothers and the distance of pollen dispersal was based on the position of the putative mothers in relation to fathers.

Results

Genetic diversity and fixation index

After Bonferroni correction, the results showed significant evidence of genotypic disequilibrium between pairwise loci HD3xHD9 and HD11xHD18 in the adults and HD9xHD11, HD9xHD18 and HD19xHD21 in both adults and seedlings (Table 1). This genotypic disequilibrium can be the result of self-pollination, correlated mating, mating among relatives, genetic bottleneck effect, founder effect, forest logging and genetic linkage between pairs of loci. Two loci, however, had a high frequency of null alleles and were therefore excluded from subsequent analyses. No significant differences in genetic parameters were observed between adults and seedlings (Table 2).

Table 1 Results of the genotypic linkage disequilibrium for samples of adults and seedlings (Bonferroni correction for multiple tests: $\alpha = 0.05 = 0.001667$).

Pairwise loci	Adults	Seedlings
HD3XHD9	0.00167	0.00500
HD3XHD11	0.16333	0.10667
HD3XHD18	0.00500	0.03833
HD3XHD19	0.16667	0.01333
HD3XHD21	0.21833	0.42500
HD9XHD11	0.00167	0.00167
HD9XHD18	0.00167	0.00167
HD9XHD19	0.12500	0.24000
HD9XHD21	0.75667	0.12500
HD11XHD18	0.00167	0.01333
HD11XHD19	0.15000	0.00667
HD11XHD21	0.78667	0.25167
HD18XHD19	0.01833	0.30833
HD18XHD21	0.02833	0.67500
HD19XHD21	0.00167	0.00167

Intrapopulation SGS

For the adult population, the θ_{xy} values were significantly higher than the upper limit of the 95% confidence interval in the 0 to 30 m distance class, whereas for seedlings

the θ_{xy} values were significantly higher in the 0 to ~40 m distance classes. When considering the total sample (adults + seedlings), the coancestry values were significant in the intervals of 0 to ~15 and 18-35 m. These results suggest that near-neighbour individuals are related (Fig. 2a, b, c). The regression slope (b_k) of the pairwise coancestry coefficient on the logarithm of spatial distance (0-100 m) was significantly negative for adults, seedlings and the total sample (Table 3), indicating the occurrence of isolation by distance.

The average coancestry coefficient ($\theta_{xy} = 0.081$) for the total sample (Fig. 2c) was significantly higher (Table 3) than that estimated for adults ($\theta_{xy} = 0.012$, Fig. 2a) and seedlings ($\theta_{xy} = 0.028$, Fig. 2b) and decreased up to the forth distance class (< 40 m). For adults, the estimates for Nb and σ_g did not converge for either of the tested effective densities ($D_e = D/10$ and $D_e = D/2$). For the seedlings, assuming $D_e = D/10$, the estimates for Nb and σ_g did not converge; however, assuming $D_e = D/2$, the historical gene dispersal distance produced a σ_g of 49.8 m and an Nb of 232 individuals. For the total sample, the historical gene dispersal distance produced a minimum σ_g of 17.8 m (assuming $D_e = D/10$) and a maximum of 51.1 (assuming $D_e = D/2$) and Nb of 90 individuals, ranging from 72 ($D_e = D/2$) to 118 ($D_e = D/10$).

Parentage analysis

The combined non-exclusion probability over six loci of the parent pair was low ($P_{Pair} = 0.0000252$; Table 2) and resulted in a low probability of cryptic pollen and seed flow of $0.005 [1 - (1 - 0.0000252)^{209}]$. The combined non-exclusion probability of genetic identity was also low ($P_{identity} = 0.00000035$; Table 2), indicating that all of the adult multilocus genotypes are unique.

A parent pair among all sampled adults, including the extra adults sampled, was assigned to 130 seedlings (87%, Table 4) and resulted in an apparent pollen immigration of 13% ($m_{pollen} = 0.13$). Additionally, eight seedlings were assigned to a single parent from all sampled adults (both within and outside the plot). Assuming that these assigned parents are the mother trees, the seed immigration was estimated to be 9% ($m_{seeds} = 0.09$). For twelve of the seedlings more than one parent pair from all sampled adults (both within and outside

the plot) was identified and these seedlings were excluded from the calculations of seed and pollen dispersal distance. Consequently, the estimate of pollen dispersal distance was based on 118 assigned seedlings and the estimate of seed dispersal distance was based on 126 assigned seedlings. Eleven seedlings were mothered and fathered by the same individual, suggesting a realised selfing rate of 11%.

The pollen dispersal distance ranged from 2.1 to 194 m, with a mean of 65 m and median of 49 m (Table 4, Fig. 3a), and approximately 81% of the pollen travelled less than 100 m (Fig. 3a). The distance between the parents explains 81% of the assigned matings ($R^2 = 0.81$, $df = 18$, $p < 0.01$) and suggests that isolation occurs as a result of distance.

When the near-neighbour parent was assumed to be the seed parent (case a), our estimates for the minimum seed dispersal distance within the plot ranged from 2.1 to 188 m, with a mean of 63 m and median of 53 m (Table 4; Fig. 3b). In this case, approximately 81% of the seedlings were found within 100 m of the seed tree (Fig. 3b). A significant association was found between the number of seedlings within a distance class ($R^2 = 0.53$, $df = 19$, $p < 0.01$) and the distance between the mother tree and seedling, which suggests short-distance seed dispersal. When the more distant parent was assumed to be the seed parent (case b), our estimates for the maximum seed dispersal distance within the plot ranged from 16 to 202 m, with a mean of 86 m and median of 82 m (Table 4; Fig. 3b). In this case, approximately 48% of the seedlings were dispersed within 100 m of the seed tree (Fig. 3b). A non-significant association was found between the number of seedlings within a distance class ($R^2 = 0.07$, $df = 19$, $p > 0.05$) and the distance between the mother tree and seedling.

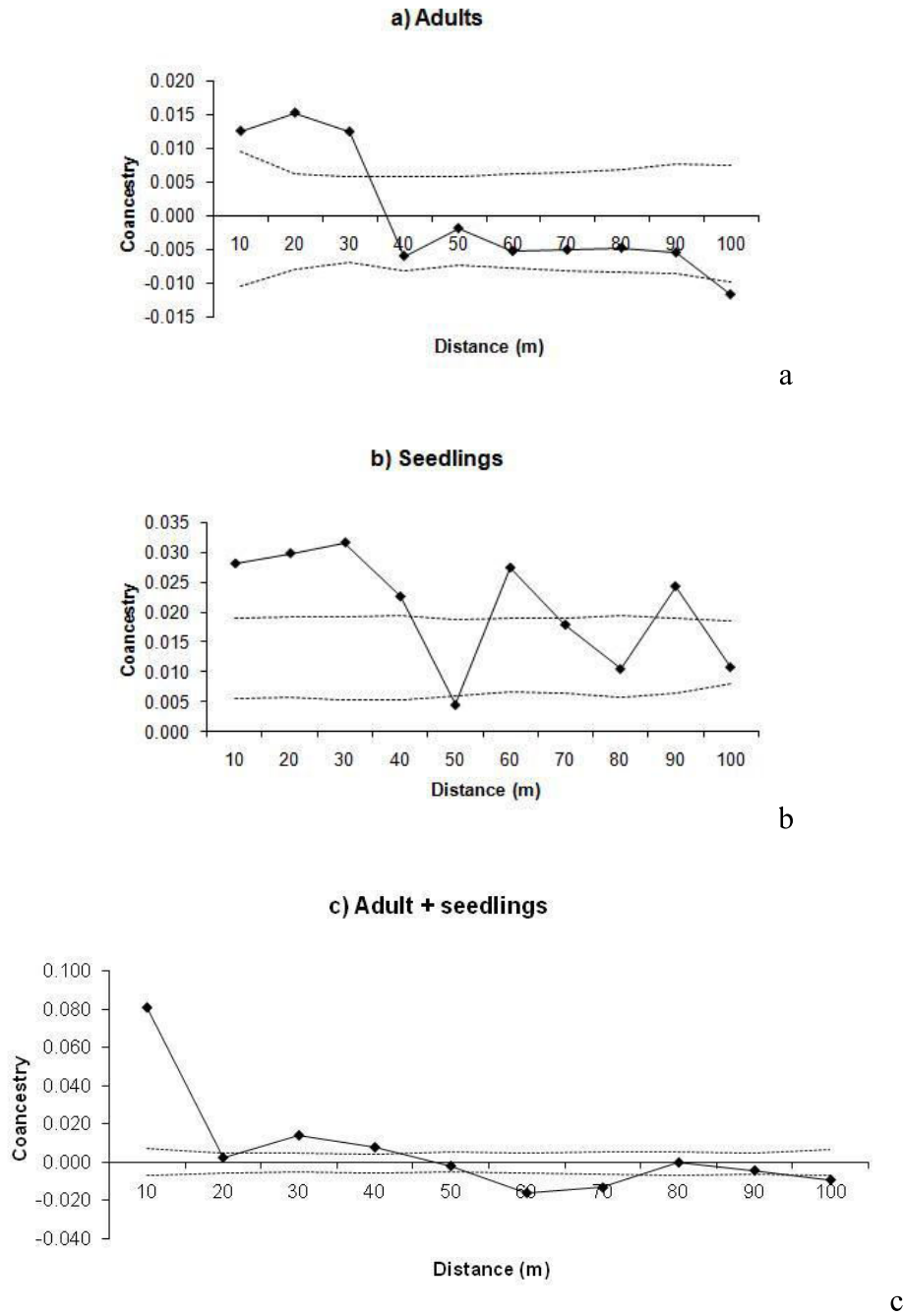


Fig 2 Correlograms of average coancestry coefficients (θ_{xy}) for adults (a), seedlings (b), and adults + seedlings (c) for 10 distance classes with intervals of 10 m for adults and seedlings of *Himatanthus drasticus*. The solid line represents the average θ_{xy} value. The dashed lines represent the 95% (two-tailed) confidence interval of the average θ_{xy} distribution calculated by 1.000 permutations of spatial distance between pairs of adults and seedlings.

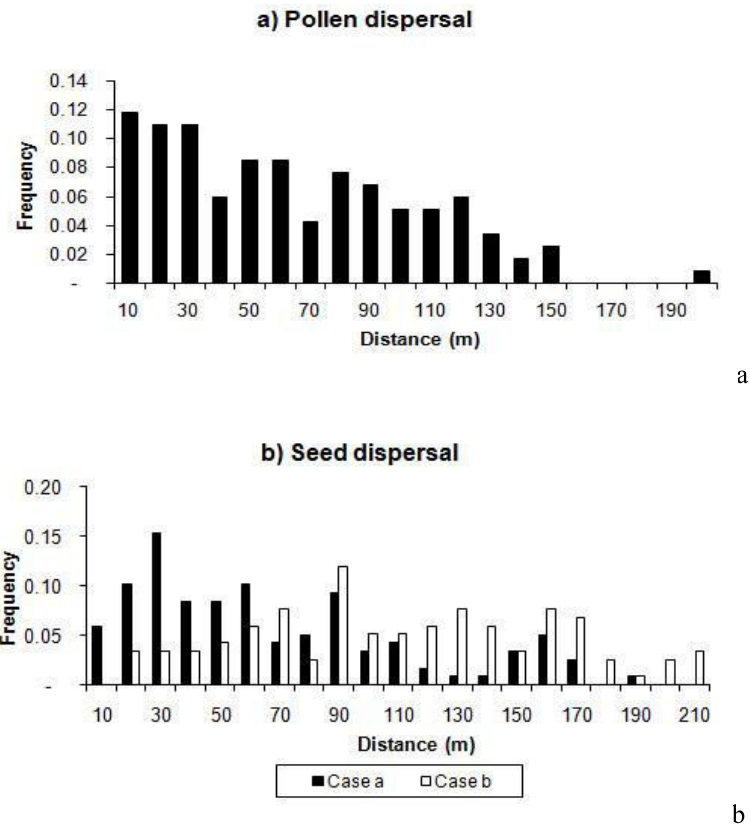


Fig 3 Frequency distributions of (a) realised (black bars) pollen dispersal distances and (b) seed dispersal distances

Table 2 Genetic diversity and fixation indexes of adult trees and seedlings in a population of *Himatanthus drasticus*.

Locus	Adults (N=209)							Seedlings (N=149)				
	A	R_{136}	H_o	H_e	F	P_{Pair}	$P_{identity}$	A	R_{136}	H_o	H_e	F^1
HD3	15	14.6	0.634	0.882	0.281*	0.089	0.026	12	12.0	0.683	0.858	0.245*
HD9	7	7	0.426	0.661	0.356*	0.346	0.147	8	7.9	0.514	0.552	0.392*
HD11	26	24.1	0.554	0.876	0.368*	0.095	0.029	16	16.0	0.662	0.870	0.213*
HD18	12	11.8	0.537	0.676	0.205*	0.368	0.154	10	10.0	0.570	0.526	0.341*
HD19	10	9.2	0.287	0.491	0.416*	0.513	0.284	12	12.0	0.338	0.524	-0.026
HD21	14	13.7	0.487	0.790	0.384*	0.208	0.073	17	16.9	0.471	0.779	0.095
Mean	14.0	13.4	0.487	0.729	0.332*	--	--	12.5	12.5	0.540	0.685	0.222*
SD	6.54	5.96	0.120	0.150	0.078	--	--	3.45	3.47	0.128	0.168	0.179
Total	84	--	--	--	--	0.00012	3.5^{-7}	75	--	--	--	--

A is the number of alleles; R_{136} is the allelic richness; H_o is the observed heterozygosity; H_e is the expected heterozygosity; F is the fixation index; P_{Pair} is the combined non-excluding probability of the parent pair; and $P_{identity}$ is the combined non-excluding probability of identity. SD is the standard deviation. * $P < 0.05$. 1: Intra-individual fixation index calculated with reference allele frequencies from the adult trees using the SPAGED1 program. Using paired t-test, the mean values of all parameters are not significant different between adults and seedlings (A : $P = 0.633$; $df = 10$; R_{136} : $P = 0.749$, $df = 10$; H_o : $P = 0.484$, $df = 10$; H_e : $P = 0.639$, $df = 10$; F : $P = 0.113$, $df = 10$).

Table 3 Estimates of intrapopulation spatial genetic structure parameters of *Himatanthus drasticus* for adults, seedlings and adults + seedlings.

Sample	θ_1	θ_1 95% Confidence interval	b_k (0-100)	Sp (SE)	Nb (SE)	$\sigma_g \pm SE$ (m) ($D_e = D/2$)	$\sigma_g \pm SE$ (m) ($D_e = D/10$)
Adults	0.012**	0.008 to 0.016	-0.0067*	0.0068 (0.0067)	NC	NC	NC
Seedlings	0.028**	0.020 to 0.036	-0.0043*	0.0044 (0.0043)	232 (120- 1977)	NC	49.8 (25 to 103)
Adults + seedlings	0.081**	0.064 to 0.098	-0.0448*	0.0448 (0.0089)	90 (72-118)	17.8 (16 to 19)	51.1 (46 to 56)

θ_1 is the coancestry coefficient in the first distance class (0-10 m); b_k is the regression slope of the coancestry coefficient value on the logarithm of spatial distance between individuals located within the distance between 0 to 100 m; Sp is the statistic that measures the extent of the spatial genetic structure in the first distance class; Nb is the neighbourhood size; σ_g is the historical gene dispersal distance for two estimated effective densities ($D_e = D/2$ and $D_e = D/10$); SE is the standard error; NC= not converged; ** $P < 0.01$, * $P < 0.05$.

Table 4 Realised pollen and seed dispersal in a *Himatanthus drasticus* population.

Sample	Dispersal (absolute number)				Dispersal distance without selfing (m)			
	N	Within	Outside	s (%)	$N_{effective}$	Mean	Median	Min/Max
Pollen	149	87% (130)	13% (19)	0.07 (11)	118	65 ± 41	49	2.1/194
Seeds – case a	149	91% (138)	9% (11)	--	126	63 ± 46	53	2.1/188
Seeds – case b	149	91% (138)	9% (11)	--	126	86 ± 53	82	16/202

Min/Max is the minimum and maximum dispersal distance, respectively. N is the sample size, and s is the selfing rate.

$N_{effective}$ is the effective sample size used for the analysis. Case a: the near-neighbour parent was considered the seed parent; Case b: the more distant parent was considered the seed parent.

Discussion

Genetic diversity and inbreeding

The estimated genetic diversity indexes averaged across all loci are comparable to the values reported for other Cerrado tree species studied using SSR markers. We found a mean of 13.9 alleles per locus in *H. drasticus* while for *Eugenia dysenterica*, Zucchi et al. (2003) reported an average of 10.4 alleles per locus, with a range from three to 22 alleles. Similar values of alleles per locus were also observed for *Caryocar brasiliense* (Collevatti et al. 2001) and *Hymenaea stigonocarpa* (Moraes and Sebbenn 2010). The highest reported average is for *Copaifera langsdorffii* with a genetic diversity index of 16 alleles per locus (Tarazi et al. 2013). In all of these studies, the reported number of alleles was considerably lower than the number of effective alleles because of the large number of low frequency alleles, which is typical of SSR markers. Furthermore, in our study eight alleles were found exclusively among seedlings, which suggests the occurrence of gene flow through pollen and/or seed immigration and the studied population receives genes from a larger area than that sampled. Among the reported values for heterozygosity in Cerrado species, the lowest value was found for *Dipterix alata* ($H_o = 0.342$) and the highest value was found for *Copaifera langsdorffii* ($H_o = 0.720$) (Tarazi et al. 2013). Again, the results obtained for *H. drasticus* ($H_o = 0.447$) were within the range typical of Cerrado species.

Despite the high level of polymorphism found for *H. drasticus*, a significant amount of inbreeding exists in both adult and seedling groups, indicating that self-fertilisation and/or mating among relatives has occurred. An alternative explanation for the substantial inbreeding is the Wahlund effect due the significant SGS found in the plot for both adults and seedlings (Fig. 2a, b). These samples are in fact a mixture of various cohorts and therefore the higher fixation index might be a reflection of cohort mixing, rather than an actual increased level of inbreeding.

Spatial genetic structure

The degree of the spatial genetic structure (SGS) depends not only on seed and pollen dispersal, but also on genetic drift. These factors are influenced by local demographic structures. The SGS can also be influenced by historical events, such as the mixing of different gene pools (Born et al. 2008). For the *H. drasticus* populations, the two

sampled groups displayed significant SGS, which confirms the isolation by distance pattern commonly reported for tree species (see Vekemans and Hardy 2004, for a review). The factors that regulate population processes vary according to the ontogenetic stage or size-class (Augspurger and Kelly 1984; Fonseca et al. 2004). Furthermore, post-dispersal events may increase SGS as the individuals move through ontogenetic stages. There are no known limitations on the dispersal and germination of *H. drasticus* (Amaro et al. 2006); however, the occurrence of compensatory mortality, which is associated with the existence of favourable sites for plant development and the presence of herbivores or plant disease, can affect spatial distribution patterns (Hutchings 1997). Therefore, high rates of seedling mortality can be a possible explanation for the reduced coancestry found in the adults when compared with the seedlings.

The correlograms for each group presented variations in SGS patterns. The average coancestry coefficient for the adult trees dramatically decreased up to the third distance class (< 30 m, Fig. 2a) and the decline in SGS was non-significant in all other distance classes. The seedlings had a significant SGS up to 40 m (Fig. 2b), followed by two successive decreases and increases in the coancestry coefficient until the final distance class (> 100 m). The average coancestry coefficient ($\theta_{xy} = 0.081$) for the total sample (adults + seedlings, Fig. 2c) was significantly higher (Table 4) than that estimated for adults ($\theta_{xy} = 0.012$, Fig. 2a) and seedlings ($\theta_{xy} = 0.028$, Fig. 2b) and it also decreased dramatically up to the forth distance class (< 40 m), indicating that there are many related individuals among near neighbours. Furthermore, the coancestry coefficient estimated in the first distance classes for seedlings was approximately two times higher and significantly different than the coancestry estimated for the adults (Table 3). The more intensive SGS and the higher coancestry coefficient estimated in the first distance classes for the total sample (adults + seedlings) reflect the large number of near neighbour individuals that are pairwise sibs. In the case of seedlings, the coancestry coefficient estimated in the first distance classes probably reflects the large number of pairwise sibs; however, this value is expected to decrease as the plants develop due to deterministic factors, such as natural selection, and stochastic factors, such as random mortality, predation, disease, and pest attacks, as discussed above.

Except for one case, the historical gene dispersal distance and neighbourhood size did not converge for either of the simulated effective densities in the adult and seedling populations. The estimates for these parameters may not converge for several reasons: low marker polymorphism, inadequate spatial scale of the sampling effort, and weak SGS because of efficient gene dispersal (Vekemans and Hardy 2004; Hardy et al. 2006). Low marker polymorphism seems unlikely because high levels of polymorphism were detected in the studied loci. An inappropriate spatial scale of the sampling could be the cause of the non-convergence, especially because of the differences in the densities of the sampled groups. The existence of weak SGS due to efficient gene dispersal appears to be more probable because even though significant SGS was found for both groups, the observed values of coancestry and Sp are considered to be very low when compared with other tree species, including species with wind-dispersed seeds (Vekemans and Hardy 2004; Jones and Hubbell 2006; Pandey and Rajora 2012).

The plant breeding system and life-history have been recognised as factors that significantly influence patterns of SGS (Vekemans and Hardy 2004). In the Brazilian Cerrado, however, there is evidence that the seed dispersal syndrome is a decisive factor in determining the degree of SGS and it is even more influential than pollination or the breeding system (Collevatti et al. 2010). Collevatti et al. (2010) compared the SGS patterns for three Cerrado species and observed that species with wind-dispersed seeds had weaker SGS, with coancestry values close to zero for all of the analysed distance classes. Our results of low but significant SGS found for *H. drasticus* support the observations described by Collevatti et al. (2010) for Cerrado tree species.

Finally, the density of *H. drasticus* in the study area was very high. In the sampled plot, the adult density is 183 individuals/ha and the seedling density was estimated to be 10,325 individuals/ha (Baldauf et al., unpublished data). According to Vekemans and Hardy (2004), the Sp statistic is expected to be lower in high-density populations and our results support this assumption. Thus, density is likely a major determining factor in the SGS patterns of *H. drasticus* populations.

Realized pollen dispersal

Flowers of the Apocynaceae family are pollinated by either butterflies or moths (Schlindwein et al. 2004). The *Himatanthus* genus has developed flowers that are adapted for hawkmoth pollination (Schlindwein et al. 2004) but no specific information about *H. drasticus* pollinators is currently available. Nevertheless, it is highly probable that this species is pollinated by hawkmoths of the genus *Isognathus* (Sphingidea), the only species whose larvae feed on the leaves (Baldauf pers. obs.). The same specificity between *Isognathus* and a sphingophyllous *Himatathus* (*H. obovatus*) was observed by Diniz et al. (1999) in the Brazilian Cerrado. Sphingid species that feed on Apocynaceae leaves would benefit from their poisonous leaves which protect the larvae from predators (Schlindwein et al. 2004). Sphingidae are known to have the capability of long-distance flight (Amorim et al. 2009). However, the movement of pollinators is primarily determined by foraging economy (Levin and Kerster 1974). In our study, we found a high density of reproductive *H. drasticus* trees in the plot, which means that pollen vectors can visit several flowers in a small area, thus limiting the pollen dispersal distance. Therefore, pollinator behaviour may explain why the majority of the assigned pollen (81%) travelled less than 100 m. Pollen vector behaviour and tree density of *H. drasticus* also provide a likely explanation for the low levels of pollen immigration ($m_{pollen} = 0.13$). A negative correlation between the tree density and pollen dispersal distance in *Symphonia globulifera* in French Guiana was also observed by Degen et al. (2004) in the French Guiana.

Realized seed dispersal

In most cases, pollen flow is considered to be more extensive than seed flow (Petit et al. 2005). In this study we observed the same pattern, although the difference is minimal (the apparent pollen immigration was 13%, and the seed immigration was estimated at 9%). The values observed for seed immigration were lower than the values reported for other species with wind-dispersed seeds (Jones et al. 2005; Bacles et al. 2006; Iwaizumi et al. 2009), with the exception of *Cercidiphyllum japonicum*, a tree found in temperate forests that has a reported seed immigration of 7% (Terakawa et al. 2009).

When we assumed the near-neighbour parent as the seed parent (case a), we identified a significant isolation by distance (IBD) (Wright 1943). However, when we

assumed case b (the more distant parent as the seed parent), the pattern of IBD was not found. The two cases used in our study represent extreme conditions. The most probable situation in the studied population is that neighbouring individuals are more likely to share alleles than distant individuals but mating between distant individuals may occur. Notably, in both of the simulated situations, the maximum dispersal distance was similar, which indicates that majority of *H. drasticus* seeds are dispersed over short-distances (188 m for case “a” and 202 m for case “b”).

The height of the seed tree is fundamental in promoting long-distance dispersal. Seeds released from lower branches are generally dispersed over shorter distances (Soons et al. 2004). This situation most likely occurs in *H. drasticus* trees because a large number of the reproductive individuals are less than 4 m in height. Furthermore, the Cerrado *sensu stricto* has more than 30% crown cover, which can constrain seed dispersal of shorter trees.

Implications for harvesting and conservation genetics

The importance of genetic diversity in the maintenance of biological diversity and evolutionary processes is well established, especially considering the predictions of climate change (Frankel and Soulé 1981; Lande 1988; Frankham 2004; Laikre et al. 2009; Konnert et al. 2011). Conservation strategies based on genetic analysis, however, are still limited for tropical trees (Assogbadjo et al. 2006; Laikre 2010). The species studied herein appears to have a high level of genetic diversity, which suggests that conservation *in situ* is a suitable strategy for *H. drasticus* genetic conservation. We also observed, however, a moderate spatial genetic structure (SGS) as a result of restricted pollen and seed dispersal. Plant populations with SGS are more likely to suffer from a loss of alleles because aggregated populations in a small area are likely to be eliminated by human activity or natural phenomena (Tarazi et al. 2010). In this study, we found no difference in the number of alleles in the sampled groups which suggests that the current levels of gene flow are sufficient to avoid the potential effects of genetic drift. In this context, short-distance pollen and seed dispersal may be a natural aspect in the life-history of *H. drasticus*.

Both trade of the species and the number of people involved in harvesting the species are increasing, which can threaten its natural populations (Baldauf and Santos 2013). Accordingly, there is a need to discuss management alternatives with all

stakeholders, such as the establishment of agroforestry systems and other stands specifically designed for exploiting the species. In this context, a complementary *ex situ* conservation strategy, such as establishing a living gene bank, as well as initiatives for participatory breeding programmes, must be considered. Because of the levels of inbreeding, the relatedness among adults associated with non-random mating, the spatial genetic structure (SGS) and short-distance pollen dispersal found in this study, we recommend collecting seeds from a large number of individuals to increase the genetic diversity and variance effective size of the sampled progeny for *ex situ* conservation and tree breeding. Furthermore, seeds should be collected from trees spaced at least 150 m apart to avoid collecting seeds from related individuals and also to decrease the probability of overlap in the pollen pools.

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CAPÍTULO 5

Harvesting Increases Reproductive Activity in *Himatanthus drasticus* (Mart.) Plumel

(Apocynaceae), a Non-timber Forest Product of the Brazilian Savanna⁷

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ABSTRACT

The harvesting of non-timber forest products (NTFP) can influence the pattern of resource allocation in plants, affecting their growth, survival and reproduction. However, only a small number of studies have addressed the impacts of NTFP harvesting on the reproductive phenology of the exploited species. The aim of this observational study was to assess the effects of harvesting and climatic variables on the reproductive phenology of *Himatanthus drasticus*, a highly exploited medicinal tree from the Brazilian Savanna (Cerrado). We evaluated the effects of two different debarking treatments (50% and 100%) in comparison to a control (no harvesting). We performed monthly counts of the number of buds, flowers in anthesis, unripe and ripe fruits in each sampled tree (intensity index), as well as the number of trees in each phenophase (activity index), over two years. We used circular statistics tools to compare the effects of each treatment on flower and fruit production and to test whether the reproductive peaks were related to climatic variables. Both the activity and intensity indexes exhibited the same patterns; flower and fruit production were lower in the control, intermediate in the 50 percent debarking treatment group and higher in the 100 percent debarking treatment group. To the best of our knowledge, this is the first study of NTFP to demonstrate an increase in reproductive activity after harvesting. All phenophases were positively correlated with higher temperature and precipitation, as it is common in the Cerrado.

Keywords: circular statistics; Cerrado; phenology; janaguba; medicinal plants; sustainable management; Chapada do Araripe

PHENOLOGICAL STUDIES ADDRESS THE TIMING OF RECURRING BIOLOGICAL EVENTS (LIETH 1974). For plants, this involves reproductive events such as bud formation and flowering, fruiting, and seed germination, along with vegetative processes such as shoot growth (Morellato *et al.* 2010). Phenological processes are fundamental traits of species and ecosystem dynamics and are likely to affect biodiversity at all levels (from genes to ecosystems) and the interactions between species (van Vliet 2008). In plants, the timing of reproductive phenology and reproductive events is determined by both biotic and abiotic factors and their interactions (Ramírez & Briceño 2012).

In savannas, the peaks of flowering and fruiting are seasonal and related to climatic variables (Silva *et al.* 2011). The phenological events that occur in Neotropical savannas are considered adaptive strategies to maximize reproductive success in seasonal environments (Monasterio & Sarmiento 1976; Oliveira 1998). In the Brazilian savanna (Cerrado), a biome with well-defined rainy and dry seasons, rainfall plays an important role in the seasonal events of plants (Gouveia & Felfili 1998; Oliveira 1998).

Despite the well-recognized influence of climatic factors on plant reproductive phenophases, other factors may affect the patterns of flowering and fruiting, such as solute concentration in the soil (Mantovani & Martins 1988, Williams *et al.* 1997), soil type (Cardoso *et al.* 2011, Ramirez & Briceño 2012), exposure to fire (Coutinho 1979), life-form (Golluscio *et al.* 2005, Ramirez & Briceño 2012), phylogenetic history (Silva *et al.* 2011), water stress (Idso *et al.* 1978, Aronson *et al.* 1992, Maes *et al.* 2009, Brachi *et al.* 2012), light availability (Killkenny & Galloway 2008; Galloway and Burgess 2012), and interactions with pollinators (Brody 1997, Aizen 2003), seed predators (Brody 1997, Collin and Shykokff 2010) and herbivores (Osada & Sugiura 2006, Kawagoe & Kudoh 2010). In addition to these ecological factors, anthropogenic pressures such as land degradation (Bisigato *et al.* 2013) and harvesting can also affect the phenological patterns and reproductive success (Peres *et al.* 2003, Ticktin 2004). Brites and Morsello (2012) reviewed the literature on non-timber forest products harvesting and stated that 67% of the studies that evaluated specific impacts on the reproductive rates of harvested species reported negative effects, whereas the remaining reported no significant effects.

Many fruits and seeds are currently harvested by felling the tree, which has led to the serious depletion of several important fruit and oil seed-producing species (Peters

1996). The harvesting of flowers or fruits may not directly affect the health or reproductive capacity of an individual plant, but if large numbers of flowers or fruits are harvested, regeneration might cease. Depending on the generation time of the species, the species and its products may be rapidly lost from the forest (Hall & Bawa 1993).

In the case of other plant parts, such as bark and exudates, harvesting might affect the pattern of resource allocation due to the expected trade-off between the regeneration of the exploited resource and growth and reproduction (Peters 1996). In addition, bark extraction could alter the conduction of the nutrients and hormones involved in the production of flower buds (Primack 1987), decreasing the number of flowers and fruits produced and altering the seasonality of the reproductive phenophases.

In some species that are harvested for bark and exudates, reductions in the number of fruits or viability of seeds are observed in overharvested trees compared with unharvested trees (Rijkers *et al.* 2006; Gaoue & Ticktin 2008; Stewart 2009), whereas in other species harvesting kept populations in a vegetative state (Cunningham & Mbenkum 1993; Cocks & Dold 2004). In contrast, some studies verified that the exploitation did not alter significantly the flower or fruit production of the harvested individuals (Bitariho *et al.* 2006; Mariot 2008; Schumann *et al.* 2010). However, the effects of bark and exudates harvesting on reproductive phenology have not been widely studied yet, thus, a general pattern of such effects cannot be described.

The aim of this study is to assess the influences of harvesting and climatic variables on the reproductive phenology of *Himatanthus drasticus* (Mart.) Plumel, a tree endemic to the Brazilian Savanna that is harvested for its medicinal latex. To exploit this latex, large strips of bark are removed, which might affect the phenology of the species, especially with regard to the seasonality and intensity of the reproductive phenophases. We specifically tested the following hypotheses: (1) intense harvesting will decrease the production of flowers and fruits in *H. drasticus*; and (2) the reproductive phenophases of the species will be positively correlated with climatic variables (temperature and rainfall), as is expected in the species of the Cerrado biome.

METHODS

SPECIES AND STUDY AREA—*Himatanthus drasticus* (Mart.) Plumel (Apocynaceae) is a widespread and endemic tree species of Brazil (Koch & Rapini 2012). It is found in the Brazilian savanna and has a maximum height of less than 7 m; its young branches are dark brown with light brown spots, and its older branches are pale brown (Spina 2004). It has dense foliage at the top of its branches. Its leaves are obovate, shiny and glabrous, and its flowers are small, white and fragrant. Its fruits are horn-shaped follicles measuring between 15 and 20 cm long and 2.5 cm wide containing many concentric winged seeds. The bark is rough and exudes white latex when removed (Plumel 1991, Lorenzi & Matos 2002, Spina 2004).

A mixture of the latex of *H. drasticus* and water (“leite de janaguba”) is popularly used to treat several diseases, such as gastritis, anemia, inflammation, worms, and many types of cancer (Fernandes *et al.* 2000, Lucetti *et al.* 2008). The preliminary results of pharmacological studies of the leaves, bark and latex of this species have validated its popular use as an analgesic, antitumor and immunostimulant (Lucetti *et al.* 2008, Colares *et al.* 2008, Souza *et al.* 2010, Mousinho *et al.* 2011).

The study area is situated in the Cariri region, Ceará State, northeastern Brazil. This region encompasses a plateau called Chapada do Araripe, recognized as a priority area for the conservation of the Cerrado biome in Brazil (Cavalcanti & Joly 2002). The cerrado of the Chapada do Araripe is a disjunction located at an altitude of 800 to 900 m, characterized as a habitat island because of its higher rainfall and lower temperature relative to the surrounding Caatinga biome (Seasonally Dry Tropical Forest) (Costa 2004).

The survey was carried out in the main protected area of the Chapada do Araripe, the Araripe National Forest (07°11'S, 39°13'W, 938 m height), which is considered the only preserved Cerrado area in Ceará State. The study area is located in the municipality of Crato. The area is covered by the “cerradão” (savanna woodland), an ecosystem of the Cerrado biome that has been described as “*an almost closed woodland with crown cover of 50 percent to 90 percent, made up of eight–12-m-tall trees casting considerable shade so that the ground layer is much reduced*” (Oliveira & Marquis 2002). The most common plant families in the Araripe National Forest are the Fabaceae, Myrtaceae, Poaceae,

Apocynaceae, Euphorbiaceae, and Malpighiaceae (Costa *et al.* 2004). The soil is classified as red-yellow *dystrophic latosoil* (Cavalcanti & Lopes 1994), and the climate is tropical wet and dry or Savanna climate (Aw), according to the Köppen classification system (Köppen 1948). The rainfall is concentrated between November and May.

Since the 1970s, the harvesting of janaguba latex has become a source of income for many communities in the Araripe Plateau, northeastern Brazil. This practice led the now defunct Brazilian Institute for Forestry Development (IBDF) to prohibit the harvesting of janaguba to avoid overharvesting of the species, which resulted in the establishment of an illegal market (Bezerra 2004). In 1989, the office of the IBAMA (Brazilian Institute for Environment and Natural Renewable Resources) in the Araripe region decided to legalize the harvesting of janaguba milk in the FLONA Araripe, where the species occurs in high densities; the managers of the FLONA developed a system to register harvesters and a participatory mapping that defines harvesting areas for each family (Baldauf & Santos 2013).

DATA COLLECTION.— The study has been conducted across an area managed mainly by a single family of harvesters for more than 20 years. There is no particular season for harvest and the intervals between exploitations in the same individual are of about two years. This particular area was selected because of its high number of non-exploited trees, a hard to find condition in the region; therefore, the study cannot be spatially replicated. These harvesters believe that it is important to keep a number of non-exploited trees to ensure continued extraction, even though some members of this family feel deceived, because members of other families eventually harvest latex in the same area and this “theft” usually includes the non-exploited individuals (D. Pessoa, pers.comm.).

With the support of a local expert (an ex-harvester, member of this family), we identified the trees that were already being harvested with different strategies (observational study). We selected individuals that had been harvested at least three years before the beginning of the phenological evaluation. This measure was taken because our objective was to assess the medium-term effects of harvesting; increases in reproductive activity are common shortly after damage or stress (Romero 2006). The selection was done with the assistance of the local expert, who had harvested janaguba for more than 30 years and has a considerable knowledge of the regeneration process in the target species. In most

individuals, a significant structural modification of the inner bark, indicating tissue regeneration, occurs only in the second year of regeneration, and by the end of the third year after harvesting only the inner bark is recovered (Baldauf & Santos, in press a), consequently, the signs of debarking can be identified in the trees.

The bark removed was mainly the “outer bark” (rhytidome), therefore, the living phloem was probably not damaged. There were no changes in survival as a result of harvesting in the study area (Baldauf & Santos, in press a), nor was there increase in mortality or reduction in the individual growth rates of heavily exploited trees (C. Baldauf, unpubl. data). The details of the regeneration process of the inner bark are still unclear, but it apparently occurs through growth in all directions from the thin layer of inner bark that is left by the harvesters when collecting the latex (Baldauf & Santos, in press a). An important variation in the management systems is the percentage of bark removed from each tree, which harvesters call the “number of sides” exploited. Exploiting one side refers to the removal of approximately 25% of the bark; two sides, 50% of the bark; three sides, 75% of the bark; and four sides, 100% of the bark (Baldauf & Santos 2013).

To evaluate the reproductive phenology, we considered two different debarking levels (level 1 - approximately 50 percent debarking; level 2 - approximately 100 percent debarking). Vertical cuts of approximately 2 meters have been made in the selected trees in the past, using a machete or a scythe. These levels of harvesting and practices were chosen to represent the most common management systems employed in the region (Baldauf & Santos 2013). A third group of individuals that did not suffer harvesting was evaluated as a control. The diameter at breast height (DBH) of the sampled trees ranged from 8.4 to 27.6 cm. All individuals were reproductive. Because the tree diameter may affect its reproductive output (Hall & Bawa 1993), we included reproductive individuals of all range of diameters in each group. We also selected plants under similar light intensity for inclusion in each group (level 3 of the crown illumination index (CII)) (Clark & Clark 1992).

Despite the fact that in the “cerradão” ecosystem the number of exploitable trees is relatively high (from 28.5 to 33.3 individuals per hectare) (IBAMA 2004); a contiguous area of about 30 hectares was sampled to ensure an appropriate number of trees and a random distribution of harvesting levels and control individuals. We selected 55 individuals

of *H. drasticus* in each treatment and 55 non-harvested individuals as controls, and evaluated the following reproductive phenophases: buds, anthesis, and unripe and ripe fruits. Monthly evaluations were performed to count the number of flowers and fruits in each phenophase with the assistance of binoculars and stairs. All flowers and fruits in each sampled tree were counted, without subsampling. The assessment of reproductive phenology began in April 2009 and ended in March 2011, totaling 24 months of observation. We obtained the climate information (accumulated monthly precipitation and monthly mean temperature) from the INMET (National Meteorological Institute of Brazil) for the municipality of Barbalha, which neighbors the municipality of Crato, Ceará State.

DATA ANALYSIS—We used circular statistics because it is the recommended tool for formally testing hypotheses in plant phenology and characterizing peaks in reproductive events for one of a few cycles (Morellato *et al.* 2010). We converted months to angles, from 0° (corresponding to January) to 330° (corresponding to December). For each angle, we considered the number of trees with buds, flowers in anthesis, unripe and ripe fruits to identify and compare the peaks of activity and the total number of flowers and fruits in each phenophase as a measure of intensity (Bencke & Morellato 2002). After an exploratory analysis of climatic and harvesting data, we opted for pooling the information about the two sampled years, as we have not found significant differences between years.

We calculated the mean angle (μ), the circular statistical deviation (CSD) and the mean vector (r) for each phenophase as described by Zar (2010). The μ and the CSD have similar interpretation in non-circular statistic, whereas r is a dispersion measurement around the mean (Morellato *et al.* 2010). We tested the uniformity of each treatment using the Kuiper test. The null hypothesis is that data are uniformly distributed throughout the year, *i.e.*, there is no seasonality. The von Mises distribution is equivalent to the normal distribution of linear data (Morellato *et al.* 2010) and is a proxy for the phenophase seasonality. To test each treatment for the von Mises distribution, we used the Watson test (U^2) (Jammalamadaka & SenGupta 2001).

Because all distributions were non-normal, we employed the non-parametric Rao tests for homogeneity (Rao) to analyze the vector direction and dispersion of the same phenophase among the three treatments (Jammalamadaka & SenGupta 2001). To test for differences in flower and fruit production between two treatments, we used the Watson's

Two-Sample test of homogeneity for non-normal data (W), which is equivalent to the Mann-Whitney test of linear data. (Jammalamadaka & SenGupta, 2001).

We carried out circular-circular type regression analyses (Jammalamadaka & SenGupta, 2001) between climatic variables and all phenophases in each treatment and control. Because the distributions were non-normal, we randomized the values of the climatic variables and the phenophases in each treatment 1,000 times. We extracted the rho value (*“square root of the average of the squares of the estimated conditional concentration parameters of y given x”*, Agostinelli & Lund, 2011) for each randomization and used it to represent significance in two-tailed regression tests. All analyses were performed using the “CircStats” (Jammalamadaka & SenGupta, 2001) and “circular” (Agostinelli & Lund, 2011) packages for R.

RESULTS

The data were not uniform (Kuiper test; $p < 0.05$; Table 1) nor normally distributed throughout the year (Watson test; $p < 0.05$; Table 1). All reproductive phenophases were concentrated in few months, regardless of treatment (Fig. 1). The presence of buds, flowers in anthesis and ripe fruits were observed mainly in December, in the beginning of the wet season, whereas unripe fruits were observed earlier (September-October). Buds, anthesis, and ripe fruit phenophases showed higher and similar values for the mean vector lengths (r), indicating less variance within groups. Conversely, the lowest values for this parameter were found in the unripe phenophase, suggesting a higher dispersion within groups. Consequently, two different mean angles were observed in this phenophase (Table 1).

Considering the number of trees, significant differences in vector direction were found in all but the bud phenophase (Table 2). When data dispersion was tested, we found differences in bud and anthesis, suggesting that both phenophases can last longer in at least one of the sampled groups. The comparison between treatments revealed differences in the number of trees in each phenophase (Table 2, Fig 1). The number of individuals that produced buds, flowers, unripe and ripe fruits was higher in the group with 100% of debarking than in the group with 50% of debarking and in control plants.

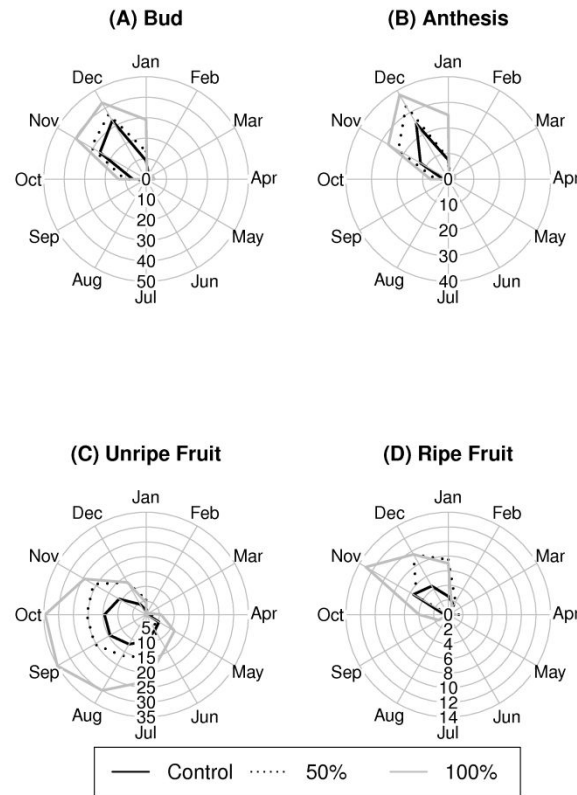


FIGURE 1. The number of *H. drasticus* trees in each phenophase during the study. The graphs summarize data from two years of observations.

A small number of individuals produced a large quantity of fruits, while the majority of individuals exhibited low production or did not produce any reproductive structures during the peak months of each phenophase. The trees produced fruits annually; however, we noticed that, in most cases, the same trees fruited in both sampled years. We found differences among harvesting levels in the number of trees that did not produce fruit. This number was higher in the control group than in the groups with 50% and 100% debarking for unripe ($\chi^2 = 10.75$; $gl = 2$; $p < 0.001$) and ripe fruits ($\chi^2 = 10.67$; $gl = 2$; $p < 0.001$).

The results considering the total numbers of flowers and fruits (intensity) were similar to those previously described for the number of individuals expressing the phenophase (activity). The vector direction and dispersion were different among treatments (Table 3, Fig 2). Trees with 100% of bark removal produced more buds, flowers, unripe and ripe fruits than the trees with 50% or no bark removal (Table 3, Fig 2).

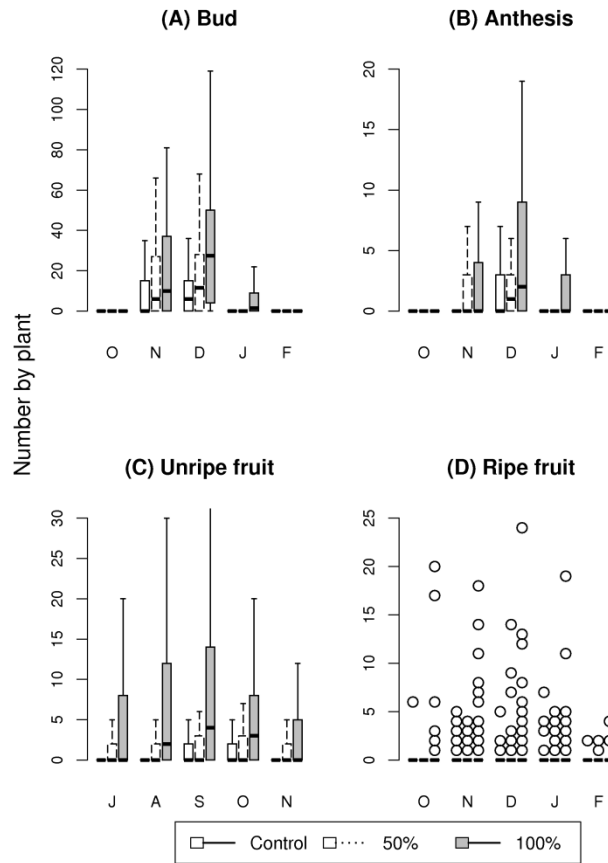


FIGURE 2. The numbers of buds (A), flowers in anthesis, (B), unripe fruits (C) and ripe fruits (D) produced by each *H. drasticus* tree during the peak months of reproductive activity. The top bar indicates the maximum observed value, and the black bar corresponds to the median value. Outliers were excluded. The number of ripe fruits produced was too low and most of the plants did not fruit. Therefore, we showed the outliers only for the ripe fruit phenophase.

Clear patterns of both activity and intensity in the different harvesting levels and the control group emerged: flower and fruit production was lower in the control group, increasing in the 50-percent debarking group, and significantly higher in the 100-percent debarking group.

With regard to climate variables, all phenophases were positively correlated with precipitation and temperature. However, the harvesting level did not alter this pattern, indicating a low influence of bark removal on tree seasonality (Table 4).

TABLE 1. Mean angle (μ), circular standard deviation (CSD), approximate month corresponding to the mean angle), mean vector length (r), Kuiper test (uniformity and Watson test for the number of trees in each treatment (Activity Index). For Kuiper and Watson tests, all values were significant at 0.05.

	Control	50%	100%
Buds			
$\mu \pm \text{CSD}$	319.99 \pm 27.08	319.94 \pm 35.22	325.36 \pm 39.41
Mean month	December	December	December
R	0.89	0.82	0.79
Kuiper test	9.02	9.64	11.22
Watson test	1.59	1.41	1.56
Anthesis			
$\mu \pm \text{CSD}$	325.60 \pm 24.06	320.26 \pm 28.23	329.18 \pm 35.84
Mean month	December	December	December
R	0.91	0.88	0.82
Kuiper test	7.69	8.75	10.23
Watson test	1.34	1.57	1.42
Unripe fruits			
$\mu \pm \text{CSD}$	236.24 \pm 66.88	252.53 \pm 68.21	236.84 \pm 66.69
Mean month	September	October	September
R	0.50	0.49	0.50
Kuiper test	5.36	6.71	8.26
Watson test	0.30	0.46	0.70
Ripe fruits			
$\mu \pm \text{CSD}$	330.24 \pm 42.67	340.41 \pm 37.78	318.75 \pm 40.01
Mean month	December	December	December
R	0.75	0.80	0.78
Kuiper test	3.76	5.02	5.69
Watson test	0.21	0.36	0.42

TABLE 2. *Rao's tests for homogeneity for vector direction and data dispersion; Watson's tests for pairwise comparisons of the number of trees in each phenophase between treatments.*

	Bud	Anthesis	Unripe fruit	Ripe fruit
Rao (vector)	5.42 *	10.99	19.27	17.47
Rao (dispersion)	60.04	38.80	0.43 *	1.07 *
Degrees of freedom	2.00	2.00	2.00	2.00
Watson's test				
Control vs. 50%	1.13	0.95	0.39	0.34
Control vs. 100%	1.37	1.18	0.25	0.16
50 vs. 100%	1.25	1.53	0.25	0.25

* non-significant at 0.05 level

TABLE 3. *Rao's tests for homogeneity for vector direction and data dispersion; Watson's tests for the pairwise comparisons of the numbers of flowers and fruits produced in each treatment and the control. All values are significant at 0.05 level.*

	Bud	Anthesis	Unripe fruit	Ripe fruit
Rao (vector)	544.83	11.41	693.42	86.55
Rao (dispersion)	1236.69	63.43	7.56	58.12
d.f.	2.00	2.00	2.00	2.00
Watson test				
Control vs. 50%	35.35	5.82	3.33	1.24
Control vs. 100%	44.87	6.55	1.87	0.59
50 vs 100%	76.75	11.50	3.25	2.42

TABLE 4. *The regression parameters rho (equivalent to the r^2 in linear regression) and kappa (maximum likelihood estimate of concentration parameter) for the relationships between phenophases and climatic variables (temperature and precipitation). All values are significant at 0.05.*

		Temperature		Precipitation	
	Treatment	rho	kappa	Rho	kappa
Bud	Control	0.94	9.30	0.92	6.95
	50%	0.89	5.17	0.89	4.61
	100%	0.87	4.23	0.86	3.94
Flower	Control	0.96	13.41	0.94	8.06
	50%	0.92	7.10	0.93	7.35
	100%	0.90	5.25	0.88	4.45
Unripe fruit	Control	0.66	1.66	0.63	1.55
	50%	0.68	1.70	0.71	1.96
	100%	0.65	1.63	0.66	1.71
Ripe fruit	Control	0.81	3.01	0.78	2.58
	50%	0.87	4.18	0.82	2.94
	100%	0.88	4.62	0.86	3.91

DISCUSSION

HARVESTING EFFECTS.— These results contradicted the hypothesis that extraction would decrease the reproductive potential of exploited individuals (Perez *et al.* 2003, Ticktin 2004). In fact, some studies have tested this hypothesis, and their results indicated negative effects or no effects of harvesting on the reproductive phenology of the exploited species. Mariot (2008), studying the effect of debarking on the reproductive phenology of the species *Drimys brasiliensis*, concluded that the harvesting did not significantly alter the

flower or fruit production of the individuals studied. In the same way, Schumann *et al.* (2010) verified that debarking had no effects on the fruit production of the multipurpose baobab tree (*Adansonia digitata*).

Stewart *et al.* (2009) reported that fruit production in the species *Prunus africana* was reduced by debarking. The same phenomenon was found in heavily harvested populations of *Khaya senegalensis* (Gaoue & Ticktin 2008). Another possible consequence of harvesting is the maintenance of populations in a vegetative state (Cunningham & Mbenkum 1993), which was confirmed by Cocks and Dold (2004) for the African species *Cassipourea flanaganii*. In a study of exudate harvesting in *Boswellia papyrifera*, Rijkers *et al.* (2006) showed that resin tapping impacted the formation of flower buds and seed viability of the species.

However, because the number of papers using this approach is very small, especially in the case of exudates, it is difficult to generalize the responses to bark/exudate harvesting in different species. Moreover, the impact of harvesting depends considerably on the intensity of management applied, as well as the life history of each plant species (Ticktin 2004), so the responses tend to be species specific.

In the case of *H. drasticus*, it would be reasonable to assume that harvesting would have some impact on the reproductive system, especially because the joint harvesting of latex and bark could have a greater impact than the exploitation of only one product. Therefore, the results obtained in this study are surprising; to the best of our knowledge, this is the first NTFPs to report an increase in reproductive activity as a result of medium-term harvesting.

The practice of damaging the bark of trees is commonly used in horticulture to stimulate fruit production. This practice is useful, because the damage to the phloem prevents photosynthates from being translocated from sources in leaves to the parts located below the damage, at least until wound healing (Romero 2006). Thus, bark harvesting may have the indirect effect of increasing the amount of photosynthates available for the formation of fruits and meristems in the regions above the damage, which is why many species produce more flowers and fruits in response to wounding (Romero 2006). This could be one explanation for the pattern found in *H. drasticus* populations. However, this phenomenon is a physiological response during wound healing. In this study, in contrast,

we sampled individuals that were debarked at least three years prior to the experiment, which is approximately the time needed to recover from the wound (Baldauf & Santos, in press a). Therefore, other factors may be involved in the relationship between management intensity and the production of reproductive structures.

These results may also indicate that the effects of harvesting have a long-term influence on the exploited individuals. Furthermore, reproductive success might be limited by traits that were not considered here, such as the number of seeds per fruit, seed viability or germination rate. In a study of the effects of bark damage caused by herbivores in *Salix myrsinifolia-phylicifolia*, Elmqvist *et al.* (1987) observed a significant reduction in the average seed number per individual and a decrease in the germination ability of damaged plants. Similar studies on herbivory suggested that increases in flower numbers are not necessarily followed by increases in seed numbers; thus, compensation for herbivore damage is rarely complete (Hendrix 1979, Hendrix 1984, Isla & Crawley 1984). Similarly, the increases in flower and fruit numbers observed after harvesting in *H. drasticus* do not necessarily guarantee an increased reproductive output; we have not considered seed viability or germination rates, topics that deserve further investigation.

CLIMATIC FACTORS.—All phenophases were positively correlated with climatic variables, as it is common in savanna species (Oliveira 1998, Felfili *et al.* 1998, Batalha & Mantovani 2000, Oliveira & Gibbs 2000, Batalha & Martins 2004, Silva *et al.* 2011). Moreover, all other parameters related to the distribution of phenophases over the year (Tables 1, 2, and 3) clearly demonstrated the seasonality of the flower and fruit production, regardless the harvest intensity.

The peaks of flowering (bud and anthesis) and ripe fruit stages occurred in the late dry and early wet season, respectively. Batalha and Martins (2004) studied the reproductive phenological patterns of a Cerrado plant community in central Brazil and confirmed a striking seasonality in the community-wide pattern of flowering and fruiting. These authors observed that woody species flowered mainly during late dry and early wet seasons (Batalha & Martins 2004). Flowering during the dry season is advantageous, because there are no heavy rains that could damage the flowers, and leaf abscission facilitates the visualization of flowers by pollinators (Janzen 1967).

Fruiting is also influenced by rainfall. In general, the fruits of wind- and self-dispersed species ripen during the dry season, when their diaspores can be dispersed more efficiently. On the other hand, the diaspores of animal-dispersed species are dispersed during the rainy season, when water is more abundant and fruits remain fleshy for a longer time (Mantovani & Martins 1988, Batalha *et al.* 1997, Batalha & Mantovani 2000; Batalha & Martins 2004). However, we found a different pattern for *H. drasticus*, in which the diaspores are dispersed during the rainy season (December-April). This may occur because the moisture provided by rain and the temporary abundance of nutrients from the decomposition of litter during the dry season allow seedlings to develop a deep root system before the next drought, increasing their chances of survival.

We observed that the reproductive activity in *H. drasticus* is strongly influenced by climatic seasonality, as expected for a savanna species. Nevertheless, the results concerning the effects of harvesting are surprising; although we expected to observe reductions in the reproductive activity, our results demonstrated the opposite. However, an increase in reproductive activity does not necessarily indicate greater reproductive success. In fact, exactly the opposite might be happening in *H. drasticus* due to pollinator behavior. Because the movement of pollinators is primarily determined by foraging economy (Levin & Kerster, 1974), the existence of trees with a higher than average number of flowers would reduce the chances of long-distance pollen dispersal. This hypothesis was confirmed by genetic studies of species in which allele loss was detected in extensively harvested populations as a result of increased short-distance pollination (Baldauf *et al.*, in press b). Moreover, the physiological processes that might be causing increased flower and fruit production are not completely clear and a long-term monitoring would be desirable to confirm whether this surprising pattern remains the same over time.

It is important to highlight the fact that the administration of the Araripe National Forest implemented a closed season during which latex extraction is prohibited (two months) to ensure the sustainability of harvesting (IBAMA 2004). This annual ban aims to avoid exploitation during the species' reproductive period. Since in the literature there is no information available about the species flowering and fruiting, the administration of this protected area consulted with the harvesters to define the appropriate period for the closed season and, based on their knowledge, suggested the months of November and December.

This rule was implemented approximately five years ago and may be contributing to the reproductive success and consequently the persistence of the *H. drasticus* populations over time. However, our data is not suitable to evaluate the success of the closed season for harvesting.

A limitation of our study is that the frequency of harvesting (the intervals between exploitation) in the region varies; thus, different regimes could produce results other than those we found. Moreover, the species occurs in another ecosystem of the Cerrado biome, called *cerrado stricto sensu*, where bark regeneration is faster than in the *cerradão* (Baldauf & Santos, in press a). This difference in bark recovery rates could influence the resources allocation pattern and, consequently, the reproductive phenology of exploited plants. Therefore, further studies considering different intervals between exploitation and ecosystems are recommended in order to draw a broader picture of the effects of harvesting on the reproductive phenology of *H. drasticus*.

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CAPÍTULO 6

The effect of management systems and ecosystem types on bark regeneration in *Himatanthus drasticus* (Apocynaceae): recommendations for sustainable harvesting⁸

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Abstract

Bark and exudates are widely commercialized non-timber forest products. However, the ecological impacts of the harvesting of these products have seldom been studied. The aim of this study is to investigate the relationship of tree resilience to harvesting intensity in *Himatanthus drasticus*, a tree that is highly exploited in the Brazilian savanna (Cerrado) for its medicinal latex. Although the traded product is the latex, the traditional harvesting systems involve the removal of the bark of the trees to allow exploitation. A three-year experiment was conducted in two different Cerrado ecosystems (open savanna and savanna woodland). Trees were debarked at four debarking intensities to simulate the effects of traditional management systems. Measurements of bark growth were taken every six months, and quantitative and qualitative indexes of bark regeneration were obtained. The mortality of the debarked trees was low and could not be related to the intensity of harvesting. No signs of attack by fungi or insects were recorded. Compared with other species exploited for bark, *H. drasticus* is very resilient to harvesting, however, bark regeneration is relatively slow. In both analyzed ecosystems, the regeneration indexes showed that three years are not sufficient for total recovery of the rhytidome. Bark regeneration occurred primarily by sheet growth and was more rapid in open savanna than in savanna woodland. No differences in the rate of bark recovery were found among management treatments. Based on the results, sustainable harvesting guidelines are suggested for the species.

Keywords: non-timber forest products, bark stripping, Cerrado, Brazilian savanna, medicinal plants

1. Introduction

Humans have gathered useful plant species from diverse ecosystems from the earliest times (Schipmann et al. 2006; Laird et al. 2010). Among the potential uses of plants, those related to medicine play a central role because they are essential to human survival (Toledo et al. 2009). Approximately 80% of the global population depends on medicinal plants (MPs) for healthcare (WHO 2001). In developing countries, especially in rural areas, the use of MPs is particularly relevant due to the difficulty of access to modern medicine (Rani and Kullar 2004; Haq 2011), the prohibitive cost of pharmaceutical products (Shanley and Luz 2003; Malviya et al. 2012) or as a result of cultural preferences (Cocks and Dold 2006; Cocks et al. 2011; Suleman and Alemu 2012).

Currently, phytotherapy is experiencing a revival in the developed countries (Efferth and Greten 2012). One of the reasons for this popularity is that natural products are preferred to products of industrial origin (Di Stasi 1996; Efferth and Greten 2012). The growing demand for herbal products has led to a significant increase in the volume of plant materials traded within and between countries (Pandey et al. 2010; Freitas et al. 2012). Most MPs products are derived from natural populations; relatively few species are cultivated (Leaman 2004; Geldenhuys and Mitchel 2006). For this reason, there is an increasing concern about the sustainable management of MPs because many species are sensitive to high levels of harvest or to ecosystem changes (Diederichs et al. 2006; Pandey et al. 2010).

Despite all the valuable efforts to establish ecological and socioeconomic criteria for the certification of non-timber forest products (Shanley et al. 2002) or specific guidelines for desirable field collection practices for MPs (WHO 1993; WHO 2002; WHO 2003; Kathe 2006), a lack of knowledge about sustainable harvest rates and practices remains one of the major challenges to the development of sustainable wild collection (Schipmann et al. 2006; Ticktin and Shackleton 2011). Therefore, there is an urgent need to obtain ecological data on species that are currently under exploitation, primarily MPs for which the market demand is high.

In the Brazilian savanna (Cerrado), the demand for marketable MPs is producing a decrease in their natural populations (Felfili and Silva Junior, 1988; Borges-Filho and Felfili, 2003; Zardo and Henriques, 2011). *Himatanthus drasticus* (Apocynaceae),

commonly known as *janaguba*, is one of the most commonly harvested tree species in the Cerrado biome. The medicinal value of its latex for the treatment of cancer was recently recognized based on pharmacological studies (Souza et al., 2010; Mousinho et al., 2011), and this finding has caused the trade in the latex to increase, with potential negative impacts on the natural populations of the species. Although the traded product is the latex, the traditional harvesting systems involve the removal of the bark of the trees to allow exploitation, and ringbarking of the tree is common.

In species harvested for bark, the definition of a maximum sustainable harvesting limit for the bark is necessary to ensure the persistence of individuals and populations (Delvaux et al. 2010). Overall, it is important to consider the response of bark regeneration not only under several intensities of debarking but also in different environments. Few studies, however, have investigated how different environments or ecosystems affect harvest impacts in non-timber forest products (Gaoue and Ticktin 2007).

To evaluate the impacts of current harvesting activities on natural populations of *H. drasticus* as well as to suggest limits for the sustainable harvesting of the species, we addressed the following specific questions:

- a. Does harvesting cause an increase in mortality in *H. drasticus*?
- b. What are the effects of different debarking intensities on the time needed for bark regrowth?
- c. Do the bark regrowth rates depend on the type of ecosystem?
- d. What is the most appropriate management regime for *H. drasticus* in each studied ecosystem?

2. Materials and Methods

2.1 Study area and species

The study area is located in the Araripe mesoregion, in the center of northeastern Brazil. This region is characterized by a particular territorial pattern, including socially and economically underdeveloped areas as well as relatively developed areas supporting activities that integrate the social and economic dynamics of the country (Cardoso 2010; MDA 2010).

The Chapada do Araripe is a plateau in the Araripe mesoregion. The highest areas of this plateau support savanna vegetation (Cerrado); humid and dry forest and caatinga dry and moist forests occur on the slopes. This region is an area of extremely high priority for the conservation of the Cerrado (Oliveira and Marquis 2002) and Caatinga biomes (MMA 2007). The Araripe National Forest (FLONA-Araripe, Figure 1), in the Araripe mesoregion, is the first National Forest in Brazil, established in 1946. This protected area includes parts of five municipalities of Ceará State (Barbalha, Crato, Jardim, Missão Velha, and Santana do Cariri).

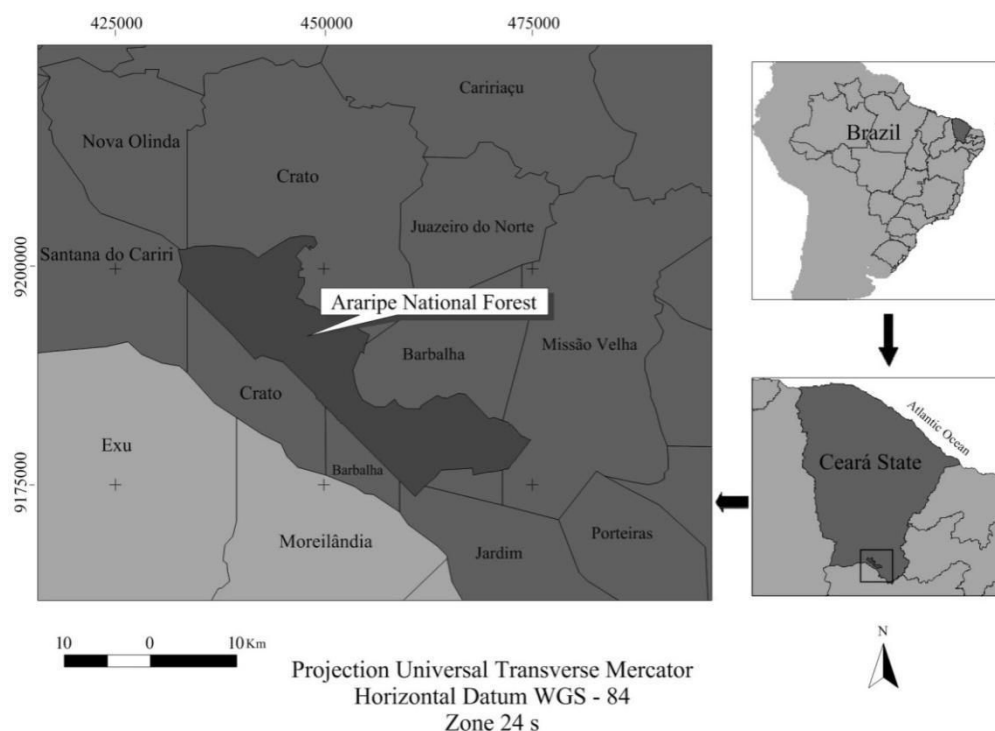


Fig 1 Location of the Araripe National Forest (FLONA-Araripe), Ceará State, northeastern Brazil

FLONA-Araripe is considered a habitat island, with higher rainfall and lower temperature than the surrounding Caatinga biome (semi-arid forest) (Costa 2004). The soil is a red-yellow dystrophic latosol (Cavalcanti and Lopes 1994), and the climate is Tropical wet and dry or Savanna climate (Aw) (Köppen 1948). The biodiversity of this area is high and includes an endemic and critically endangered bird, the Araripe manakin (*Antilophia bookermanni*). Several species with socioeconomic importance are found in FLONA-

Araripe, including pequi (*Caryocar coriaceum*), faveira (*Dimorphandra mollis*) and janaguba (*Himantanthus drasticus*).

H. drasticus is a tree species endemic to Brazil. Its height ranges from 3 to 7 m. Young branches are dark brown with light brown spots; older branches are pale brown (Spina 2004). This species occurs in the Cerrado and Caatinga (semi-arid forest) biomes, and its distribution includes all of northeastern Brazil (Spina 2004). Mixed with water, the latex of *H. drasticus* (“leite de janaguba”) is popularly used to treat gastritis, hemorrhoids, anemia, inflammations and many types of cancer (Fernandes et al. 2000; Lucetti et al. 2010).

2.2 Experimental design, data collection and analyses

For study, we selected two ecosystems of Araripe National Forest: “cerrado *sensu stricto*” (hereafter cerrado) and “cerradão”. Cerrado is defined as “a vegetation dominated by 3–8-m tall trees and shrubs with more than 30% crown cover but with still a fair amount of herbaceous vegetation between them”, whereas “cerradão” is described as “an almost closed woodland with crown cover of 50% to 90%, made up of 8–12-m-tall trees casting considerable shade so that the ground layer is much reduced” (Oliveira and Marquis 2002).

The experiments involved the removal of strips 2.0 meters in length. Four levels of debarking were used. The approximate percentage of the circumference at breast height subjected to debarking was identified as follows: approximately 25% - “one side”, 50% - “two sides”, 75% - “three sides” and 100% - “four sides”). These treatments were used to represent the management systems employed by the harvesters in the study area. These values are approximate because bark is removed with a scythe or machete. Even in the “four sides” treatment, a narrow strip of bark remained on the individual tree. These remaining strips were measured with a bark gauge and used as a control to be compared with the values obtained for regeneration.

Each treatment was applied to 15 individuals, with a total of 120 sampled trees (60 in each ecosystem). All trees were exploited by the same harvester to eliminate the additional variation that could result from differences among harvesters. The latex was extracted after the bark was removed. The exposed area was measured on the next day, after the drained latex had dried.

The term “bark” is somewhat generic, and the consideration of bark as a single unit may obscure important aspects of the biochemistry, physiology, ecology and evolutionary biology of the species of interest (Romero 2006). Thus, a more detailed definition would recognize the "inner bark" as a product of the vascular cambium composed of live phloem, whereas the "outer bark", or rhytidome, would be a complex of tissues including the products of phellogen, epidermis, cortex, primary and secondary phloem, as well as all tissues external to the phellogen dead at maturity (Romero 2006). In this experiment, the rhytidome was removed to simulate the traditional management systems. The depth of the bark removed was variable, with higher values in trees with greater diameters because their rhytidome was thicker.

Evaluations were made every six months for three years. The mortality in each area/treatment was also recorded. In the first evaluation, it was observed that regeneration occurs through sheet growth (regrowth on the surface of the wound). For this reason, a bark gauge was used to obtain data on the thickness of the recovered bark (TRB). Three measurements were performed on each tree, and the average of these measurements (cm) was used in the analyses. A two-way ANOVA was performed to compare TRB values between ecosystems and treatments. The average TRB values were compared with the controls (strips of bark that remained on the each tree), and confidence intervals were obtained from 10,000 bootstrap runs.

A qualitative evaluation of bark regeneration was also conducted. For this purpose, the bark regeneration of each individual was classified using an increasing scale that ranged from zero to five, zero indicating the absence of regeneration and five indicating complete recovery of the bark, including the rhytidome (Figure 2). This measure was termed the "bark regeneration index" (BRI). The BRI scores were determined by one researcher and one harvester. The BRI values were compared among treatments and between areas with a Scheirer-Ray-Hare test (Dytham 2011).

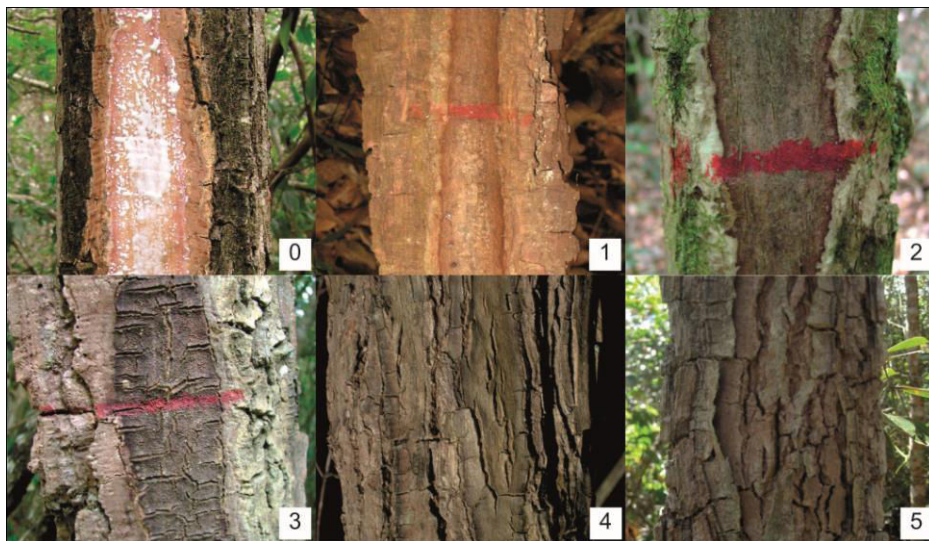


Fig 2 Characterization of stages of bark regeneration in *H. drasticus*. The numbers represent values of the bark regeneration index (BRI)

To test the effect of tree size on the bark recovery rates, the trees were assigned to three classes according to their diameter at ground level (DGL): class 1 ($7.0 \leq \text{DGL} < 10.0$), class 2 ($10.0 \leq \text{DGL} < 13.0$) and class 3 (≥ 13.0). The indexes obtained were subjected to an ANOVA and a Tukey test. To verify the possible influence of light on the bark regeneration process, the crown illumination index (CII) (Clark and Clark 1992) was used. This index is a visual estimate of the proportion of the canopy of a tree that receives light, ranging from one (completely shaded canopy) to five (fully exposed canopy). A Spearman correlation coefficient (r_s) was subsequently calculated to associate the TRB and BRI values with the CII. All analyses were performed in the R environment (R Development Core Team 2012).

3. Results

3.1 Regeneration patterns

Only two individuals initiated bark regrowth from the center of the exposed area. The regeneration was most likely triggered by living cells remaining on the xylem at the time of debarking. A single individual showed edge growth. These exceptions excluded, all individuals showed sheet growth, characterized by the simultaneous regeneration of tissues throughout the surface of the exposed area. The details of the regeneration process of the inner bark are still unclear, but it apparently occurs through growth in all directions from the thin layer of inner bark that is left by the harvesters when collecting the latex.

In most individuals, a significant structural modification of the internal bark, indicating tissue regeneration, occurred only in the second year of the experiment. These results contrast with the information provided by many harvesters, who consider one year sufficient for bark regeneration (Baldauf and Santos, 2013). However, this issue is controversial among the harvesters because some of them believe that more than 18 months are needed for bark regrowth.

Over the three-year study period, nine of the 120 harvested trees died (seven in the cerradão and two in the cerrado). In terms of the degree of debarking of the dead trees, one tree had 25% debarking, two had 50%, four had 75% and only one had 100% debarking.

3.2 Effects of the environment and the management system

The values of bark recovery (thickness) ranged from 0.15 to 0.65 cm (mean = 0.32; sd = 0.09). Bark regrowth varied between ecosystems, with higher average values in the cerrado than in the cerradão (Table 1 and Figure 3). However, the bark recovery values obtained in each management treatment did not differ (Table 1, Figure 3a). The same pattern was found for the BRI (Table 1, Figure 3b).

Table 1: Comparison of bark regeneration in *H. drasticus* in different ecosystems and management systems

	Df	Mean square	F-value/H	P
<i>Two-way ANOVA (TRB)</i>				
Factor 1 (Ecosystem)	1	0.1654	23.3665	4.643e-06
Factor 2 (Management)	3	0.0115	1.6315	0.1866
Interaction	3	0.0061	0.8655	0.4616
<i>Scheirer- Ray-Hare test (BRI)</i>				
Factor 1 (Ecosystem)	1	78560	689.01	<0.0001
Factor 2 (Management)	3	104	2.75	0.4321
Interaction	3	87	2.30	0.5123

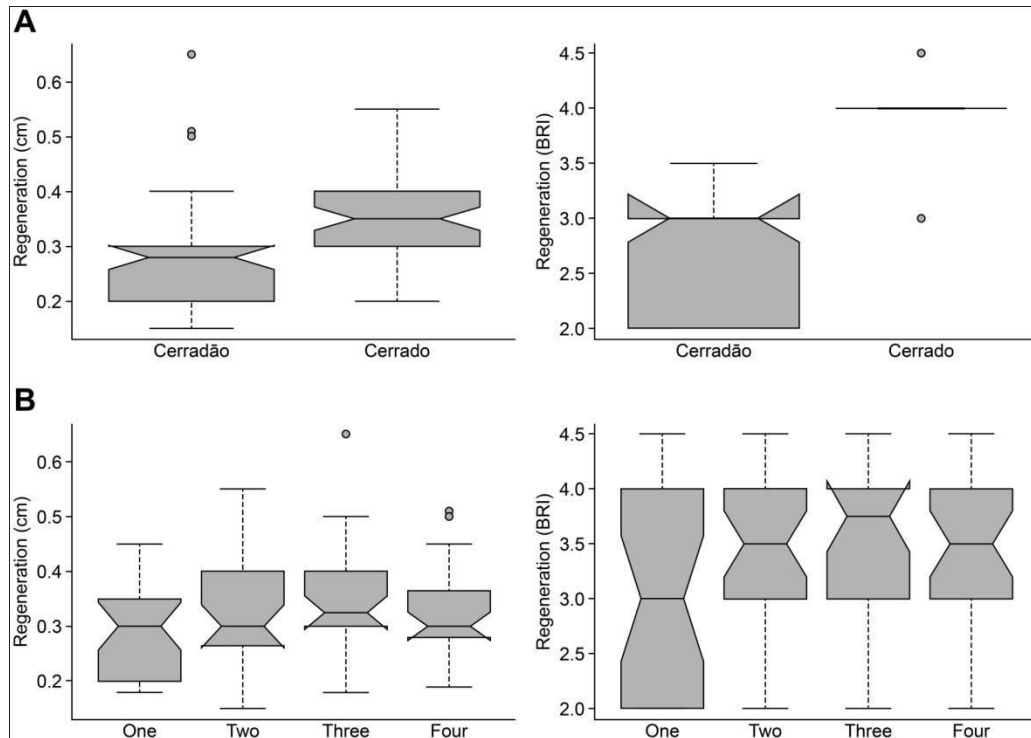


Fig 3 Thickness of recovered bark (left) and bark regeneration index (right) after harvesting in different ecosystems (A) and management systems (B). Top and bottom bars are the maximum and minimum values, respectively. Black bars correspond to the median value. The dots represent outliers.

In both analyzed ecosystems, a comparison of the TRB values showed higher values in the controls than in the treatments, indicating that three years are not sufficient for total recovery of the rhytidome (Table 2). A recovery of 60.2% and 46.0% of the rhytidome was observed in the cerrado and cerradão ecosystems, respectively.

No association was found between bark regeneration and the diameter of the harvested trees in the cerrado. However, the regeneration in the cerradão was higher in diameter class 3 than in the smaller trees, diameter class 1 ($F = 4.485$; $p = 0.015$ and $F = 6.991$; $p = 0.002$ for BRI and TRB, respectively). No differences were found in the other contrasts.

Finally, a positive correlation was found between the TRB and the CII ($r_s = 0.43$; $df = 109$; $p < 0.01$), as well as between the BRI and the CII ($r_s = 0.57$, $df = 109$; $p < 0.01$), indicating greater regeneration in the trees that received more sunlight.

Table 2: Mean values of bark thickness after three years of debarking (treatment) and control values in each studied ecosystem. The values in parentheses are the confidence intervals obtained after 10,000 bootstrap runs.

	Treatment	Control
Cerrado	0.357 (0.339-0.372)	0.593 (0.566-0.616)
Cerradão	0.280 (0.255-0.302)	0.608 (0.551-0.667)

4. Discussion

4.1 Regeneration patterns and mortality

Bark regeneration can result from edge growth and/or sheet growth. Regeneration from the edges is the most common pattern. In this case, regeneration originates from the bark of living tissues (vascular cambium, phloem and phellogen) along the edge of the exploited area toward the center (Mariot 2008). In sheet growth, the living tissue regenerates simultaneously over the entire exposed surface area from the damaged xylem. Certain species may also show regeneration from the center of the wound. However, this pattern is relatively uncommon in trees (Mariot 2008).

A wide variety of tissues may be involved in the regeneration of the bark. These tissues include the phloem parenchyma, xylem parenchyma, immature xylem zone and cambium (Delvaux 2009). The details of the regeneration process in *H. drasticus* and the tissues involved are still unknown. Throughout the experiment, regrowth occurred as sheet growth, and only three individuals showed a different pattern. In these three individuals, however, the harvester accidentally penetrated the bark during exploitation of the latex, reaching the heartwood. Thus, the pattern of regeneration from the edges or center appears to be a response to deeper damage and thus represents an exception to the commonly observed pattern of sheet growth.

Responses to damage in the bark and, consequently, the formulation of criteria for sustainable management depend on a number of factors, such as the type of damaged tissue and the extent of damage, the morphology and anatomy of bark and the presence of

exudates (Guariguata and Gilbert 1996; Schoonenberg et al. 2003; Romero and Bolker 2008; Pandey et al. 2012). Because of these multiple factors, the effect of bark removal and the sustainability of harvesting practices can be considered species-specific (Chungu et al. 2007; Delvaux et al. 2009).

Romero and Bolker (2008) studied the effects of debarking in seven species in the Bolivian Amazon. Their study found that species that produce some type of exudate showed more efficient bark recovery and that wound healing occurs more readily in species with thick bark than in species with thin bark. These findings are important in the case of *H. drasticus* because it produces exudates and has relatively thick bark. These characteristics indicate that *H. drasticus* is potentially capable of showing efficient bark regeneration and, consequently, that it is potentially suitable for sustainable harvesting.

4.2 Effects of environment and management systems

Several experiments have shown that the most important factor for successful recovery of the bark is the humidity at the exposed layer (Neely 1988; Stobbe et al. 2002; Juan 2006), which may be related to the occurrence of a rainy season. Moreover, seasonality can influence the regeneration of the bark due to variations in tissue water content and in the activity of the cambium (Puritch and Mullick 1975; Dujesiefken and Liese 1990).

Mariot et al. (2008) tested the effect of different harvest seasons on the regenerative ability of the species *Drimys brasiliensis* (Winteraceae) in an Atlantic Forest area and found no seasonal differences in the speed of biomass regeneration. In *Myracrodruon urundeuva* (Anacardiaceae), a medicinal species whose bark is exploited in the Brazilian dry forest (Caatinga), no significant correlation was found between the monthly percent regeneration and the average monthly precipitation (Monteiro et al. 2011). However, Delvaux et al. (2010), assessing the bark regrowth patterns of 12 species in Benin, found that the rate of regeneration was higher during the spring. Other species in which bark recovery is influenced by the season are *Terminalia arjuna* (Combretaceae) and *Litsea glutinosa* (Lauraceae), a highly exploited Indian medicinal trees (Pandey and Mandal 2012).

Vermeulen and Geldenhuys (2004) studied the effects of bark harvesting in three tree species in South Africa and observed that debarking in the winter (dry season and low

temperatures) favored edge growth, whereas exploiting the same species in the summer (rainy season and high temperatures) promoted sheet growth. Because bark growth in *H. drasticus* occurs through sheet growth, it is possible that the rainy season is favorable for harvesting. Thus, seasonality is an important variable to be tested in future studies on the bark regeneration potential of the species.

The results showed that the regeneration of the bark was favored by open environments because the correlation between TRB/BRI and light was significant and positive and the regeneration of bark was more rapid in the cerrado than in the cerradão. These data apparently contradict the trend cited before, in which humidity is considered the most important factor for the regeneration of the bark through laminar growth, because the cerrado has lower levels of humidity than the cerradão. However, it is possible that the latex that remains in the trees after extraction serves to provide the necessary humidity to trigger the cellular division process that will culminate in the recovery of injured xylem and phloem. Plants of certain families, such as Apocynaceae, Euphorbiaceae, Moraceae and Canellaceae, show great resilience after debarking, in part because the cambium is protected by exudates after the bark is removed (Cunningham 2001).

The absence of differences among groups with different percentages of debarking and the lack of signs of morbidity in the trees with approximately 100% removal of the bark represents a contrast with the results of several previous studies on bark harvesting. In many species studied to date, overexploited individuals died shortly following the exploitation, most likely due to the disruption of water and/or nutrient flows (Cunningham and Mbenkum 1993; Borges Filho and Felfili 2003; Geldenhuys et al. 2007; Guedje et al. 2003; Guedje et al. 2007; Delvaux et al. 2010).

Species in which individuals can survive ringbarking are rare. *Walburgia salutaris* is an African medicinal plant that strongly and intensely regenerates bark after ringbarking (Cunningham and Mbenkum 1993; Botha et al. 2004). Other species that can recover after complete debarking are *Prunus africana* (Cunningham and Mbenkum 1993) and *Adansonia digitata* (Fasola and Egunyomi 2005). In *A. digitata*, the wood parenchyma immediately below the exposed surface dedifferentiates and regenerates the xylem and phloem (Fisher 1981); however, the bark regrowth is considered to occur slowly (Fasola and Egunyomi 2005).

A possible explanation for the resilience of *H. drasticus* is that most of the harvesters remove only the rhytidome. This type of bark removal does not impede the flow of water and photosynthates. Vermeulen and Geldenhuys (2004) found a high rate of regeneration if a thin layer of bark and cambium was left on the exploited trees, whereas little or no regeneration was observed if the bark was completely removed in three species. This trend was also verified by Delvaux et al. (2009), who detected greater sheet regeneration if a layer of bark was left on harvested trees than if all the bark was removed. In *Prunus africana*, trees that were harvested without disrupting the vascular cambium showed an increased probability of survival (Stewart 2009).

Debarking may also favor attack by diseases caused by insects and fungi, especially if a large amount of bark is removed (Fasola and Egunyomi 2005; Mariot et al. 2007; Chungu et al. 2010). Nevertheless, the current study found no evidence of attacks by insects or fungi, even in plants with approximately 100% of the bark removed. The absence of pathogen attacks might be related to the secondary metabolites with antimicrobial activity found in *Himatanthus* bark and latex (Silva et al. 1998; Souza et al. 2004). Furthermore, no evidence of crown senescence was observed in the harvested trees of *H. drasticus*. Crown health has been used as an indicator of tree health after bark harvesting because the crowns of the exploited trees frequently display signs of senescence (Sunderland and Tako 1999; Hall et al. 2000; Stewart 2009; Uniyal 2013).

4.3 Suggestions for sustainable management

Delvaux et al. (2010) found that bark recovery rates varied greatly among species. The bark of several species (e.g., *Khaya senegalensis* and *Lannea kerstingii*) recovered rapidly after harvesting, whereas others, such as *Azelaia africana* and *Maranthes polyandra*, showed no increase in the recovery rate over a 24-month experimental period.

The results obtained for *H. drasticus* indicate that the species is extremely tolerant to debarking. However, bark regeneration is relatively slow. Regardless of the ecosystem or management system, the bark of the exploited trees had not recovered completely by the end of the three-year experimental period.

The recovery of the inner bark in *H. drasticus* progressed to an advanced stage or was complete by the end of the three-year experimental period. However, the formation of rhytidome appears to be a slower process (pers. obs.). Rhytidome plays an important role

because it serves as a physical barrier to protect against attacks by herbivores, insects, fungi, and fire (Catry et al. 2012). Thus, even if the inner bark recovers, the exploited individuals in cerrado areas are more vulnerable because the thickness of the bark is an important determinant of survival probabilities if fires occur (Hare 1965; Prance and Prance 1993). In *Quercus suber*, bark thickness and bark harvesting are the major factors limiting resistance to fire (Catry et al. 2012).

In this context, despite the resilience of *H. drasticus* to latex exploitation, a balance must be reached between product demand and the recoverability of the bark. This is especially the case in the cerradão, where the regeneration of the bark is slower than in the cerrado. Another factor that should be considered is that bark regeneration is slower in the smaller plants in the cerradão. This result is surprising because the literature has reported faster regeneration in younger and middle-aged trees (Delvaux et al. 2010; Pandey and Mandal 2012).

The results obtained in the cerrado and cerradão show that specific management regimes are needed for each distinct ecosystem. To maintain protection against fire, the removal of a maximum of 50% of the bark (two sides) is suggested. It is recommended that the exploitation of individuals less than 10 cm in DGL (class 1) in cerradão areas be avoided because bark regeneration is slower in these individuals.

Under the assumption that the regeneration of the bark is linear in other size classes, the optimal interval between harvesting events (to allow the bark to recover completely) would be five years in cerrado areas and six and one-half years in cerradão areas. This suggestion considers that complete recuperation of the bark is needed for sustainable management, which may not be the case. However, there is no doubt that partial recovery is essential for plant protection and survival if fires occur. Therefore, the intervals suggested can serve as reference values for the process of developing participatory management plans for *H. drasticus*. The opinions and knowledge of the multiple stakeholders must be considered in this process so that the socioeconomic and cultural dimensions of sustainability can also be addressed.

CONCLUSIONS

Compared with other species exploited for bark, *Himatanthus drasticus* is very resilient to harvesting. No relationships between harvesting intensity and mortality or bark recovery speed were found in this species by the current study. However, bark regeneration is relatively slow and ecosystem dependent. It is more rapid in the open areas than in the woodlands. The establishment of criteria for the harvesting of this species is of the utmost importance because the demand for the product has been increasing substantially. These increasing demands can potentially cause overharvesting. In this context, the results obtained in this study are key elements for the development of a sustainable management plan for the species.

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CAPÍTULO 7

Effects of harvesting intensity and ecosystem type on the *Himatanthus drasticus* (Apocynaceae) population ecology

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Abstract

The extent of the demographic impacts of non-timber forest product harvesting is influenced by factors such as management intensity and environmental conditions. We assessed harvesting impacts on the population dynamics of *janaguba* (*Himatanthus drasticus* (Mart.) Plumel), an indigenous Brazilian savanna tree that has been exploited for its medicinal latex. We studied 12 Northeast Brazil populations, six in the *cerrado* (open savanna) and six in the *cerradão* (woodland), for three years (2008-2010). In each ecosystem, we surveyed populations subjected to different harvesting intensities (high-harvest and low-harvest). We used matrix population models and a factorial life-table response experiment (LTRE) to distinguish between the effects of harvesting intensity and ecosystem type on *H. drasticus* population dynamics. The different factor combinations revealed population stability in most cases, indicating that the species may be sustainably maintained. We observed a population reduction under only one condition (*cerradão* x high-harvest) in one of the two demographic intervals. In this particular case, our results revealed that the stasis of the adult trees was the most important contributor to the reduced population growth rate. Because this population reduction occurred in a year where rainfall from the previous period was greatly reduced, the environmental conditions may have influenced this result. In summary, our data indicated that the *cerradão* populations were vulnerable when high levels of exploitation were considered, indicating that management criteria specific to each studied ecosystem needed to achieve conservation and sustainable harvesting goals.

Keywords: matrix models, non-timber forest products, Cerrado, population dynamics, sustainable management, latex

Introduction

The Brazilian savanna (Cerrado) is one of the most threatened biomes in South America, primarily due to the rapid expansion of agriculture frontiers and cattle husbandry (Oliveira and Marquis 2002, Machado et al. 2004). A lack of familiarity with this biome at the international level persists even though the Cerrado is the most biodiverse savanna and is considered a biodiversity hotspot because of its threatened status, species richness and high endemism levels (Oliveira and Marquis 2002, Mittermeier et al. 2005).

Some of the recommendations for conserving the Cerrado include the consolidation of existing conservation units and the creation of new large protected areas to protect the remaining blocks of natural Cerrado (MMA 2006). Nevertheless, the creation of protected areas is not only costly but is also insufficient to conserve the Cerrado on its own (ISPN 2006). Complementary strategies that employ community-managed areas should be considered because they can be more effective than protected areas in reducing deforestation (Porter-Bolland et al. 2012).

The Cerrado houses a variety of traditional and local populations, such as indigenous groups, *quilombolas* (descendants of slaves), harvesters and small family farmers (ISPN 2006). These populations have been collecting wild biological resources based on traditional ecological knowledge passed down over numerous generations. Some of the exploited species include several non-timber forest products (NTFPs) that may be sustainably harvested, such as the buriti palm (*Mauritia flexuosa*), butiá (*Butia capitata*) and capim-dourado (*Syngonanthus nitens*) (Lima 2011, Schmidt 2011, Schmidt and Ticktin 2012, Sampaio 2012). Conversely, a demand for marketable species, such as barbatimão (*Stryphnodendron* spp.), arnica (*Lychnophora ericoides*) and pequi (*Caryocar brasiliense*), has led to a decrease in their populations, especially in the case of arnica because the entire plant is harvested (Felfili and Silva Junior 1988, Borges-Filho and Felfili 2003, Zardo and Henriques 2011).

The ecological impacts of harvesting on NTFPs are influenced by several factors, including the plant parts that are harvested, management intensity, species' life history and environmental conditions (Ticktin 2004). The collection of leaves, flowers and fruits are generally assumed to possess a low ecological impact on natural populations, whereas exudate tapping exerts an intermediate impact and harvesting bark, roots and apical meristems may highly impact the ecology and, therefore, cannot be sustainably managed (Boot and Gullison

1995, Cunningham 2001, Ticktin and Shackleton 2011). Abiotic variables, such as light, nutrients, water availability and climate, can alter the ecological impacts of harvesting (Martinez-Ramos et al. 2009, Ticktin and Shackleton 2011). However, the environmental impact on population vital rates has been poorly studied (Crone et al. 2011), which can lead to suboptimal or even erroneous conclusions about sustainability (Gaoue and Ticktin 2010, Schmidt et al. 2011).

To quantify the effect of NTFP harvesting at a population level, matrix population models (Caswell 2001) have been employed (see Schmidt et al. 2011 for a review). An important use of these models is demonstrated in the family of techniques known as perturbation analyses, which compare the importance of different model components to the model output, usually the population growth (Zuidema 2000). Using this approach, the harvesting effect on the population growth rate depends on its effects on matrix parameters and the population growth sensitivity to parameter changes (Gaoue et al. 2011).

In this study, we assessed the harvesting impacts on populations of janaguba (*Himatanthus drasticus* (Mart.) Plumel), a tree native to the Cerrado biome that is harvested for its medicinal latex. To harvest the latex, large strips of bark are removed, which can decrease the survival, growth and/or reproduction of the harvested individuals due to a reallocation of resources to wound healing and bark/latex regeneration. Previous NTFP ecological studies confirmed this resource trade-off and demonstrated that plants that suffered high levels of bark removal usually died due to the interruption of water and/or nutrient flow (Borges-Filho and Felfili 2003, Langenhein et al. 2003, Delvaux et al. 2007, Geldenhuys et al. 2007, Guedje et al. 2007). The impacts can be more extensive when the species possesses multiple uses and, thus, more than one product is harvested, which is the case for *H. drasticus*, a tree that is also harvested for its bark to exploit the latex.

We aimed to evaluate the impacts of harvesting and to suggest regulations for sustainably managing the target species. We addressed the following specific questions: (1) Do the bark and latex harvest intensities affect the population growth rates of *H. drasticus*? (2) Do the harvesting effects depend on the type of ecosystem considered? (3) What is the potential for sustainably harvesting the studied species in two different Cerrado ecosystems?

2. Methods

2.1 The species and study area

Himatanthus drasticus is tree endemic to Brazil that displays a height of 1 to 7 m and has young, dark brown branches with light brown spots and older, pale brown branches (Spina 2004). It thrives in the Cerrado (savanna) and Caatinga (dry forest) biomes and is distributed throughout Northeast Brazil (Spina 2004, Spina 2013). The latex of *H. drasticus* mixed with water (“leite de janaguba”) is popularly used to treat gastritis, hemorrhoids, anemia, inflammation and numerous cancer types (Baldauf and Santos, 2013). The preliminary results from pharmacological studies assessing this species have confirmed its uses as an analgesic, anticarcinogen and immunostimulant (Colares et al. 2008, Lucetti et al. 2010, Sousa et al. 2010, Mousinho et al. 2011).

The study area is in the Cariri region, Ceará State, northeastern Brazil (Figure 1). In this region, the plateau Chapada do Araripe is recognized as one of the priority areas for conserving the Brazilian Cerrado biome (Cavalcanti and Joly 2002). The Cerrado of the Chapada do Araripe is a disjunction at an altitude of 800 to 900 m and is characterized as a habitat island due to the higher rainfall and lower temperature than its surrounding Caatinga biome (Costa 2004). The soil is a red-yellow dystrophic latosol (Cavalcanti and Lopes 1994), and the climate is a wet and dry tropical or a savanna climate (Aw) according to the Köppen classification system.

The survey was performed in one of the protected areas of Chapada do Araripe, the Araripe National Forest (07°11’S, 39°13’W, 938 m high), which is considered the only preserved Cerrado area in Ceará State. The area is covered by two different ecosystems: “cerrado *sensu stricto*” (hereafter *cerrado*), defined as “a vegetation dominated by 3–8-m-tall trees and shrubs with more than 30% crown cover but with still a fair amount of herbaceous vegetation between them” and “*cerradão*”, which is described as “an almost closed woodland with crown cover of 50% to 90%, made up of 8–12-m-tall trees casting considerable shade so that the ground layer is much reduced” (Oliveira and Marquis 2002).

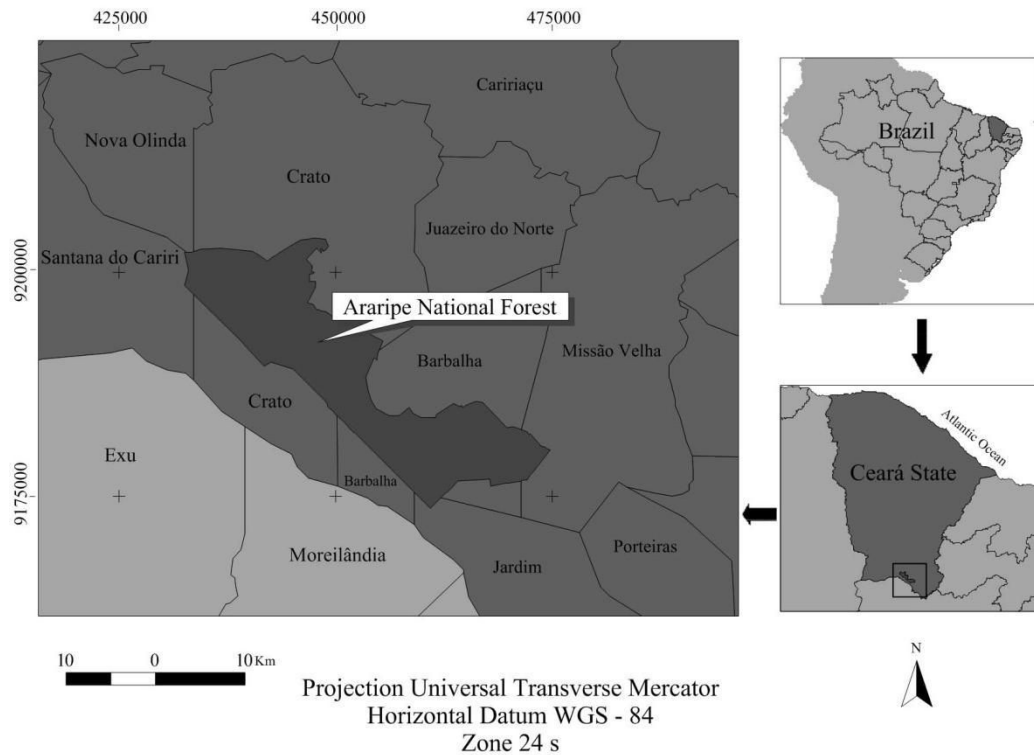


Figure 1- Location of the Araripe National Forest

2.2 Sampling and data collection

We sampled 12 populations, six in the *cerrado* (open savanna) and six in the *cerradão* (woodland). In each ecosystem, we surveyed three populations subject to high rates of bark harvesting (high-harvest populations) and three populations subject to low harvest rates (low-harvest populations). More than 50% of the adult trees are debarked in the high-harvest populations, and generally, more than 50% of the bark circumference is removed from these individuals. In contrast, less than 20% of the adults trees are debarked in the low-harvest populations, and a maximum of 50% of the bark circumference is removed.

In each population, we established a plot of 1 ha (200 x 50 m) divided into subplots of 10 x 10 m in which all individuals with a diameter at ground level (DGL) > 1 cm were sampled. Due to the high abundance of individuals with a $DGL \leq 1$ cm, these were sampled in a 2 x 2 m area inside each subplot of 10 x 10 m. Between August and December 2008, we sampled and tagged 8,897 individuals in the *cerrado* and 5,437 in the *cerradão*. The diameter at ground level (DGL), height and reproductive status were recorded for each tagged plant.

Evaluations were repeated annually between August and December 2009 and August and December 2010.

2.3 Data analysis

For data analysis, we divided the sampled individuals into six diameter classes according to the DGL recorded in 2008: C1: $\text{DGL} < 1 \text{ cm}$, C2: $1 \text{ cm} \leq \text{DGL} < 3 \text{ cm}$, C3: $3 \text{ cm} \leq \text{DGL} < 5 \text{ cm}$, C4: $5 \text{ cm} \leq \text{DGL} < 7 \text{ cm}$, C5: $7 \text{ cm} \leq \text{DGL} < 10 \text{ cm}$ and C6: $\text{DGL} \geq 10 \text{ cm}$. We defined the ranges and the diameter class number based on a combination of biological criteria (the DGL range and presence of reproductive structures) and harvesting patterns (the DGL of tapped trees).

The fertility rates were calculated by considering the number of recruited seedlings of each reproductive class (C5 and C6) according to their proportional reproductive effort. To assess the reproductive effort of each diameter class, we multiplied the number of fruits produced by the individual, the number of individuals in each diameter class and the probability of the individual being reproductive. The probability of the individuals of each diameter class being reproductive was obtained by the ratio between the DGL and the presence or absence of reproductive structures calculated by logistic regression.

We used the Lefkovich stage-structured population matrices (Lefkovich 1965) modified by Caswell (2001) to describe and compare the population dynamics of different harvest levels and ecosystems. The matrix elements demonstrate the individual's fate and traditionally represent the transition probabilities of growth, stasis, regression and fecundity. However, the elements can be decomposed into underlying vital rates (lower level vital rates - LLVR), such as survival (s - probability of survival), growth (g - probability of survival and growth into the next diameter class), regression (r - probability of survival and a return to a smaller diameter class) and fertility (f - offspring production from each class) (Morris and Doak 2002). Based on the demographic information, we calculated the underlying vital rates (Table 1). For a complete description about matrix construction, see Morris and Doak (2002).

Table 1: Vital rates used to construct the projection matrix based on the lower level vital rates. The letters indicate the vital rates (s - survival, g - growth, r - regression, f - fecundity), and the numbers indicate class size (Class 1, Class 2, Class 3, Class 4, Class 5, Class 6).

$s1*(1-g21)$	$s2*r12$	0	0	$S1*f5$	$s1*f6$
$s1*g21$	$s2*(1-g32-r12-g42)$	$s3*r23$	0	0	0
0	$s2*g32$	$s3*(1-g43-g53-r23)$	$s4*r34^a$	0	0
0	$s2*g42$	$s3*g43$	$s4*(1-g54-g64-r34)$	$s5*r45^b$	0
0	0	$s3*g53$	$s4*g54$	$s5*(1-g65)$	0
0	0	0	$s4*g64$	$s5*g65$	$s6$

^a - transition nonexistent in the interval 2009-2010; ^b - transition nonexistent in the interval 2008-2009

For each demographic interval (2008-2009 and 2009-2010), we constructed a summary matrix for each harvest level and ecosystem based on pooled data from the populations sampled in each treatment. We also built four matrices based on the combinations between the two levels of harvesting and the two sampled ecosystems and an overall summary matrix for each of the two annual transitions. We calculated the population growth rates for each treatment combination as well as the elasticities of the population growth rates for all vital rates. The confidence interval for each λ was calculated using bootstrap resampling with 10,000 iterations.

We employed all of the cited matrices (Annex 1) to perform a factorial LTRE analysis to decompose the effects of harvest, ecosystem type and their possible interactions on the population growth rates (λ). Once the LTRE was analogous to the analysis of variance (Caswell 2001), we considered “harvest level” and “ecosystems” as treatments following the approach and calculations described in Gaoue and Ticktin (2010). All matrix calculations were performed in R (version 2.12.1, R Development Core Team 2010) using the package “popbio” (Stubben and Milligan 2007).

3. Results

In the first demographic interval (2008-2009), none of the population growth rates (λ) of the *H. drasticus* populations differed, indicating population stability, regardless of the intensity of management employed or the ecosystem type (Table 2). The same pattern was observed for the populations when they were grouped based on the interactions between the factors. However, in the second demographic interval (2009-2010), the λ were lower than one in the “high harvest” and “*cerradão*” treatments as well as in the interaction between these treatments (Table 2). The confidence intervals of all λ overlapped among the periods and

treatments, indicating no differences between them. In 2008-2009, the values varied from 0.976 to 1.005, whereas the values ranged from 0.931 to 0.976 in 2009-2010 (Table 2).

Table 2: Population growth rates and their confidence values (95%) in different ecosystems and with different harvesting practices.

	2008-2009	2009-2010
<i>Harvesting intensity</i>		
High	1.005 (0.976; 1.033)	0.948 (0.896; 0.989)
Low	0.969 (0.935; 1.012)	0.966 (0.924; 1.000)
<i>Ecosystem type</i>		
<i>Cerrado</i>	0.972 (0.942; 1.008)	0.976 (0.941; 1.001)
<i>Cerradão</i>	0.999 (0.969; 1.031)	0.931 (0.883; 0.982)
<i>Interaction</i>		
High x <i>cerrado</i>	0.992 (0.953; 1.025)	0.974 (0.921; 1.000)
Low x <i>cerrado</i>	0.976 (0.922; 1.009)	0.979 (0.936; 1.003)
High x <i>cerradão</i>	1.007 (0.972; 1.041)	0.931 (0.862; 0.983)
Low x <i>cerradão</i>	0.989 (0.950; 1.033)	0.943 (0.861; 1.000)

The results of the factorial LTRE demonstrated that, in 2008-2009, the averages of the contributions of harvesting intensity and ecosystem type were 0.0038 and 0.0055, respectively, indicating that the second factor exerted a higher influence than the first one (Table 3, Figure 2). However, no factor or combination significantly altered the *H. drasticus* population growth rate. In 2009-2010, the average harvesting intensity and ecosystem contributions were 0.011 and 0.016, respectively, and the interaction between factors had a higher effect on population growth (average of 0.020) (Table 3, Figure 3). The LTRE effects on populations during the second period were higher than those previously observed, consistent with the population growth rate differences.

Retrospective (Table 4) and prospective (Table 5 and 6) analyses for all matrix models demonstrated that the stasis was the most important vital rate for changes in λ and that growth was the second most important. Fecundity and regression exerted minimal contributions on *H. drasticus* population growth rates. The higher elasticities for survival were verified in the diameter class 6 ($DGL \geq 10$ cm). Similarly, the differences detected in the LTRE were mainly driven by the stasis of the same diameter class. Both results indicate that the survival of the exploited individuals, commonly observed in class 6, is crucial to the population stability (Table 5).

Survival rates ranged from 60 to 96% in the first period and from 30 to 97% in the second period, indicating a survival reduction in 2009-2010. Despite the differences between years in both periods, the survival of C4 (the boundary between reproductive and non-reproductive classes) is lower than the survival of other classes, which can be attributed to the high probability of growth associated with that class. Notably, although the survival of the smaller size class (C1) was high, growth remained below 2%, which limited the progression of individuals throughout the life cycle.

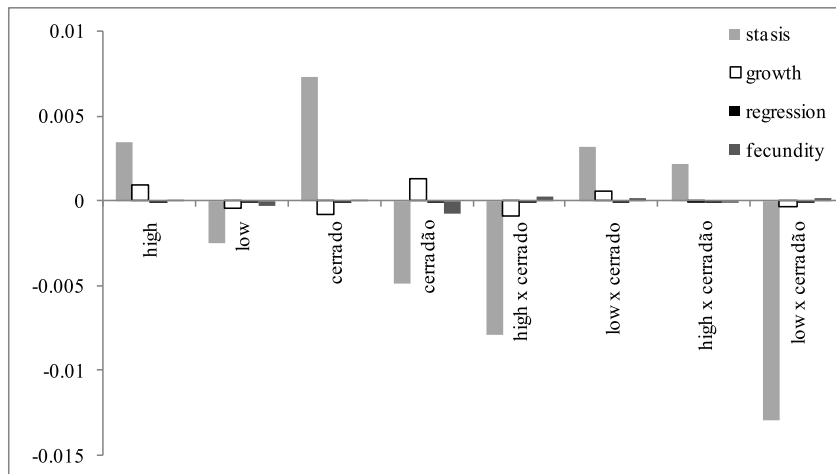


Figure 2: LTRE contributions of harvesting intensity, ecosystem type and interactions to the population growth rate differences in 2008-2009.

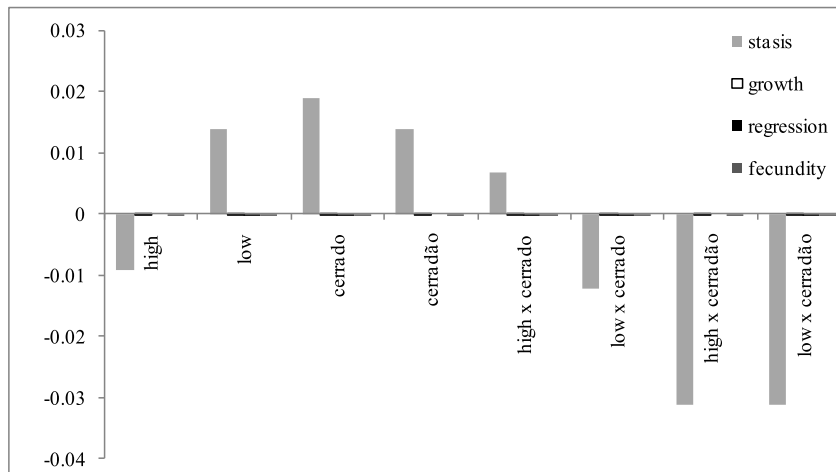


Figure 3: LTRE contributions of harvesting intensity, ecosystem type and interactions to the population growth rate differences in 2009-2010.

Table 3: Summary of the LTRE contributions of different treatments and their interactions to the *H. drasticus* population growth rate differences.

LTRE effect	2008-2009	Average	SE	2009-2010	Average	SE
Harvesting intensity						
High	0.0045	0.0039	0.0006	-0.0090	0.0115	0.0025
Low	-0.0032			0.0140		
Ecosystem type						
<i>Cerrado</i>	0.0065	0.0055	0.0010	0.0190	0.0165	0.0025
<i>Cerradão</i>	-0.0045			0.0140		
Interaction						
High x <i>cerrado</i>	-0.0086	0.0069	0.0035	0.0070	0.0203	0.0009
Low x <i>cerrado</i>	0.0040			-0.0120		
High x <i>cerradão</i>	0.0020			-0.0310		
Low x <i>cerradão</i>	-0.0131			-0.0310		

Table 4: LTRE contributions of each vital rate to the *H. drasticus* population growth rate differences.

	LTRE effect per vital rate							
	2008-2009				2009-2010			
	Stasis	Growth	Regression	Fertility	Stasis	Growth	Regression	Fertility
Harvest intensity								
High	0.00349	0.00093	0.00002	0.00004	-0.00900	-0.000001	3.18E-09	-0.000001
Low	-0.00251	-0.00042	-0.00001	-0.00029	0.01401	0.000008	-5E-08	0.000004
Ecosystem type								
<i>Cerrado</i>	0.00729	-0.00085	0.00001	0.00009	0.01900	0.000003	-3.5E-08	0.000006
<i>Cerradão</i>	-0.00493	0.00126	-0.00002	-0.00079	0.01399	-0.000001	2.55E-09	-0.000002
Interaction								
High x <i>Cerrado</i>	-0.00795	-0.00088	-0.00002	0.00026	0.00699	-0.000001	1.62E-08	-0.000003
Low x <i>Cerrado</i>	0.00323	0.00058	0.00001	0.0002	-0.01200	0.000001	4.9E-08	0.000004
High x <i>Cerradão</i>	0.00222	-0.0001	0.000001	-0.00007	-0.03100	4.43E-07	6.87E-09	0.000002
Low x <i>Cerradão</i>	-0.01297	-0.00034	-0.00002	0.00021	-0.03101	-0.000005	1.85E-08	-0.000006

Table 5: Elasticity values for the vital rates of different harvesting intensities and ecosystem types in 2008 - 2009

2008-2009	Vital rate elasticities						
	Size class						
Harvest	Vital rates	C1	C2	C3	C4	C5	C6
High harvest	Survival	0.03311	0.02581	0.01407	0.01019	0.01113	0.90567
	Growth	0.00421	0.00243	0.00228	0.00259	0.00239	-
	Regression	-	-0.00002	-0.00004	0	-	-
	Fecundity	-	-	-	-	0.00003	0.00445
Low harvest	Survival	0.0057	0.00481	0.00296	0.00179	0.00281	0.98192
	Growth	0.00094	0.00055	0.00055	0.00063	0.00057	-
	Regression	-	-0.00001	-8.07E-06	-2.01E-06	-	-
	Fecundity	-	-	-	-	2.40E-06	0.00096
Habitat							
<i>Cerrado</i>	Survival	0.00484	0.00269	0.00192	0.00144	0.00194	0.98716
	Growth	0.00072	0.00044	0.00044	0.00045	0.00042	-
	Regression	-	-6.68E-06	0	-1.76E-06	-	-
	Fecundity	-	-	-	-	8.60E-07	0.00073
<i>Cerradão</i>	Survival	0.02685	0.04359	0.01908	0.00937	0.01271	0.88839
	Growth	0.00418	0.00218	0.00252	0.00286	0.00252	-
	Regression	-	-0.00004	-0.00011	0.00000	-	-
	Fecundity	-	-	-	-	0.00005	0.00449
Interaction							
High harvest x <i>cerrado</i>	Survival	0.02383	0.00945	0.00676	0.0066	0.00615	0.94721
	Growth	0.00247	0.00146	0.00154	0.00143	0.00138	-
	Regression	-	-0.00003	0	0	-	-
	Fecundity	-	-	-	-	9.53E-06	0.00253
Low harvest x <i>cerrado</i>	Survival	0.00244	0.00156	0.00112	0.00073	0.00119	0.99296
	Growth	0.00042	0.00026	0.00025	0.00028	0.00025	-
	Regression	-	-3.86E-06	0	-1.35E-06	-	-
	Fecundity	-	-	-	-	3.07E-07	4.20E-04
High harvest x <i>cerradão</i>	Survival	0.02884	0.04121	0.02126	0.00938	0.01291	0.8864
	Growth	0.00455	0.00259	0.00272	0.00288	0.00263	-
	Regression	-	0	-0.00012	0	-	-
	Fecundity	-	-	-	-	0.00006	0.00492
Low harvest x <i>cerradão</i>	Survival	0.03311	0.06865	0.02282	0.01187	0.01632	0.84723
	Growth	0.00489	0.00179	0.00281	0.00351	0.00299	-
	Regression	-	-0.00012	-0.00009	0	-	-
	Fecundity	-	-	-	-	0.00008	0.00519

Table 6: Elasticity values for the vital rates of different harvesting intensities and ecosystem types in 2009-2010.

2009-2010		Vital rate elasticities					
		Size class					
Harvest	Vital rates	C1	C2	C3	C4	C5	C6
High harvest	Survival	2.06E-06	1.81E-06	1.49E-06	7.63E-07	1.24E-06	0.99999
	Growth	6.76E-07	5.88E-07	4.84E-07	3.89E-07	4.87E-07	-
	Regression	-	0	-6.11E-09	-	0	-
	Fecundity	-	-	-	-	7.39E-13	6.77E-07
Low harvest	Survival	0.00013	0.00009	0.00006	0.00005	0.00007	0.99960
	Growth	0.00003	0.00002	0.00003	0.00002	0.00002	-
	Regression	-	-7.12E-08	-1.89E-07	-	1.49E-07	-
	Fecundity	-	-	-	-	1.89E-09	0.00003
Habitat							
Cerrado	Survival	0.00008	0.00005	0.00003	0.00002	0.00003	0.99978
	Growth	0.00002	0.00001	0.00001	0.00001	0.00001	-
	Regression	-	-3.76E-08	-9.24E-08	-	-7.54E-08	-
	Fecundity	-	-	-	-	4.23E-10	0.00002
Cerradão	Survival	8.25E-07	7.57E-07	6.60E-07	4.87E-07	5.29E-07	0.99999
	Growth	2.68E-07	2.34E-07	2.00E-07	1.66E-07	1.80E-07	-
	Regression	-	0	-2.82E09	-	0	-
	Fecundity	-	-	-	-	1.63E-13	2.68E-07
Interaction							
High harvest x cerrado	Survival	0.00002	9.58E-06	6.82E-06	3.24E-06	6.10E-06	0.99995
	Growth	3.60E-06	2.65E-06	2.58E-06	1.92E-06	2.80E-06	-
	Regression	-	0	-3.70E-08	-	0	-
	Fecundity	-	-	-	-	2.28E-11	3.61E-06
Low harvest x cerrado	Survival	0.00027	0.00016	0.00011	0.00008	0.00012	0.99925
	Growth	0.00005	0.00004	0.00004	0.00004	0.00003	-
	Regression	-	-1.24E-07	-1.04E-07	-	-4.36E-07	-
	Fecundity	-	-	-	-	4.38E-09	0.00005
High harvest x cerradão	Survival	9.05E-08	7.48E-08	7.66E-08	4.71E-08	5.25E-08	0.99999
	Growth	2.85E-08	2.60E-08	2.10E-08	1.64E-08	1.80E-08	-
	Regression	-	0	0	-	0	-
	Fecundity	-	-	-	-	1.76E-15	2.85E-08
Low harvest x cerradão	Survival	0.00001	0.00001	9.15E-06	7.86E-06	8.48E-06	0.99995
	Growth	3.68E-06	2.79E-06	2.64E-06	2.35E-06	2.44E-06	-
	Regression	-	0	-6.40E-08	-	0	-
	Fecundity	-	-	-	-	3.02E-11	3.69E-06

4. Discussion

4.1 Effects of harvesting and the ecosystem type on population growth rates

There is a paucity of published studies that have employed matrix models to evaluate the effects of bark and exudate harvesting on natural populations, and thus, a general pattern about such effects cannot be described. Numerous studies about these resources limit their discussion to histograms of size distributions, which are not good predictors of future population trends or of their potential for sustainable management (Condit et al. 1998, Wright et al. 2003, Virillo et al. 2011).

Guedje et al. (2007) observed that bark harvesting did not affect the population growth rates of *Garcinia lucida* (Clusiaceae) in Cameroon, whereas Gaoue and Ticktin (2010) verified reductions in the population growth rates of the populations of *Khaya senegalensis* that suffered high levels of bark and leaf harvesting in Benin. Stewart et al. (2009) demonstrated that the exploitation of the bark of *Prunus africana* contributed to the mortality of reproductive adults, which caused a significant reduction in fruit production and, consequently, in the natural regeneration of harvested populations. *Hevea brasiliensis* (rubber tree) studies demonstrated that tapping its latex for exudates can reduce the diameter increases of exploited individuals (Dijkman 1951, Silpi et al. 2006). For *Boswellia papyrifera*, an African species that produces an internationally marketed resin (frankincense), a negative correlation was identified between the intensity of harvesting and the inflorescence number, number of seeds per inflorescence and seed viability (Rijkers et al. 2006). Such changes have affected recruitment patterns at the population level, with lower reproductive success associated with intensely explored populations (Rijkers et al. 2006). Furthermore, high seedling mortality can also limit their recruitment (Negussie et al. 2008).

Conversely, the harvesting of copaiba oil (*Copaifera reticulata*) did not decrease the regeneration of *C. reticulata* populations in the Eastern Amazon (Herrero-Jauregui et al. 2011). Similarly, our results did not demonstrate a clear association between harvesting practices and the regeneration of *H. drasticus* populations. We observed only one situation of population reduction, which might have been triggered by other factors that will be discussed below. Likewise, a recent study on the population dynamics of *B. papyrifera* revealed that a lack of regeneration and high mortality rates occurred both in exploited

populations and in preserved populations; thus, the population decline was not directly related to the harvesting but to other causes, such as fire occurrence, grazing and beetle attacks (Groenendijk et al. 2012).

Other factors beyond harvesting can affect the population growth. Gaoue and Ticktin (2010) observed that harvesting reduced *K. senegalensis* population growth rates, but ecological differences between regions produced a greater effect on λ than harvesting. In our study, we verified that the majority of the interactions between harvesting intensity and ecosystem type did not cause significant changes in the observed λ in both demographic intervals, except in the interaction between *cerradão* and high-harvest populations. Therefore, our results suggest that the *cerradão* populations are less resilient than the *cerrado* populations when subjected to high levels of exploitation. Moreover, the *cerradão* trees regenerate their bark at lower rates than trees in the *cerrado* (Baldauf and Santos, unpubl. data), highlighting the need for different management recommendations for each ecosystem.

We also observed high λ and confidence interval variation among the demographic intervals. The population growth rates varied more between years than between populations, which is a previously reported pattern (Buckley et al. 2010, Schmidt et al. 2011). The high intra-study λ variance observed in the majority of the NTFP studies reduces the power of this indicator to predict the sustainability of harvesting (Schmidt et al. 2011). Therefore, the results obtained in our study must be carefully interpreted in the context of the environmental variations in the study area during the sampling period to avoid erroneous conclusions about the impacts of harvesting latex from *H. drasticus* populations.

Rainfall likely promoted population changes in our study because the total precipitation in the study area in 2009-2010 was approximately half of the total precipitation registered in 2008-2009 (INMET 2012). Rainfall is a recognized important factor that might alter the demography of harvested species. Fox (2007) observed that climatic stochasticity in precipitation patterns and amounts was the major determining factor of the performance of the two NTFP species. Martínez-Ramos et al. (2009) verified that the growth and inflorescence production in the palm *Chamaedorea elegans* increased in an ENSO (El Niño Southern Oscillation) year, while survivorship and seed production

diminished significantly. ENSO years are characterized as periods of extreme water shortage and high temperatures.

In *H. drasticus*, the diaspores are dispersed during the rainy season (December-April), most likely because the moisture provided by the rainfall allows seedlings to develop a deep root system before the next drought, thereby increasing their chances of survival (Baldauf et al., unpubl. data). Rainfall is particularly important for the *cerradão* because it decreases the depth of the litterfall, thereby enhancing the chances of the seedling root system to reach the soil (Ferreira 2011). According to Buckley et al. (2010), post-seedling survival is the most vital contributor to the temporal variation in the population growth rate of perennial plants. We observed similar results in this study, in which *cerradão* seedlings exhibited a high mortality in the third survey (August-December 2010), in contrast to our previous results. Such mortality may be correlated with the extremely low precipitation levels of the previous rainy season (December 2009-April 2010).

4.2 Prospective and retrospective analysis

Bark harvest from trees is typically not sustainable, largely due to decreases in survival (Schmidt et al. 2011). The numerous species that are exploited for bark die soon after the debarking, most likely due to the disruption of water flow (Borges-Filho and Felfili 2003, Delvaux et al. 2007, Geldenhuys et al. 2007, Guedje et al. 2007). Surprisingly, *H. drasticus* survival was not affected by exploitation, except in the interaction among *cerradão* x high-harvest in 2009-2010. In this particular case, our results revealed that the stasis of the adult trees was the most important contributor to the reduction in the population growth rate. The higher contribution of adult survival to λ variations in the trees can be explained by the longer time they require to reach reproductive size (Gaoue and Ticktin 2010).

Few species can tolerate the removal of large quantities of bark, such as *H. drasticus*. *Walburgia salutaris*, an African medicinal plant, can vigorously regenerate the bark after its intense harvest (Cunningham and Mbenkum 1993, Botha et al. 2004). Some plant species from botanical families that are harvested for bark (e.g., Apocynaceae, Cannelaceae, Euphorbiaceae, Moraceae) display a great resilience partly because the

cambium is protected by exudates after the bark is removed (Cunningham 2001, Romero and Bolker 2008). This appears to be the case for *H. drasticus*, in which the latex continues to exude after its harvest and thus prevents fungal attack, promoting the survival of the exploited individuals. Moreover, most of the harvesters remove only the rhytidome without disrupting the vascular cambium; thus, the flow of water and photosynthates are not interrupted.

4.3 Implications for *H. drasticus* conservation and sustainable management

In general, we did not identify significant harvesting impacts in the two studied ecosystems. The different combinations of factors demonstrated the stability of the populations, indicating their potential for sustainable management. A unique combination of factors revealed a lower population growth rate than the one in 2009-2010. Because this population reduction occurred in a year following a greatly reduced rainfall period, environmental conditions may have influenced this result. Conversely, the LTRE data indicated a vulnerability of the *cerradão* populations under high levels of exploitation, which suggests a need for management criteria specific to each ecosystem studied.

Notably, the populations sampled represent extreme types in the study area; therefore, such high extraction levels as those observed in the high-harvest treatment are not a common pattern. However, there is a regional trend of increasing management intensity (Baldauf and Santos, 2013); thus, information about the possible impacts of high-intensity harvesting on population dynamics, such as those generated in this study, is vital.

For more than 40 years, ecologists have used matrix projection models to understand and guide the management of plant populations (Crone et al. 2011). However, these models are based on parameters typically estimated with only a few years of data and limited sample sizes, calling into question the accuracy of the results and predictions obtained (Doak et al. 2005). In contrast, a recent study that evaluated the ability of matrix models to explain plant population dynamics concluded that poor forecasts were not associated with the number of individual plants or years of data but rather with the environmental differences between the data collection and forecast periods (Crone et al. 2013). Consequently, to forecast population fates, more detailed models, such as those that

project how environments are likely to change and how these changes will affect population dynamics, should be employed (Crone et al. 2013).

In this context, the possible influence of plant succession in the dynamics of *H. drasticus* populations in FLONA-Araripe should be assessed in the future. Because the FLONA is a protected area, it has been supported by a firefighting governmental program. The absence of fire, a key element in the population dynamics of the Brazilian savanna, makes ecosystems more open, and the *cerrado* may be gradually replaced by forest ecosystems such as the *cerradão* (IBAMA 2004). Consequently, the *H. drasticus* population decline observed in the *cerradão* in 2009-2010 may represent a natural process that can be accelerated in populations enduring intense extraction. For this reason, alternatives, such as the species cultivation in agroforestry systems, must be evaluated to maintain the necessary stock of this valuable NTFP.

In conclusion, the data thus far provide the groundwork for establishing criteria for sustainably managing *H. drasticus*. Future studies that integrate environmental variables into more complex demographic models, such as the integral projection models (IPMs), may provide critical information for species management. Additionally, the criteria for sustainable management may be improved by including the traditional knowledge of the harvesters, who have been observing changes in *H. drasticus* populations and in the ecosystems of the study area for over 30 years.

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Appendix 1- *Himatanthus drasticus* matrices employed in the factorial LTRE.

	High harvest		Low harvest		Cerrado		Cerradão		Cerrado – High harvest		Cerrado - Low harvest		Cerradão – High harvest		Cerradão – Low harvest	
Vital rates	$t_0 - t_1$	$t_1 - t_2$	$t_0 - t_1$	$t_1 - t_2$	$t_0 - t_1$	$t_1 - t_2$	$t_0 - t_1$	$t_1 - t_2$	$t_0 - t_1$	$t_1 - t_2$	$t_0 - t_1$	$t_1 - t_2$	$t_0 - t_1$	$t_1 - t_2$	$t_0 - t_1$	$t_1 - t_2$
s1	0.822	0.615	0.766	0.710	0.797	0.738	0.776	0.504	0.850	0.700	0.765	0.766	0.778	0.504	0.774	0.503
s2	0.874	0.641	0.849	0.757	0.809	0.746	0.908	0.671	0.816	0.712	0.805	0.760	0.901	0.608	0.916	0.753
s3	0.788	0.644	0.781	0.661	0.747	0.633	0.834	0.681	0.742	0.610	0.750	0.646	0.844	0.676	0.827	0.685
s4	0.719	0.465	0.622	0.559	0.663	0.456	0.663	0.641	0.753	0.395	0.602	0.505	0.666	0.607	0.661	0.669
s5	0.755	0.576	0.761	0.704	0.755	0.652	0.762	0.641	0.744	0.526	0.761	0.714	0.762	0.612	0.763	0.680
s6	0.959	0.948	0.955	0.971	0.965	0.976	0.95	0.938	0.964	0.974	0.966	0.978	0.956	0.931	0.933	0.954
g21	0.011	0.001	0.006	0.004	0.003	0.002	0.02	0.002	0.004	0.001	0.003	0.004	0.022	0.001	0.017	0.004
g32	0.089	0.073	0.096	0.098	0.124	0.114	0.06	0.067	0.135	0.132	0.119	0.106	0.065	0.052	0.055	0.089
g42	0	0.001	0.002	0	0.002	0.001	0	0	0	0.003	0.003	0	0	0	0	0
g43	0.157	0.153	0.149	0.172	0.173	0.178	0.118	0.141	0.187	0.175	0.165	0.180	0.114	0.125	0.122	0.156
g53	0.006	0.013	0.004	0.002	0.006	0.006	0.003	0.007	0.005	0.016	0.006	0	0.007	0.010	0	0.004
g54	0.186	0.226	0.216	0.217	0.205	0.226	0.203	0.196	0.178	0.223	0.218	0.222	0.195	0.199	0.210	0.193
g64	0.010	0.003	0.003	0	0.006	0	0.005	0.005	0.008	0	0.005	0	0.011	0.011	0	0
g65	0.180	0.221	0.161	0.186	0.171	0.208	0.169	0.199	0.190	0.244	0.160	0.175	0.172	0.191	0.165	0.208
r12	0.001	0	0.003	0.001	0.003	0.001	0.001	0	0.004	0	0.003	0.001	0	0	0.002	0
r23	0.006	0.007	0.006	0.006	0	0.006	0.014	0.007	0	0.012	0	0.002	0.013	0	0.015	0.013
r34	0	-	0.003	-	0.003	-	0	-	0	-	0.005	-	0	-	0	-
r45	-	0	-	0.004	-	0.004	-	0	-	0	-	0.006	-	0	-	0
f5	0.674	0.056	0.807	0.221	0.721	0.2	0.756	0.025	0.798	0.149	0.671	0.239	0.764	0.006	0.738	0.065
f6	2.984	0.25	2.383	0.653	3.144	0.871	2.31	0.078	3.479	0.647	2.925	1.043	2.334	0.019	2.254	0.198

Capítulo 8

From peer-reviewed to people-reviewed: the participatory construction of a sustainable management plan for janaguba⁹

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1. INTRODUCTION

“Community management is not a panacea, but the only examples of successfully managed tropical production forest are found with local communities, and the correlates of sustainable management of common pool resources by communities are known”

Zimmerman and Kormos (2012)

Despite the considerable attention of conservationists and the international media on rainforests, particularly in the Brazilian Amazon, the Brazilian Savanna (Cerrado) remains one the most threatened biomes of South America due to the rapid expansion of the agriculture frontier characterized by soybean expansion and cattle-raising (Oliveira and Marquis 2002, Machado et al. 2004). There is a global lack of understanding of the importance of this biome at the international level despite the Cerrado being the richest savanna formation in terms of biodiversity. Because of its threatened status, species richness and high levels of endemism the Cerrado is considered a biodiversity hotspot of global significance (Oliveira and Marquis 2002, Mittermeier 2005).

Among the specific recommendations to its conservation are the consolidation of existing conservation units and the creation of new extensive protected areas in order to protect the remaining blocks of natural Cerrado (BRASIL 1999). Nevertheless, the creation of protected areas is not only costly but, as a single strategy, is insufficient to conserve the Cerrado in its entirety. Complementary strategies such as community-managed areas must be taken into consideration, since they can be equally, if not more effective in reducing deforestation than strictly protected areas (Porter-Bolland et al. 2012).

The Cerrado is home of a variety of traditional populations such as indigenous groups, *quilombolas* (descendants of slaves), harvesters and small-scale farmers (ISPN 2006). These populations have been collecting wild biological resources for many generations. Among the exploited species are many non-timber forest products (NTFP) with the potential for sustainable harvesting and long-term multiple-use.

Himatanthus drasticus (Apocynaceae) is a tree species commonly known as *janaguba* and one of the most commonly harvested NTFP in the Cerrado. It has been exploited since the 1970's due to the medicinal value of its latex for the treatment of

various forms of cancer. The harvesting of *janaguba* latex has become a source of income for many communities in the Cariri region, northeastern Brazil. This practice led the Institute for Forestry Development (IBDF) to prohibit the harvesting of *janaguba* to avoid the over-harvesting of the species. This, however, resulted in the establishment of an illegal market.

The Araripe National Forest (FLONA Araripe), located in the State of Ceará, is a site of intensive *janaguba* harvesting. In 1989, the office of IBAMA (Brazilian Institute for Environment and Natural Renewable Resources, now ICMBio - Chico Mendes Institute for Biodiversity Conservation) decided to legalize the harvesting of *janaguba* latex in the FLONA Araripe, as the species occurs in high densities and has potential for sustainable management. However, the harvesting of non-timber forest products can impact the species at different levels of biological organization; from genes to ecosystems. To examine the harvesting impact on this species, in 2008 the “Janaguba Project”*, was initiated by the Post-Graduate Program in Plant Biology - State University of Campinas (UNICAMP) and the Federal University of Semi-arid Region (UFERSA), in collaboration with the FLONA Araripe/Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) in close collaboration with local harvesters.

The main objective of “Janaguba Project” was to evaluate the ecological impacts of harvesting on natural populations of *janaguba* using different scales (from genes to ecosystems) and participatory approaches. Overall, the results suggest that there are no significant impacts caused by harvesting at the individual, population or ecosystem scales. At genetic level, it was observed a decrease in allelic richness in the seedlings of the high-harvest populations. However, the majority of the genetic diversity is maintained. In short, the harvesting remains potentially sustainable even after 40 years of exploitation and some adjustments can be made to avoid genetic losses in an adaptive management process.

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2. THE WORKSHOP

Developing a sustainable management plan for any NTFP will require the understanding of the synergies and trade-offs between different stakeholder opinions and conservation objectives. For that reason we held the following event: “Workshop on sustainable harvesting on non-timber forest products in the Brazilian Savanna” that took place on November 12 -13, 2012 in the municipality of Crato, Ceará State, Brazil (Appendix 1).

The main goal of the workshop was to discuss strategies to improve livelihoods and reduce anthropogenic pressure on the target NTFP species. Besides the *janaguba*, many NTFP’s such as the *pequi* (*Caryocar coriaceum*), the *faveira* (*Dimorphandra mollis*) and the *mangaba* (*Hancornia speciosa*) are harvested in the Araripe Plateau region. These products represent an important source of income for the local communities as well as food security for the families of the harvesters. The process of developing of a sustainable management plan for *janaguba* will serve as a framework for the future establishment of a broader strategy of use and conservation of forest products in the region.

During the workshop, aspects of the supply chain of *janaguba* were discussed and new commercialization rules were defined aimed at improving income for the direct harvesters. Valuing forest resources in this way is particularly important in avoiding deforestation since NTFP management can establish positive economic values for standing forest and discourage forest clearance for other destructive land uses such as gypsum mining that results in major changes in the landscape in the region.

2.1 Organizers and participants

The workshop was co-organized by the UFERSA (Federal University of Semi-arid), UNICAMP (State University of Campinas) and the FLONA Araripe/Regional Office of ICMBio (Chico Mendes Institute for Biodiversity Conservation). Among the partners were the Federal University of Minas Gerais (UFMG), the Brazilian Institute for Animal Rights and Environment (IBDVAMA) and the APA – Araripe (Environmental Protection Area of Chapada do Araripe).

The event brought together a wide range of stakeholders: resource users (harvesters), different members of the supply chain, government, technical and

administrative persons at local level, decision-makers, including official representatives of local and state government levels, local NGOs, researchers and students of local and regional universities, FLONA Araripe interns and volunteers, members of local communities and social movements. The total number of participants was 78.

2.2 Detailed activities and outcomes

2.2.1 First day

The programme of the workshop is presented in the Appendix 2. After the opening session and introduction of the participants (Figure 1), the main results of the “Janaguba project” were presented in accessible language so the harvesters had the opportunity of participate and discuss the results (Figure 2).

Based on the research, some adaptations in the current management systems were suggested in order to ensure the resilience and the reproduction of the species. The suggestions were the following: a) increase the minimum diameter for extraction from 7cm to 10cm; b) standardize the tool used for latex tapping avoiding the use of the machete, c) change the period of the closed season (*defeso*) to the months of November and December when the plant presents three reproductive phenological phases simultaneously; d) debark only two “sides” of each tree using a minimum interval of three years to exploit the other “sides” to guarantee the recovery of bark and the plant protection against external agents (mainly fire); e) consider the differences between areas since the bark regeneration in the cerrado (open savanna) is faster than in the cerradão (woody savanna). The time needed for total recovery is 5.0 and 6.5 years in the cerrado and cerradão, respectively; f) avoid intensive latex harvesting in the cerradão areas once the populations of *janaguba* are more vulnerable in this ecosystem due to the low natural regeneration and recruitment as well as slower bark recovery.



Figure 1. Chief of FLONA Araripe/Regional Office of ICMBio (Chico Mendes Institute for Biodiversity Conservation) opening the event.



A



B

Figure 2. Leader of “Janaguba Project” presenting (A) and discussing (B) the main results obtained in the research

The second presentation was about the management plan of the FLONA Araripe with emphasis on the aspects concerned with harvesting activities. The details of the management plan that were discussed are the following: a) the FLONA Araripe has no potential for timber exploitation; b) the collection of fuelwood for cooking is allowed once a week only for the registered harvesters; c) the controlled collection of non-timber forest products is permitted. After that, it was presented a detailed map with the zoning of the FLONA Araripe. The specific zones where the harvesting of *janaguba* is allowed were highlighted and the participants suggested that the zoning for the harvesting of *janaguba* should be redefined taking into consideration the results of the research with regard to the resilience of each ecosystem of the FLONA Araripe.

In the third presentation an expert of the Public Health Surveillance Department of the municipality of Crato (Ceará State) presented information about the existing legislation for production and marketing of pharmaceuticals and food products, and shared two

alternative frameworks to obtain trade licenses for *janaguba* products (Figure 3). He also described the steps needed to start the regularization process of the production and marketing of *janaguba* products and suggested that extractors and traders should organize themselves into an association or cooperative, as was done by other communities that are exploiting honey and *pequi* fruits (*Caryocar coriaceum*) in the same region.



Figure 3. Expert of the Public Health Surveillance Department of Crato talking about legislation issues (A) and participants questioning the speaker (B)

In the afternoon, the participants were divided in three groups to facilitate the debate and enhance the participation of all stakeholders (Figure 4). The first group was responsible for discussing the ecological aspects of the management and the adequacy of the environmental zoning proposed in the FLONA Management Plan. After the discussion, the group suggested that the zoning must include only the harvesters and communities who live in the vicinity of the FLONA. Also, according to the group the harvest should not take place in the area known as “strictly controlled” in the zoning, once the priority for this area is the conservation of the main ecosystems of the FLONA. Participants in this group also recommended reducing the number of harvesters in the region of *Belmonte* due to overharvesting in the region and the low resilience of the *cerradão* in relation to harvesting. They also emphasized the need for better supervision of the harvesting activity and training courses for registered harvesters. The group concluded that the amount of cuts and frequency should respect the type of vegetation; however, four cuts (sides) should not be undertaken in any context. The plants must be over 3m high before being exploited and the interval between extractions must be two and a half years for open areas and three years for shade areas. These intervals were proposed to ensure that nearly 50% of the bark would be

recovered before the exploitation of the other “two sides” since the time needed for total recovery is 5.0 and 6.5 years in the cerrado and cerradão, respectively.



Figure 4. Group debating the criteria for sustainable management of *janaguba* (A) and discussion concerning the legislation about medicinal plants trade in Brazil (B)

The second group discussed issues related to registration of the harvesters, the rules of access to the resource in the FLONA, standardization of methods of latex harvesting and the amount of latex removed daily. The group recommended that latex extraction should only be performed by people that have the authorization of the local environmental agencies. They suggested having a new zoning to include the data obtained by the “Janaguba Project” and thus determine whether there are conditions whereby new people are registering to extract the product. The group believes that the area already has reached its maximum carrying capacity. Participants suggested that it is necessary to standardize the tools used for tapping the latex and highlighted the need of hygiene measures to avoid latex contamination by microorganisms. The amount of latex collected will depend on the environment but a maximum of three liters of pure latex per day was suggested by the group.

The third group discussed the collective organization of the communities into an association or cooperative. Other issues debated by this group were the standardization of the harvesting process and good hygiene and handling practices. The group argued that the preparation of the product for consumer use must be undertaken with care and cleanliness of the manufacturing place and the container where the latex is stored. For the production of one liter of *janaguba* milk it was defined that a standard mixture should consist of 25% of pure latex and 75% of water. The group concluded that good manufacturing practices are a pre-requisite to ensure product quality and the approval of the *janaguba* products by

ANVISA (National Health Surveillance Agency). Some members of the group did not agree with the creation of an association so this issue was discussed again in the second day of the workshop.

2.2.2 Second day

The second day of the workshop consisted of a field activity in a highly harvested area of *cerradão*. The event organizers provided information about the area and divided the participants into groups where they were informed about the measurements methods used during “Janaguba project” (three groups). Participants were able to learn the techniques and use the equipments (calipers, measuring tape and bark gauges) employed to produce the ecological information on reproductive phenology, demography and biomass recovery (Figure 5). The other two groups were led by local harvesters who shared their knowledge about the autoecology of the species and the traditional management systems of *janaguba*. The participants had the opportunity of learning the practical methods of *janaguba* latex harvest, checked the minimum sizes to harvest and also observed examples of practices that can damage the plant and increase the risk of mortality by pathogens and fire.

In the afternoon a questionnaire was applied to discuss the management practices and the details of the supply chain of the *janaguba* (Figure 6). Participants answered 15 questions and a subsequent discussion was conducted based on this information to establish a general consensus on the discussed issues (Table 1 and Appendix 3).



A



B



Figure 5. Field activity in the FLONA Araripe. Explanation about the experiments performed during “Janaguba Project” (A), student measuring bark regeneration (B); harvesters sharing their knowledge about the autoecology of *janaguba* (C) and traditional management systems (D).



Figure 6. Participants filling out the questionnaire (A) and voting about management practices and supply chain details (B).

Table 1- Summary of the questions and votes of the participants of the workshop

Subject	Voting				Total voting	Notes
	Options	FA ^a	AG ^a	AB ^a		
1. Collective or individual management	Organization of an association or cooperative.	34	13	0	47	
2. Maximum number of harvesters exploiting <i>janaguba</i> in FLONA Araripe.	Not discussed	-	-	-	-	Insufficient time to discuss this issue.
3. Maximum amount of latex collected per month in FLONA Araripe	Not discussed	-	-	-	-	Insufficient time to discuss this issue.
4. Value paid for a liter of <i>janaguba</i> milk, ready for consumption.	R\$ 20.00 R\$ 25.00 R\$ 30.00	1 14 16	30 17 15	16	47	After discussion it was decided to charge R\$ 25.00 per liter.
5. Percentage of market price to be paid for local harvesters.	40% 50% 60% 70%	2 14 15 3	32 20 19 31	13	47	After discussion it was agreed to pay 60% of market price to local harvesters.
6. Percentage of market price to be paid to ICMBio for raw material.	10% 15% 20% Payment by harvesters Payment by trader Payment by both	22 1 5 2 2 24	8 29 25 26 26 4	17 19	47 47	
7. Trader profit	-	-	-	-	-	Is implied in the voting of items 4, 5 and 6.
8. Minimum height for harvest	3m for cerrado and 4m for cerradão	35	8	4	47	
9. Minimum thickness for harvest	10cm in diameter or 31cm in perimeter measured at breast height.	35	8	4	47	Suggestion: use a ring made of plastic bottle for the measurement of the thickness standard.
10. Maximum debarking width	About 7cm width	31	10	6	47	
11. Maximum debarking extension	About 0.50m above ground until 2m height.	36	5	6	47	
12. Time lag between harvest events	2.5 years in the cerrado and 3 years in the cerradão	41	2	4	47	

13. Extension of debarking in each plant	Two “sides” per plant	39	2	6	47	
14. Local harvesters cooperation on FLONA Araripe protection.	Not discussed	-	-	-	-	Insufficient time to discuss this issue.
15. Monitoring and surveillance on <i>janaguba</i> latex harvesting	Not discussed	-	-	-	-	Insufficient time to discuss this issue.

^a FA – Favorable, AG – Against, AB - Abstentions

3. Evaluation of the workshop and future perspectives

The expected outcomes of the workshop were achieved. The challenges and possibilities of sustainable harvesting of *janaguba* were identified considering the results obtained in the “Janaguba Project” and multiple dimensions of sustainability (ecological, socio-economic, cultural). Based on the discussions it was possible to develop guidelines for the sustainable harvesting of the species through a deliberative and participatory approach. The decisions about the questions 8-13 of the Table 1 will constitute the core of the “Sustainable Management Plan” for the harvesting of *janaguba*. The plan will be send to the central office of ICMBio in Brasília (Federal capital) for publication. The “Sustainable Management Plan” will also be published in the form of “Cordel literature”^{*}, a very popular literature genre in Northeastern Brazil. The publication of a “cordel” about the *janaguba* will serve as an educational tool to be used in schools, universities and community associations among other institutions.

In addition to the advances achieved in ecological terms, an important outcome of the workshop was the negotiation and approval of the increase in the profit of the harvesters on the final product. In general, the income of the non-timber forest products harvesters is minimal and most of the profit is obtained by the other members of the supply chain. In the case of trade of *janaguba* milk, the profit of the harvesters used to be about 20-40% of the value of the market value. After the workshop an agreement was reached and the profit of the harvesters will be at least 60% from January 2013, which represents a significant gain for the harvesters.

^{*} http://en.wikipedia.org/wiki/Cordel_literature

Some questions of the questionnaire were not debated during the meeting due to the lack of time (Table 1). These questions will be addressed during the periodic meetings of the “Advisory Council of the FLONA Araripe” where there are representatives of the harvesters. However, the main objectives of the workshop were accomplished including the discussion of the main results obtained by the “Janaguba Project” with all stakeholders. Moreover, the most important issues related to the ecological and economical dimensions of sustainability were debated and relevant decisions were taken in order to guarantee the resilience of *janaguba* natural populations and improve livelihoods in the Cariri region.

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5. Appendix

1. Workshop poster



**1º ENCONTRO
SOBRE A JANAGUBA**

**Para tiradores de leite de janaguba, pesquisadores,
estudantes, representantes do poder público,
ONGs e interessados em geral**

**Venha conhecer os resultados das pesquisas e
participar da construção do plano de manejo
sustentável para a janaguba!**

**Dias 13 e 14 de novembro
às 8h**

**Local: Creche São Miguel
Crato, CE**


lebensministerium.at

 
INSTITUTO CENSO MENDES
MMA

Diagramação: Karina Hmeljevski



2. Programme of the workshop

FIRST DAY	Coordination	Timeframe
Registration	- FLONA Araripe volunteers	30 minutes
Breakfast		30 minutes
Opening session/participants introduction	- Cristina Baldauf (UFERSA) - Willian Brito (FLONA Araripe)	30 minutes
“Janaguba project”: results of the ecological studies/discussion	- Cristina Baldauf (UFERSA)	45 minutes
Management Plan and Zoning of FLONA Araripe with emphasis in harvesting activities/discussion	- Pedro Monteiro (FLONA Araripe)	30 minutes
Legislation about food and medicinal plants trade/discussion	- Assilou Freitas (Public Health Surveillance Department- Municipality of Crato- Ceará)	60 minutes
LUNCH		
Thematic group discussion	- Willian Brito (FLONA Araripe) - Pedro Monteiro (FLONA Araripe)	120 minutes
Coffee break		20 minutes
Presentation of the groups synthesis	- Cristina Baldauf (UFERSA)	60 minutes
SECOND DAY		
Field activity	- Cristina Baldauf (UFERSA) - Willian Brito (FLONA Araripe) - Local specialists (harvesters) - Interns of “Janaguba Project”	4 hours
LUNCH		
Participatory and deliberative discussion: ecological aspects	- Cristina Baldauf (UFERSA) - Christiane Corrêa (UFMG)	90 minutes
Participatory and deliberative discussion: economical aspects	- Willian Brito (FLONA Araripe) - Pedro Monteiro (APA Araripe)	90 minutes
Evaluation of the meeting and future perspectives	- Cristina Baldauf (UFERSA) - Willian Brito (FLONA Araripe)	30 minutes

3. Questionnaire

INSTITUTO CHICO MENDES DE CONSERVAÇÃO DA BIODIVERSIDADE – ICMBio
FLORESTA NACIONAL DO ARARIPE

Consulta pública para aprimoramento do manejo da Janaguba (*Himatantus drasticus*)

Responda por gentileza as questões abaixo, da forma mais precisa possível para que possamos aprimorar o manejo da Janaguba, assegurando a conservação desta espécie, bem como a geração de ocupação e renda.

1. No seu entendimento o manejo da Janaguba deve ser coletivo ou individual? Por que?
2. Qual o limite suportável de coletores para a FLONA Araripe de modo a evitar a extinção da Janaguba?
3. Qual a cota máxima para extração de leite de Janaguba por coletor a cada mês?
4. Qual o valor justo para um litro de leite de Janaguba manipulado, pronto para o consumo?
5. Deste valor quanto deve ser pago ao extrator?
6. Quanto deve ser pago ao ICMBio pela matéria-prima?
7. Qual deve ser o lucro do comerciante?
8. Qual a altura mínima da planta para que ela seja desleitada?
9. Qual a espessura (grossura) mínima da planta para que ela seja desleitada?
10. Qual a largura máxima do corte?
11. Qual a extensão (comprimento) máximo do corte?
12. Qual o intervalo de tempo entre um corte e outro?
13. Qual o limite para o número de cortes (talhos) por planta?
14. Como conseguir a colaboração dos extratores na proteção da Floresta Nacional?
15. Como monitorar e fiscalizar a extração do leite da Janaguba?

CONSIDERAÇÕES FINAIS

Esta tese teve como objetivo principal a avaliação da sustentabilidade do extrativismo de janaguba em áreas de Cerrado. É mister ressaltar que a chefia da FLONA Araripe teve um papel central na definição deste objetivo, uma vez que a proposta inicial da tese era o estudo do extrativismo de outra espécie, a mangaba (*Hancornia speciosa*). Visando atender as demandas de pesquisa mais urgentes desta unidade de conservação, aceitamos a sugestão de estudar o extrativismo de janaguba, por esta representar um dos recursos mais explorados e pouco estudados na FLONA Araripe. Destaca-se ainda o pioneirismo da FLONA ao exigir o compromisso dos pesquisadores em relação ao retorno dos resultados das pesquisas, a qual é exemplificada nas palavras de Willian Brito, atual chefe da unidade:

*“O pessoal da janaguba também quer saber se vai haver mudanças no manejo da espécie. E eu estou precisando de suas conclusões e recomendações. Como já lhe falei muitas vezes, a pesquisa é fundamental para a gestão ambiental (...). Conto com sua colaboração para **ordenar ou proibir** (grifo meu) o extrativismo da janaguba.”*

A consciência de que nosso trabalho poderia afetar a geração de renda e o sustento de um número considerável de famílias na área de estudo nos motivou a fazer uma análise abrangente da atividade extrativista e de seus impactos, utilizando diversas abordagens e metodologias complementares.

Com base nos principais resultados obtidos, pode-se afirmar que a janaguba possui amplo potencial para manejo sustentável. Uma série de atributos da espécie favorecem a sustentabilidade da coleta na área de estudo, tais como: alta densidade, regeneração abundante, bem como as propriedades antimicrobianas e cicatrizantes do seu látex, as quais possivelmente impedem o ataque fúngico após a retirada da casca, auxiliando ainda na sua regeneração. Adicionalmente, algumas características do sistema de manejo tradicional, como a retirada superficial da casca sem causar lesões nos tecidos vasculares e a remoção de baixas quantidades de casca tendem a diminuir os impactos ecológicos do extrativismo.

Por outro lado, verificamos que a combinação entre fatores climáticos, sucessão vegetal e alta intensidade de extrativismo pode ter causado uma redução populacional nas áreas de cerradão em um dos períodos amostrados neste estudo. Além disso, registramos

uma pequena redução da diversidade genética em populações sofrendo intensa exploração. Ainda que as populações que consideramos altamente exploradas neste trabalho representem tipos extremos e pouco comuns na área de estudo, é preciso que se levem em consideração os potenciais impactos descritos. Desta forma, é fundamental que o plano de manejo sustentável de atividade seja cumprido a fim de garantir a conservação das populações de janaguba. Para tanto, a fiscalização da atividade extrativista por parte da FLONA Araripe, em parceria com os agentes locais, é de suma importância.

Um aspecto bastante controverso ao longo desse estudo foi a adoção de intervalos adequados entre as coletas. Nossos dados apontaram para intervalos de 5 e 6,5 anos para a completa regeneração das cascas dos indivíduos explorados em áreas de cerrado e cerradão, respectivamente. Do ponto de vista ecológico, seria importante a recuperação das cascas antes de um novo evento de extração no mesmo indivíduo, devido aos aspectos discutidos no capítulo 6. No entanto, nossos dados de etnobotânica (capítulo 1) revelaram que os intervalos praticados pelos extratores são muito menores, alguns deles inferiores a um ano. De fato, quando apresentamos nossos resultados, os extratores consideraram os intervalos ideais obtidos em nosso estudo como economicamente inviáveis. Assim, após um intenso debate com a participação de todos os interessados, chegou-se ao consenso de intervalos de 2,5 e 3 anos para o cerrado e cerradão, respectivamente, bem como a exploração máxima de “dois lados” de cada indivíduo. Estes intervalos permitiriam a regeneração de aproximadamente 50% da profundidade da casca antes de uma nova coleta, o que garantiria uma proteção (ainda que parcial) dos indivíduos explorados e, simultaneamente, contemplaria a dimensão econômica do manejo sustentável da espécie.

Destaca-se ainda que, mesmo com a implementação do plano de manejo sustentável na região, o aumento do interesse na espécie e a crescente comprovação das suas propriedades medicinais poderá acarretar futuramente em sobre-exploração das suas populações, tendência já observada em um grande número de PFNM. Neste contexto, alternativas como o cultivo de janaguba, especialmente em sistemas agroflorestais, merecem ser avaliadas quanto a sua viabilidade em estudos futuros. Nossos resultados representam um ponto de partida para os processos de cultivo e conservação *on farm*, ao fornecerem diretrizes para a coleta de sementes visando à manutenção da diversidade genética das populações.

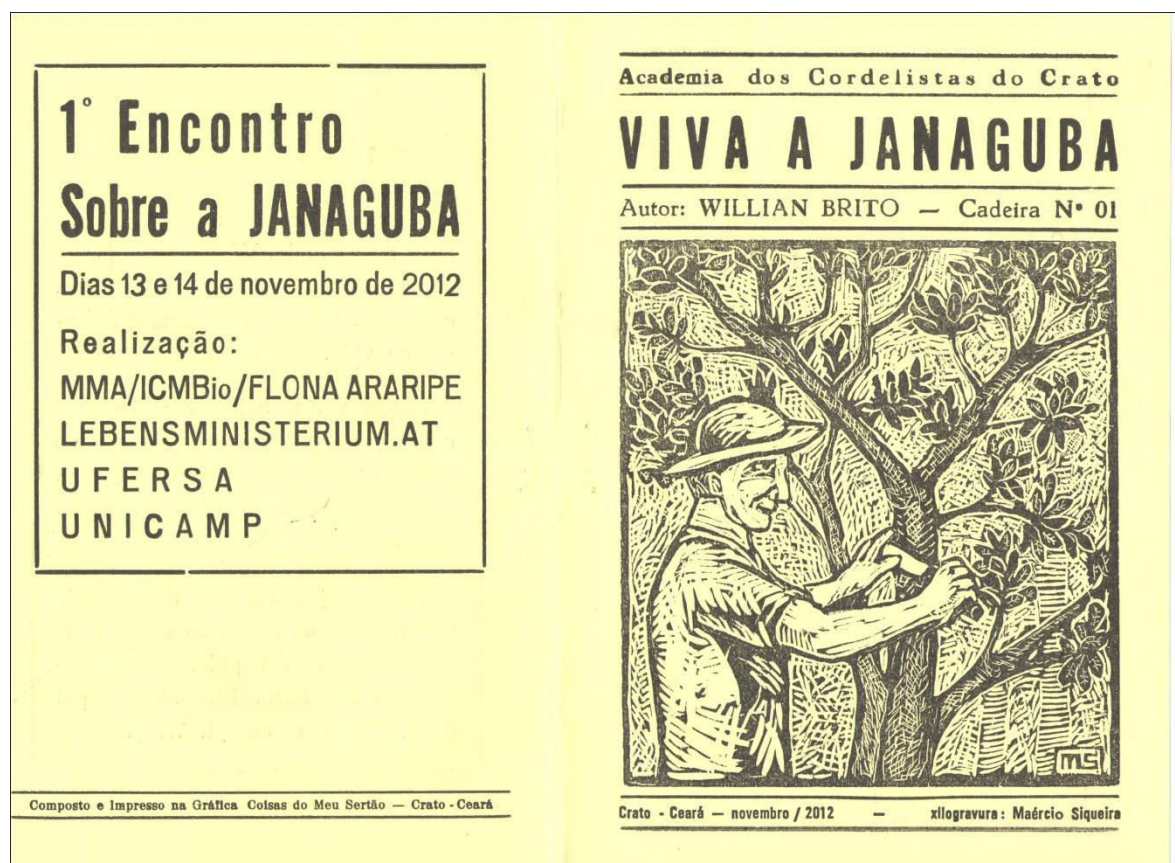
Outro aspecto importante, o qual não foi abordado nesse trabalho e merece maior atenção se refere à dinâmica populacional da espécie na presença de fogo, uma vez que nossa pesquisa foi realizada em uma unidade de conservação, a qual conta com o auxílio do programa “Prevfogo” do IBAMA. Este programa tem atuado no controle de queimadas e prevenção e combate aos incêndios florestais. Consequentemente seria importante avaliar a sustentabilidade da coleta da espécie em outras regiões, sujeitas às queimadas, para que os resultados possam ser corroborados ou adaptados a esta situação. De todo modo, procuramos considerar a possibilidade de queimadas ao discutir e deliberar coletivamente os critérios do plano de manejo sustentável da espécie. Dentre as medidas aprovadas, encontra-se a adoção de uma altura mínima para a remoção da casca, a fim de proteger os indivíduos explorados de prováveis danos causados pelo fogo rasteiro.

Tendo em vista a escassez de estudos avaliando a sustentabilidade do extrativismo de PFNM no Cerrado, temos consciência de que nossa contribuição foi ínfima frente às imensas lacunas que existem, tanto na pesquisa científica quanto na implementação de estratégias de manejo sustentável e geração de renda neste bioma. Não obstante, frente ao cenário pessimista global em relação ao papel dos PFNM na conservação da biodiversidade, o manejo tradicional da janaguba exemplifica o que educador Paulo Freire chamaria de “inédito viável”, oferecendo resistência à visão fatalista dominante na literatura da área. Além disso, avaliamos que a renegociação e o decorrente aumento dos valores recebidos pelos extratores pelo produto final (capítulo 8) foi outra consequência positiva deste trabalho, representando não apenas uma melhoria das condições de vida destes atores sociais, mas também uma valorização de suas práticas sustentáveis de manejo. Neste contexto, encerramos o relato dessa jornada de cinco anos no Cariri Cearense e de suas pequenas contribuições com as palavras de Eduardo Galeano:

“São coisas pequenas. Não acabam com a pobreza, não nos tiram do subdesenvolvimento, não socializam os meios de produção, não desapropriam as covas de Ali Babá. Mas talvez desencadeiem a alegria de fazer e a traduza em atos. Afinal de contas, atuar sobre a realidade e mudá-la, ainda que seja um pouquinho, é a única maneira de provar que a realidade pode ser transformada.”

ANEXOS

1. Capa do cordel “Viva a janaguba”, composto pelo poeta Willian Brito com a finalidade de divulgação dos resultados da tese e do plano de manejo sustentável da janaguba



2. Texto do cordel “Viva a Janaguba” de autoria do poeta Willian Brito

Viva a Janaguba!

Willian Brito

1
Na chapada do Araripe,
Na Floresta Nacional
De tantas plantas queridas
Uma é muito especial,
Chamada de Janaguba,
Pau-de-leite, ou Sucuuba,
Merece espaço em jornal.

2
Da família Apocinácea,
A mesma da mangabeira,
A Janaguba é um tesouro,
Uma planta de primeira;
Desde muito admirada,
Conhecida e utilizada
Pela gente brasileira.

3
A Janaguba é nativa
De Sergipe ao Ceará;
Da Bahia até Roraima,
Das Gerais até o Pará;
Existe até na Guiana,
Salvando a pessoa humana
Aqui, ali, acolá.

4
Em meados de setenta
Do século 20 passado,
Um paciente com câncer
Até já desenganado
Em casa ouviu de um vaqueiro
Que a Janaguba ligeiro
Melhorava o seu estado.

5
Ele consultou no Crato
Doutor Ulisses, famoso,
Se tomava esse remédio
Ou se acaso era reimoso;
E ele disse vá em frente,
Acredite, lute, tente,
Pois seu caso é perigoso.

6
E o homem mandou raspar
Janaguba na chapada,
O Doutor foi calibrando
A dose recomendada,
Até que o homem sarou,
E a notícia se espalhou
Pra tudo quanto é quebrada.

7
Foi aquele ribuliço,
Muita gente quis provar.
E como dava dinheiro
Danou-se o povo a talhar,
Fazer o leite e vender,
Sem ligar como ia ser
Pra Janaguba escapar.

8
Como a saída era grande
Teve muito espertalhão
Que passou gato por lebre
Lesando a população,
Leite de mangaba e goma,
Para paciente em coma
Venderam na região.

9
Na Floresta Nacional
A coisa ficou terrível.
O povo raspando as plantas
Numa proporção incrível,
Com uma sanha assassina,
Mesmo à luz de lamparina
Perigando um fogo horrível.

10
Jackson Nuvens, do IBAMA,
Um funcionário ativo,
Pedi ao chefe da FLONA
Um recurso criativo
Que acabasse com a guerra
E desse ao povo da terra
Um salário alternativo.

11
Willian Brito propôs
Fazer um experimento:
Organizar a coleta,
Oferecer treinamento,
Definir um dia certo
E o território mais perto
Evitando o sofrimento.

12
Willian foi pro mestrado,
Da chefia se afastou,
Mas Verônica assumiu
E a extração prosperou,
Só faltava a Academia
Isenta e sem agonia
Ver como a coisa ficou.

13
Cristina Baldauf veio,
Pesquisar pro doutorado,
Feito lá na UNICAMP,
E chegou com um gás danado;
Marcou 14 mil plantas
E sem achar que eram tantas
Acompanhou seu estado.

14
Com uma pequena equipe,
Vencendo muita barreira,
Cristina mediu a casca,
Observou a madeira,
Viu toda reprodução,
E o dano da extração
Por nossa Floresta inteira.

15
Mundô, Galdino, Damásio...
Muita gente ela escutou
E o saber do nosso povo
Com a ciência ela juntou,
De modo que a pesquisa,
Mais completa, mais precisa,
Mais exata 'inda ficou.

16
E com o resultado em mãos
Ela fez questão de vir
Promover um belo encontro
E com todos repartir
O jeito, a forma, a maneira,
Da nação caririzeira
Explorar sem destruir.

17
Disse ela: “minha gente,
Não caia no triste ardil,
De explorar sua riqueza
Duma maneira febril,
Como se fez no passado
Reparem no triste estado
Do mogno e do pau-Brasil”.

18
“Araucária, violeta,
Imbuia e jequitibá,
Angico, cedro, pau-ferro,
Angelim e jatobá,
Minguaram como a peroba,
Os ipês e a andiroba,
Por ganância, coisa má”.

19

“A Janaguba explorada
De maneira racional
Sem excesso é coisa boa
Pra Floresta Nacional.
Mas é preciso um acordo
Que evite o olho gordo
Que tem efeito fatal”.

20

E o acordo foi firmado
Com base na confiança
Na pesquisa, na justiça,
Na lei e na governança;
No respeito e no seguro
Do presente e do futuro
Que tem direito a herança.

21

Um bom plano de negócio
Sustentável, eficiente,
Deve ser negociado
Entre a Floresta e a gente
Da cadeia produtiva,
De modo que a planta viva
Pra muito além do presente.

22

O manejo deve ser
Legal e comunitário;
E todo extrator precisa
Ter cadastro e vestuário
Que fácil lhe identifique;
E a coleta que pratique,
Bom estado sanitário.

23

Planta mais fina que um litro
Não pode ser desleitada,
E a reprodução da espécie
Deve ser facilitada.
Tem defeso de novembro
Até o mês de dezembro
Antevendo a trovoada.

24

Não se deve usar no corte
Faca, trinchete ou facão,
Porque vão até o cerne,
Causam profunda lesão;
Sendo foice ou roçadeira
A planta sara ligeira
E mantém a produção.

25

Só dois lados cada vez
O extrator deve cortar
E precisa dar um tempo
Até o corte fechar.
Se for com muita ganância
E fizer extravagância,
Muita planta vai matar.

26

Todo corte tem limite
Na porção inferior
A altura do joelho
Do cidadão extrator,
Para evitar mortandade
Quando fogo a humanidade
Causa a FLONA, meu senhor.

27

Pra garantir que haverá
Justiça e paz social,
Ficou também acordado
Que do produto final,
Será 60%
O direito, o pagamento,
Do extrator tradicional.

28

A Floresta tem direito
A receber 10%,
Como uma compensação
Não tanto por pagamento,
Pois vale mais do que isso
O cuidado, o compromisso,
Do extrator bom elemento.

29

A coleta deve ser
Muito bem monitorada
Pra evitar que a espécie
Venha a ser ameaçada
Em sua conservação,
E que a população
Tenha a saúde lesada.

30

Mas o ICMBio
Sozinho não vai poder
Dar conta dessa missão,
Daí precisa envolver,
Vigilância sanitária
E a ação comunitária
Pra todo excesso conter.

31

Seja você um fiscal
Da saúde e do ambiente,
Faça valer seu direito
Não aceite, não aguente,
Abuso de explorador,
A lei do consumidor
Exija daqui pra frente.

32

Se você colaborar
Um dia será viável
Nós termos no Cariri
Sociedade notável,
Digna de admiração
Da nossa grande nação
Porque será sustentável.