UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA VEGETAL

EDITO ROMÃO DA SILVA NETO

CONSEQUÊNCIAS DA PARTILHA DE POLINIZADORES USANDO DUAS ESPÉCIES DE *CNIDOSCOLUS* (EUPHORBIACEAE) COMO MODELO

Recife
2023

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Dissertação apresentada ao Programa de Pós-Graduação em Biologia Vegetal da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de Mestre em Biologia Vegetal; Área de concentração: Ecologia e Conservação.

Orientadora: Profa. Dra. Isabel Cristina Sobreira Machado
Coorientadora: Profa. Dra. Zelma Glebya Maciel Quirino

Recife
2023

Dados Internacionais de Catalogação na Publicação (CIP) de acordo com ISBD

Silva Neto, Edito Romão
Consequências da partilha de polinizadores usando duas espécies de *Cnidoscolus* (Euphorbiaceae) como modelo / Edito Romão Silva Neto– 2023.

52 f. : il., fig., tab.

Orientadora: Isabel Cristina Sobreira Machado
Coorientadora: Zelma Glebya Maciel Quirino

Dissertação (mestrado) – Universidade Federal de Pernambuco. Centro de Biociências. Programa de Pós-Graduação em Biologia Vegetal, Recife, 2023.
Inclui referências.

1. Polinização por insetos 2. Angiosperma 3. Caatinga I. Machado, Isabel Cristina Sobreira (orient.) II. Quirino, Zelma Glebya Maciel (coorient.) III. Título

571.8642 CDD (22.ed.) UFPE/CB – 2023 -214

Elaborado por Elaine C Barroso - CRB-4/1728

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Aprovada em 25/08/2020.

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AGRADECIMENTOS

Gostaria de agradecer imensamente a minha Orientadora, Profa. Dra. Isabel Machado, e a minha Coorientadora, Profa. Dra. Zelma Quirino, por toda a fundamental ajuda que me foi dada, por todos os ensinamentos, o apoio, incentivo, paciência, compreensão, sobretudo por terem acreditado em mim. Por mais consciente que eu possa ser ou vir a ser, jamais conseguirei ter completa noção da minha sorte em tê-las como orientadoras. Sou profunda e eternamente grato.

Ao Ilton Neto, por toda a amizade cultivada desde que nos conhecemos na graduação, por toda a ajuda durante a fase de campo do mestrado, mais que isso, foi minha dupla, parceiro de campo, sofreu tanto quanto eu nos tricomas urticantes das espécies que estudei, por todas as ideias compartilhadas, os incontáveis momentos que sempre tornavam as idas a campo tão alegres, a sua grande habilidade como piloto que nos manteve saudáveis pelos caminhos arenosos, rochosos e tortuosos até à Serra. O tenho como um irmão. Não tenho dúvidas que eu não conseguiria ter feito tanto sem a sua ajuda.

À família do Ilton, Geane, Tenório, Tainá, Sra. Marluce, Sr. Ilton, por terem me recebido de maneira tão fantástica em suas casas. Todo o carinho da recepção e o suporte que sempre ofereceram aos alunos que conduziam pesquisas na Serra do Jatobá fizeram das idas à Serra Branca sempre muito confortáveis. Tenho-os como uma segunda família.

À Dra. Dora, por ter disponibilizado sua propriedade e instalações na Serra do Jatobá para que pudéssemos ter realizado nossas pesquisas e estabelecido uma base de operações, o que foi essencial para termos melhor acesso ao local de estudo e permanecer mais livremente no campo.

Aos alunos e amigas do Laboratório de Ecologia Vegetal da UFPB, Alinny, Josefa, Ellen, Thayana, pela ajuda em campo e/ou em laboratório, obrigado por terem compartilhado o sofrimento causado pelos tricomas das plantas comigo.

A todos que de maneiras tão distintas me ajudaram ao longo do percurso até aqui, que torceram por mim, estiveram ao meu lado me dando forças. Muito obrigado.

À CAPES, pela concessão da bolsa e financiamento da pesquisa.

RESUMO

Angiospermas com atributos florais semelhantes frequentemente atraem os mesmos grupos de polinizadores e, em espécies sincronopátricas, a partilha de polinizadores pode resultar em competição, facilitação ou ter efeitos neutros. Analisamos, portanto, os efeitos da partilha de polinizadores entre duas espécies de Euphorbiaceae (*Cnidoscolus infestus* e *C. urens*) com populações em isolamento e coocorrência na Serra do Jatobá, um afloramento rochoso na Caatinga localizado no município de Serra Branca-PB. Utilizamos a floração, os atributos morfométricos, os visitantes florais e o sucesso reprodutivo das diferentes espécies em manchas de isolamento e coocorrência para determinar as consequências da partilha. A floração das duas espécies possui alta sobreposição, porém difere quanto à duração do período de pico. Verificamos diferenças significativas nos atributos florais, como tamanho das flores e da antera entre as espécies. Nas flores pistiladas, a abertura, o comprimento do tubo e do pistilo diferiram entre as espécies e entre as manchas. A produção de frutos variou significativamente entre as manchas. O visitante floral e polinizador mais frequente em ambas as espécies foi o beija-flor *Chlorostilbon lucidus*. O número de flores visitadas foi estatisticamente diferente entre as manchas, com as populações em coocorrência recebendo menor quantidade de visitas. Concluímos que não ocorre facilitação entre as espécies. A competição é enfraquecida em função das diferenças de estratégia de floração e atributos morfométricos, permitindo, dessa maneira, a coexistência entre as populações.

Palavras-chave: Caatinga; Competição; Facilitação; Polinização; Sucesso reprodutivo.

ABSTRACT

Flowering plants share pollinators when they occur, bloom at the same time and area, and have a similar set of floral traits. We aimed to assess the effects of pollinator sharing between two species of Euphorbiaceae (*Cnidoscolus infestus* and *C. urens*) when they occurred associated and non-associated in patches from the semiarid Caatinga. We used flowering, morphometric attributes, floral visitors, and the reproductive success of both species to determine the consequences of pollinator sharing. We found high overlap in the flowering period of the two species, however it differs in terms of the duration of the peak. We found significant differences in floral attributes as the length of the pistil of the populations between the patches. Fruit production was significantly different between patches. The most frequent pollinator in both species was the hummingbird *Chlorostilbon lucidus.* The number of flowers visited by pollinators was statistically different between the patches, with populations in co-occurrence receiving fewer visits. We conclude that there is no facilitation between *C. infestus* and *C. urens*. Competition is weakened due to differences in flowering strategy and floral morphometric attributes by character displacement, thus allowing the coexistence among the two studied species.

Keywords – Caatinga; Competition; Facilitation; Pollination; Reproductive success.

SUMÁRIO

1 APRESENTAÇÃO

O sentido da luta pela existência, abordado por Darwin (1859, p. 103), leva em consideração a capacidade do próprio organismo em se sobressair às dificuldades impostas pelo ambiente. Mais que isso, retrata a dependência da vida pra se manter viva, ou seja, a necessidade das relações entre os próprios indivíduos na perpetuação da vida. Essas relações, chamadas de interações ecológicas, definem de modo simples o que representa para as populações a influência de uma espécie sobre a outra, podendo ser da mesma ou de outra espécie. É importante, portanto, entender como ocorre as interações ecológicas para que possamos compreender processos evolutivos (Thompson, 1989), a distribuição geográfica dos organismos (Munguía et al., 2008) e até mesmo o equilíbrio de ecossistemas (Tylianakis et al., 2008).

Quando as populações interagem entre si, o resultado pode gerar efeitos positivos mútuos ou não, negativos ou até mesmo nulos (Arthur and Mitchell, 1989). Populações que interagem de forma mutuamente negativa são vistas como competidoras (Arthur and Mitchell, 1989). As principais classes de interações ecológicas são a competição, o mutualismo, predação ou herbivoria, parasitismo, entre outras. As plantas formam todos esses tipos de interações, elas competem entre si por nutrientes, água, luz solar; estabelecem mutualismos com animais polinizadores e dispersores de sementes, estão submetidas a predadores de plântulas/sementes e herbívoros, havendo, inclusive, famílias de plantas que parasitam árvores e arbustos.

Neste estudo, avaliamos as consequências das interações entre plantas mediadas por polinizadores, as quais, de maneira geral, podem apresentar efeitos negativos (competição) ou positivos (facilitação). As plantas podem competir por polinizadores segundo dois principais processos, por meio da superioridade na atração dos visitantes, que pode levar ao monopólio dos polinizadores, ou por meio da transferência interespecífica de pólen, em que uma espécie pode ocupar a superfície estigmática das flores de uma outra, tornando menos provável a recepção de grãos coespecíficos, de modo que o sucesso reprodutivo seja reduzido em ao menos uma espécie. Interações positivas entre plantas caracterizam a facilitação, que ocorre quando uma espécie modifica condições ambientais estressantes de modo que favorece o estabelecimento e ciclo de vida das outras plantas, e as espécies capazes de produzir esses efeitos são chamadas de facilitadoras (Callaway, 1995). Outra forma de expressão do mecanismo de facilitação é quando espécies que florescem simultaneamente e possuem o mesmo grupo de polinizadores atuam em conjunto para atrair mais visitantes, o que pode resultar em uma maior produção de frutos juntas, em vez de isoladas (Feldman et al., 2004).

A primeira parte desta dissertação consiste na Fundamentação Teórica, onde apresentamos a base conceitual que suporta e orienta nossa investigação e mostramos os estudos e achados relevantes presentes na literatura científica sobre a temática da pesquisa. A segunda parte representa o artigo que será submetido à revista para publicação.

O artigo intitulado “Does pollinator sharing as a result of flowering overlap and similarity of floral attributes provide effects on reproductive success in two species of *Cnidoscolus* (Euphorbiaceae)?” investiga a reprodução de duas espécies congêneres (*C. infestus* e *C. urens*) em condições associadas e não-associadas, testando as hipóteses de facilitação e competição entre elas e descrevendo características fenológicas, morfológicas e o sucesso reprodutivo.

2 FUNDAMENTAÇÃO TEÓRICA

2.1 Partilha de polinizadores

O papel de vetores bióticos na reprodução de plantas anteriores às angiospermas pode ter sido mais comum do que previamente especulado (Ren et al., 2009). Os registros fósseis indicam que as relações dos insetos com as estruturas reprodutivas das plantas remontam ao Siluriano e início do Devoniano (Labandeira, 2013, 1998). Tais evidências sugerem que a transferência de pólen exercida por insetos, possivelmente, já acontecia no Permiano, cerca de 300 Ma (Labandeira, 2013). Porém, foi com o surgimento das plantas com flores que aconteceu uma rápida diversificação nas angiospermas e em alguns grupos de insetos (van der Kooi and Ollerton, 2020), o que causou uma verdadeira revolução entre as relações planta-animal e inseriu os insetos no patamar de principais polinizadores. A origem das angiospermas ainda é um tópico misterioso, bem como os modos de polinização presentes nas plantas com flores primitivas (Hu et al., 2012; van der Kooi and Ollerton, 2020). Hu et al. (2012), com base em características polínicas e análises filogenéticas dos grãos de pólen, sustentam a hipótese de ambofilia, em que, tanto insetos quanto o vento foram os responsáveis pelo transporte de pólen nas primeiras plantas floríferas.

Atualmente, com mais de 360.000 espécies, Angiospermae representa o principal grupo de plantas da atualidade (Lughadha et al., 2016) e uma das razões desse sucesso é o seu complexo modo de reprodução, o qual envolve interações entre flores e animais (Heath et al. 1992, Armbruster 2014). Aproximadamente 76% das angiospermas em zonas temperadas são polinizadas por animais, e essa proporção alcança 94% na região tropical (Ollerton et al., 2011). Os grupos de animais polinizadores são bastante diversificados, e tanto invertebrados quanto vertebrados fazem parte dessa galeria. As abelhas merecem destaque pelo elevado número de plantas que polinizam, além disso, consiste no grupo polinizador mais representativo na maioria dos ecossistemas (Bawa et al., 1985; Corlett, 2004; Dafni and O'Toole, 1994; Quirino and Machado, 2014). A miríade de polinizadores ainda comporta lepidópteros, besouros, moscas, vespas, aves, morcegos, entre outros animais (Bawa, 1990; Machado and Lopes, 2004).

Concomitante à diversidade de espécies vegetais, há uma grande variedade de formas e estruturas florais, geralmente associadas a diferentes mecanismos de polinização (Fenster et al., 2004). Muitas plantas possuem flores que são especializadas na polinização por determinado grupo animal (Wolfe and Sowell, 2006) ou apresentam especializações para algum grupo funcional, como por exemplo abelhas grandes de língua longa (Fenster et al., 2004). No entanto, é importante destacar que esta abordagem tradicional da especialização floral tem sido intensamente debatida, em face o surgimento de observações sobre a existência de plantas com flores que são polinizadas por um amplo espectro de vetores de pólen, conhecidas como generalistas (Johnson and Steiner, 2000; Ollerton et al., 2007).

Diferentes espécies que apresentam sobreposição da floração e características florais semelhantes podem partilhar os mesmos grupos de polinizadores (Campbell, 1985). Embora exista uma grande diversidade de características florais, algumas vezes duas ou mais espécies que coexistam no mesmo local possuem semelhanças morfológicas como: cor, tamanho e forma; sobreposição do período de floração e, nestas circunstâncias, espera-se que ocorra partilha de polinizadores (Botes et al., 2008; Moeller, 2004). Tendo em vista que a polinização é uma etapa crucial no ciclo de vida das plantas, os polinizadores podem ser considerados recursos limitantes para espécies que os têm em comum (Ingvarsson and Lundberg, 1995).

A partilha de polinizadores está relacionada com diversos processos ecológicos e evolutivos como a produção de híbridos, seleção de características florais, tais como o local de deposição dos grãos de pólen, cor, oferta de recursos e a variação temporal da disponibilidade de pólen (Christianini et al., 2013; Grossenbacher and Stanton, 2014; Hopkins, 2013; Huang and Shi, 2013; Miyake and Inoue, 2003). O papel estruturador da partilha de polinizadores nas comunidades, atuando como um mecanismo de coexistência, também é objetivo de pesquisas (Botes et al., 2008).

O resultado final do processo é o sucesso reprodutivo das espécies que usam os mesmos polinizadores. A produção de frutos e sementes representa um forte indicador dos processos dominantes na interação entre as plantas na obtenção do polinizador como recurso. Onde a redução na produção de frutos pode indicar competição por polinizadores (Bell et al., 2005) e o aumento revela uma possível facilitação entre as plantas (Moeller, 2004). Ainda podem ser encontrados efeitos neutros, quando não há interferência no sucesso reprodutivo na interação planta-planta (Moragues and Traveset, 2005).

2.2 Competição

A competição ocorre quando uma espécie reduz o sucesso reprodutivo de uma outra espécie, interferindo na capacidade da outra espécie em obter determinado recurso limitante (Palmer et al., 2003). No caso dos polinizadores como recurso, implica na diminuição da frequência do polinizador e transferência polínica. A disputa pelo polinizador pode ocorrer seguindo duas maneiras: I - quando uma espécie é atrativamente superior à outra, por conseguinte recebendo maior número de visitas (Feinsinger et al., 1988); II - quando ocorre transferência interespecífica de pólen, nesse caso ambas espécies sofrem redução no sucesso reprodutivo (Feinsinger and Tiebout, 1991). Em ambas as maneiras o fluxo polínico é afetado pela partilha de polinizadores.

Grande parte dos estudos sobre partilha de polinizadores foram, inicialmente, analisados sob a ótica da competição interespecífica (Campbell, 1985; Lack, 1976; Levin, 1972). Tem sido observado que os efeitos da competição por polinizadores reduzem o sucesso reprodutivo de ao menos uma das espécies competidoras (Arceo-Gómez and Ashman, 2011; Bell et al., 2005; Flanagan et al., 2011; Runquist, 2012). Tais achados são frequentes em experimentos análogos aos de laboratório, em que são manipulados a proporção e a distância entre indivíduos em cada espécie, o número de flores ofertadas e, em muitos casos, o sucesso reprodutivo é obtido por meio de polinizações manuais, onde são transferidas misturas de grãos de pólen hetero e coespecíficos. Neste sentido, é necessário verificar se os mesmos efeitos ocorrem sob circunstâncias naturais, permitindo assim avaliar quão acurados os experimentos representam na natureza a competição por polinizadores. Estudos que verificaram a partilha de polinizadores sem interferências na estrutura populacional das espécies em campo e que observaram o fluxo polínico promovida por polinizadores são raros, como por exemplo o realizado por Nadia et al. (2007), que não encontrou redução do sucesso reprodutivo entre plantas que partilham polinizadores.

2.2.1 Transferência interespecífica de pólen

Sabendo-se que a efetividade da polinização depende da quantidade de grãos de pólen transferidos e também da qualidade dos grãos depositados nos estigmas (Herrera, 1989, 1987), cargas polínicas de outras espécies podem ocupar a região estigmática, resultando em menor área para os grãos de pólen coespecíficos se aderirem (Morales and Traveset, 2008). Plantas que partilham polinizadores estão diretamente sujeitas à deposição heteroespecífica de pólen (Da Fonseca et al., 2016). Por consequência, a Transferência Interespecífica de Pólen (TIP) afeta negativamente o sucesso reprodutivo das espécies (Waser, 1978).

A TIP ocorre quando os visitantes florais movimentam-se indiscriminadamente entre as flores das espécies, o que pode acentuar a chegada de grãos de pólen estrangeiros em estigmas coespecíficos, resultando em diminuição do sucesso reprodutivo (Waser, 1978). A reprodução é afetada por dois subcomponentes da TIP: a deposição heteroespecífica – que interfere na fertilização de pólen nos estigmas coespecíficos – e a perda de carga polínica coespecífica – a qual reduz a quantidade de pólen transferidos.

Por se tratar do principal mecanismo pelo qual as plantas podem interferir na formação de sementes umas às outras, a Transferência Interespecífica de Pólen (TIP) é frequentemente analisada nos estudos de partilha de polinizadores (Morales and Traveset, 2008). Os primeiros esforços foram direcionados aos impactos que plantas exóticas exerciam nas culturas (Free, 1968; Galen and Gregory, 1989; Mosquin, 1971). Com a evolução do campo de pesquisa, começaram a ser analisadas as estratégias das plantas para evitar a transferência interespecífica e as consequências da deposição heteroespecífica de pólen, como alterações no fenótipo floral em função da competição, o deslocamento fenológico e a coexistência entre espécies (Aizen and Rovere, 2010; Botes et al., 2008; Muchhala and Potts, 2007; Stone et al., 1998).

2.2.2 Vantagens na atração por polinizadores

Em um complexo sincronopátrico onde ocorre partilha de polinizadores, o “display floral” pode exercer grande influência no sucesso reprodutivo de uma espécie (Waser, 1986). Quando uma espécie possui flores atrativamente superiores – as quais apresentam de cores vistosas e grande quantidade de recursos - os visitantes florais podem ter preferência por ela (Gibson et al., 2013). Tal preferência eleva a taxa de visitação de um tipo floral em detrimento do outro (Flanagan et al., 2011).

Outra forma de obter vantagens na atração por polinizadores é o efeito denso-dependente (Hegland, 2014). Uma espécie de população maior e distribuição agregada pode apresentar grande quantidade de flores, assim aumentando o próprio “display” e recebendo a maioria das visitas (Feldman, 2006). Existe uma linha tênue entre competição e facilitação, no que se refere ao resultado do efeito denso-dependente (Hegland et al., 2009). Em espécies com características florais menos atrativas aos polinizadores, portanto inferiores competitivamente na partilha de polinizadores, a densidade representa um fator que aumenta as suas taxas de sobrevivência na comunidade (Hanoteaux et al., 2013). Ao examinar as taxas de visitação e o efeito da densidade em uma espécie de orquídea numa comunidade, Duffy & Stout (2008), verificaram que em altas densidades as inflorescências de *Spiranthes romanzoffian* competem intraespecificamente por polinizadores, no entanto, quando analisada a densidade total de flores na comunidade, há uma tendência de aumento no número de visitas, o que indica facilitação na atração de polinizadores.

A taxa de visitação é um componente determinante para a avaliação da preferência floral, nesse sentido há uma preocupação prática quanto aos efeitos das espécies exóticas, que possuem flores vistosas e maior “display floral” em relação às nativas, nas interações entre plantas e polinizadores na comunidade (Beverly et al., 2002). A extensão da interferência de espécies exóticas na taxa de visitação sobre as plantas nativas ainda precisa de mais dados para uma avaliação mais efetiva do processo. Estudos têm registrado efeitos diferentes em cada caso, como Gibson et al. (2013), que verificaram efeitos negativos de *Acacia saligna* sobre a taxa de visitação de *Roepera fulva*. Lopezaraiza-Mikel et al. (2007), não encontraram efeitos negativos da espécie exótica, *Impatiens glandulífera*, na rede de polinização da comunidade de plantas nativas, de modo inverso, os autores relataram indícios de efeitos positivos na atração de polinizadores.

2.3 Facilitação

A partilha de polinizadores nem sempre resulta em competição, uma vez que a maioria das plantas exploram os polinizadores diferencialmente por meio de características morfológicas e/ou fisiológicas isolantes (Devaux and Lande, 2009; Miyake and Inoue, 2003). Deslocamento fenológico e de mudanças na morfologia floral impedem que as espécies que partilham polinizadores compitam ou hibridizem, tornando os sistemas reprodutivos isolados entre si. Por exemplo, a deposição de pólen em diferentes partes do corpo do polinizador (Muchhala and Potts, 2007).

Diante do colocado acima, outro resultado da partilha de polinizadores seria a facilitação. Tal processo ocorre quando uma planta modifica condições ambientais ou a disponibilidade de recursos, beneficiando o crescimento e reprodução de outras espécies vegetais (Callaway, 1995). A facilitação na atração de polinizadores ocorre por meio do incremento na atratividade das populações, i.e. aumento no “display floral” (Kipling and Warren, 2014). O aumento no “display” floral afeta o número e a diversidade de polinizadores nas comunidades (Ghazoul, 2006). Quando as espécies apresentam morfologia floral similar, o “display floral” pode exercer um efeito denso-dependente, uma vez que quanto maior a densidade de plantas com flores, maior será a taxa de visitação (Feldman, 2006). Nesse caso, os custos reprodutivos do efeito denso-dependente conjuntamente à transferência interespecífica de pólen são menores que os benefícios.

Alguns estudos encontraram efeitos positivos na interação entre plantas que partilham polinizadores. Para Feldman et al. (2004), a facilitação incrementa o sucesso reprodutivo das espécies, havendo dois tipos de facilitação, sendo uma forte e outra fraca. Nos modelos de Feldman et al. (2004), uma facilitação forte permite que a segunda espécie coexista indeterminadamente na presença da facilitadora, enquanto na fraca favorece a coexistência por maior período de tempo, quando comparado com a condição de isolamento. Yang et al. (2013) encontraram forte facilitação em parcelas mistas de *Pedicularis densispica* (Orobanchaceae) com *Astragalus pastorius* (Fabaceae). Outros modelos também produziram resultados positivos para a facilitação numa comunidade de plantas nos Andes (Tur et al., 2016).

Em face ao desenvolvimento do conhecimento da facilitação na atração de polinizadores entre plantas, os estudos têm especulado sobre a sua importância na dinâmica da comunidade e o papel desse mecanismo favorecendo a coexistência estável entre espécies no mesmo nível trófico (Fantinato et al., 2018; Pauw and Johnson, 2018).

Parece claro, portanto, que existem efeitos de vizinhança no processo de polinização em espécies sincronopátricas, com efeitos diretos na ecologia reprodutiva das espécies, ora negativo por meio da competição, ora positivo por causa da facilitação (Mesgaran et al., 2017).

3 ARTIGO – DOES POLLINATOR SHARING AS A RESULT OF FLOWERING OVERLAP AND SIMILARITY OF FLORAL ATTRIBUTES PROVIDE EFFECTS ON REPRODUCTIVE SUCCESS IN TWO SPECIES OF *CNIDOSCOLUS* (EUPHORBIACEAE)?

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Abstract - Flowering plants share pollinators when they occur and bloom at the same time and area and have a similar set of floral traits. We aimed to assess the effects of pollinator sharing between two species of Euphorbiaceae (*Cnidoscolus infestus* and *C. urens*) when they occurred associated and non-associated in patches from the semiarid Caatinga. We used flowering, morphometric attributes, floral visitors, and the reproductive success of both species to analyze and determine the consequences of pollinator sharing. We found high overlap in the flowering period between the two species, however it differs in terms of the duration of the peak. We found significant differences in floral attributes as the length of the pistil of the populations, as well as fruit set between different patches. The most frequent pollinator in both species was the hummingbird *Chlorostilbon lucidus.* The number of flowers visited by pollinators was statistically different between the patches, with associated populations receiving fewer visits. We conclude that there are negative effects on the pollination sharing between *C. infestus* and *C. urens*. However, the competition can be weakened due to differences in flowering strategy and floral morphometric attributes by character displacement, thus allowing the coexistence among the two studied species.

Keywords - pollination, flowering phenology, facilitation, reproductive success, character displacement, Tropical Dry Forest, Caatinga, *Cnidoscolus infestus, Cnidoscolus urens*, *Chlorostilbon lucidus*.

INTRODUCTION

One of the most intriguing aspects of the phenomenon of life is certainly the interactions of its representative forms, which we organize in species, with the environment, but also, and specially, with each other. Biotic interactions have major implications for the organization and structure of communities (Heithaus, 1974) and are essential for maintaining biodiversity in various ecosystems. The need for these associations is evident, for example, in modern angiosperms. It is estimated that nearly 90% of them establish associations with animals for the transport of pollen grains, an essential process in their reproduction (Ollerton et al., 2011). Still, the community of pollinating animals, which play different roles in pollination, exhibits the same degree of diversity as flowering plants (Ollerton, 2017). And yet, it is not uncommon to find pollinator species shared among simultaneously flowering species (Moeller, 2004).

Pollinator sharing often occurs when two or more plants coexist in the same location, show synchronous flowering, and have similar floral traits (Pinto and Schlindwein, 2015). From this point of view, in which the pollinator is considered a limiting resource, plant-plant interactions assume a competitive or facilitative character, depending on the context in which the interaction occurs (Mesgaran et al., 2017). Competition has been traditionally more explored in studies on pollinator sharing, resulting in a plethora of information on this process and the mechanisms involved in it, such as interspecific pollen transfer (IPT) and density-dependent effects. Interspecific pollen transfer represents a direct interference, since heterospecific pollen grains may accumulate on the stigmas and prevent the deposition and germination of conspecific pollen (Lara-Romero et al., 2016; Streher et al., 2020; Waser, 1978). At high densities, species with showy flowers may monopolize the pollinator, leading to lower visitation rates to neighboring plants (Seifan et al., 2014). In both mechanisms, the ultimate result is a decrease in the reproductive success of the competitors.

With the growth of the research field and the finding of results at odds with the competitive hypothesis, new perceptions started to emerge. In some cases, the neighborhood effect can be beneficial to plants due to an improvement they produce in the environmental conditions and, analogously, also in the attraction of pollinators (Callaway, 1995; Moeller, 2004). The facilitation hypothesis assumes that two or more co-occurring species increase the floral display, which leads to an increase in visitation rates, chances of pollination and, consequently, reproductive success (Braun and Lortie, 2019). Different models have been used in recent studies to investigate the facilitation hypothesis. For example, in diffuse models, a very attractive plant benefits neighboring species by attracting pollinators to the community, which is known as the magnet species effect (Seifan et al., 2014), and in sequential flowering models, plants that flower earlier attract, maintain, or increase pollinator populations, which then pollinate late-flowering species (Braun and Lortie, 2019).

However, investigations of diffuse and indirect interactions are highly complex and, therefore, less likely to reach significant and conclusive results (e.g. Ghazoul, 2006). In this sense, studying species pairs in direct interaction provides advantages by allowing more concrete reflections (e.g. Landry, 2013). This is important because the boundary between competition and facilitation is often subtle and confusing, even in works with species pairs (e.g. Campbell and Motten, 1985). Furthermore, these interactions seem to be rather dynamic than static, that is, they may alternate depending on the density of the populations (Mesgaran et al., 2017).

A possible solution, in view of this uncertainty, is to carry out investigations of pairs of plants with similar traits, as they are expected to provide straightforward results on the effects of pollinator sharing. In this sense, in the present study we used two species of the genus *Cnidoscolus*, *C. urens* and *C. infestus*, with similar floral traits and synchronous blooming to analyze which of the two hypotheses - competition or facilitation - best explains the consequences of pollinator sharing.

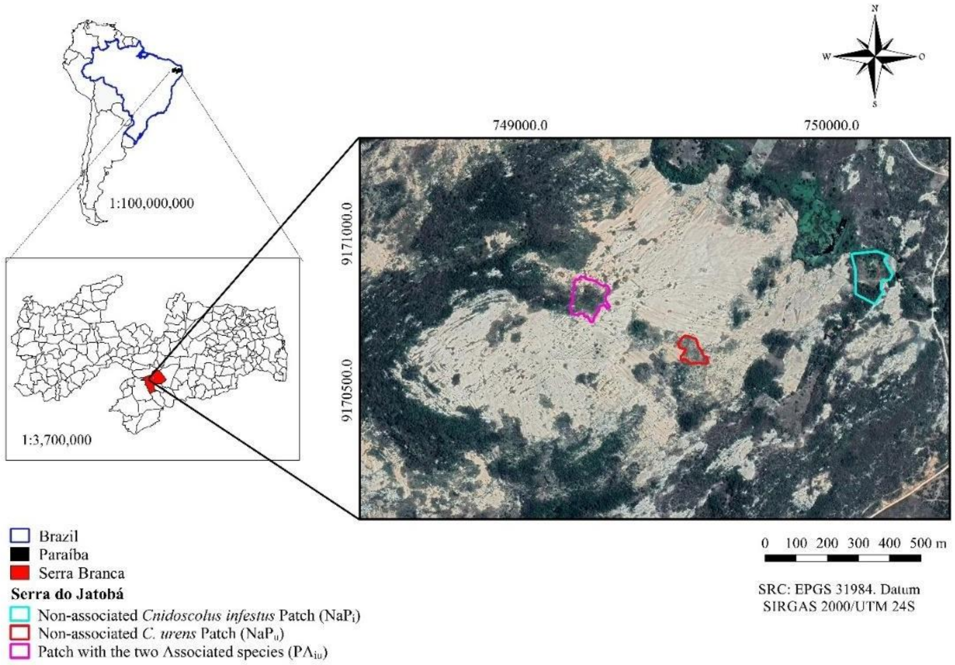
We determined the possible effects of pollinator sharing through analyses of floral phenology and morphology, visitation frequency, and reproductive success of the two species considering the following predictions: I – the flowerings of the two species overlap and peak during the same periods, leading to an aggregated pattern (facilitation), or are displaced, resulting in a segregated pattern (competition); II – the associated species are close in terms of floral traits (facilitation) or are phenotypically distant from each other through character displacement (competition); III – a greater number of pollinators is attracted when the two species are associated (facilitation) or not associated (competition); IV – the reproductive success of the species is greater when the two species are associated (facilitation) than separated (competition).

MATERIAL AND METHODS

Study site

The study was developed in Serra do Jatobá, located in the municipality of Serra Branca in Paraíba, Northeastern Brazil. Serra do Jatobá is a granitic rocky outcrop of approximately 2.4 km², reaching approximately 737 m in elevation, located between the coordinates 07°29'46" S and 36°44'36" W (Figure 1). Serra do Jatobá is an important tourist spot in the municipality and the destination of many visitors, who use the area for sport, religious, educational, recreational purposes, among others.

Figure 1. Location map of Serra do Jatobá, municipality of Serra Branca-PB, Northeast Brazil and delimitation of the studied patches. Blue = Non-associated *Cnidoscolus infestus* patch (NaP_i); Red = Non-associated *C. urens* patch (NaP_u); Lilac = Patch with the two species *C. infestus* and *C. urens* associated (PA_{iu}). NaP_i = Non-associated *C. infestus* patch; NaP_u = Non-associated *C. urens* patch; PA_{iu} = Patch with the two associated species.



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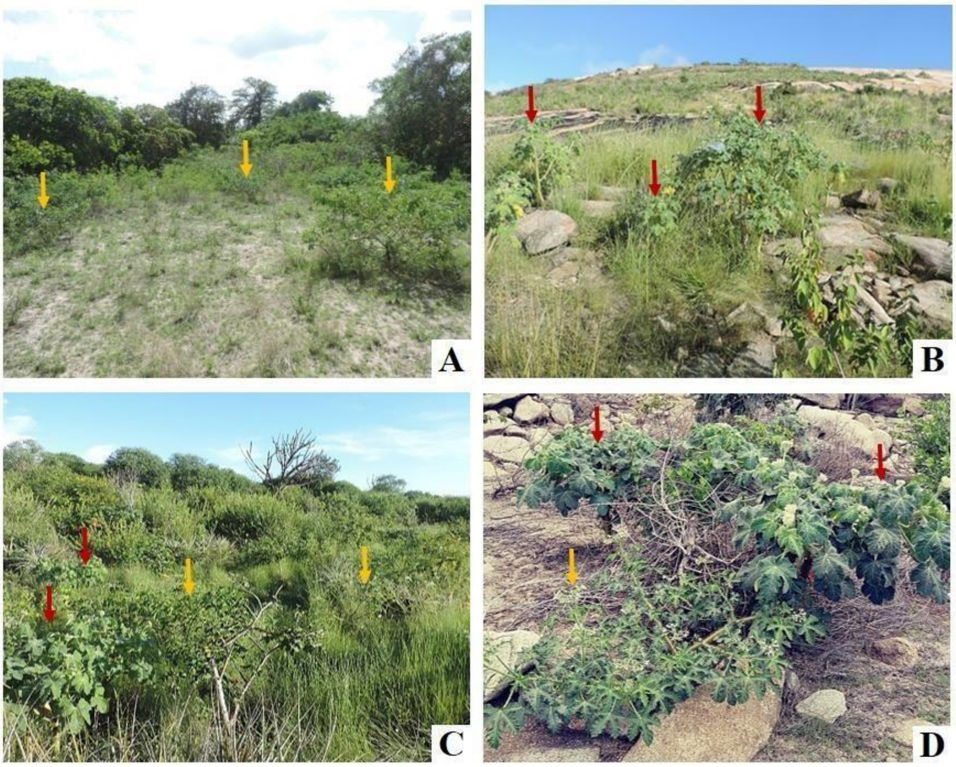
The region where Serra do Jatobá is inserted has BSh climate type, according to the Köppen classification, updated by Alvares et al. (2013). It is a semi-arid area characterized by a relatively high average temperature, around 27 °C, average annual precipitation rates close to 600 mm, and irregular rainfall over the years, concentrated in 3-4 months (Alvares et al., 2013).

Rocky outcrops are unique environments within the Caatinga because, despite the influence of the surrounding vegetation, the flora that is able to thrive on these places is adapted to severe abiotic conditions, such as extreme water deficit and high incidence of solar radiation (*e.g.* Queiroz et al., 2017). The notorious environmental heterogeneity in Serra do Jatobá further contributes to produce a variety of phytophysiognomies on the rocky outcrop and its surroundings. These phytophysiognomies vary from rupicolous vegetation growing on exposed rock, formed by species of *Encholirium* (Bromeliaceae), *Melocactus* and *Pilosocereus* (Cactaceae), to typical vegetation of Crystalline Caatinga consisting of a deciduous arboreal formation growing on shallow, stony and nutrient-rich soils, and finally small patches of Sedimentary Caatinga present in dunes and sedimentary areas around the rocky outcrop, on deeper and poorer soils (Queiroz et al., 2017).

Species studied

Cnidoscolus urens and *C. infestus* (Euphorbiaceae) are bisexual shrubs up to 2.5 m tall that bloom throughout the year (Araújo et al., 2012). Both species are spread over Serra do Jatobá. *Cnidoscolus urens* is found abundantly in vegetation islands formed in fissures and potholes on rocks along the altitudinal gradient of Serra do Jatobá, while *C. infestus* is commonly observed in the understory of the tree-shrub formations on the top and around the rocky outcrop. Despite the different niches, populations of the two species co-occur in sites with intermediate soil conditions (Figure 2).

Figure 2. Overview of the populations of the two species of *Cnidoscolus* in the studied patches, located in Serra do Jatobá, a rocky outcrop in the Caatinga area in the municipality of Serra Branca-PB, Brazil. A = Non-associated (NaP$_i$) *C. infestus* patch; B = Non-associated (NaP$_u$) *C. urens* patch; C = Patch of associated (PA$_{iu}$) *C. infestus* and *C. urens*; D = Individuals of *Cnidoscolus* associated (PA$_{iu}$). Orange arrow (⬇) = indicates individuals of *C. infestus*; Red arrow (⬇) = indicates individuals of *C. urens*.



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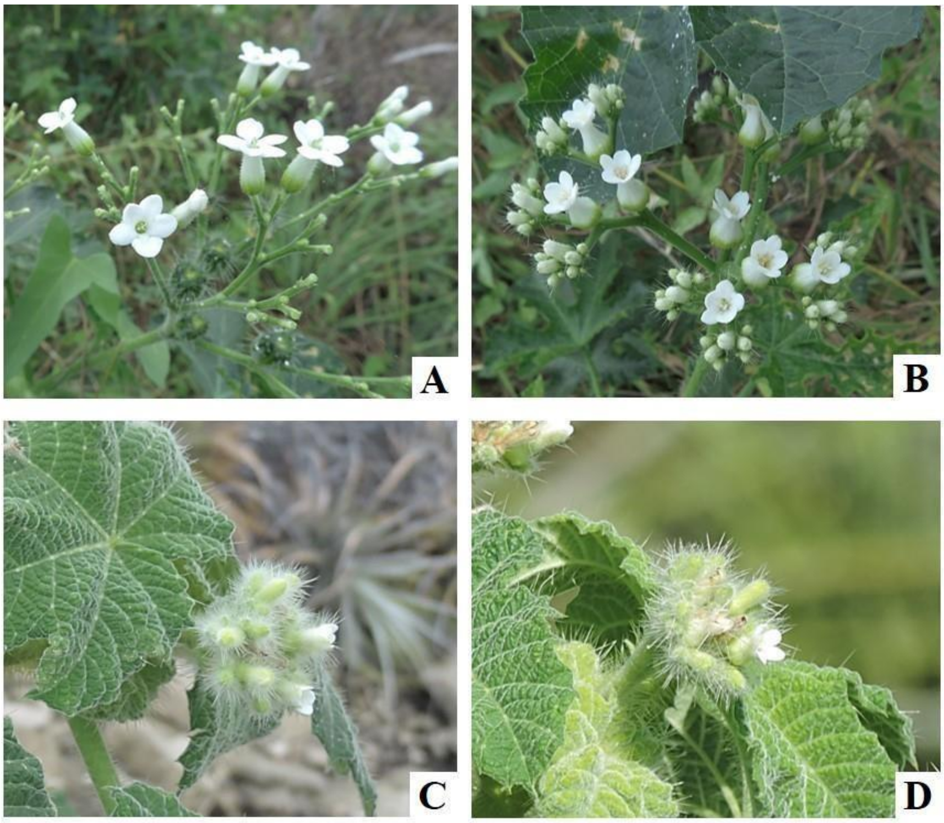
The flowers of both species are gathered in terminal and axillary inflorescences of the dichasium type, are unisexual, pentamerous, white, sessile, tubular, odorless, diurnal, with 10 stamens, 5 of them larger and located at the height of the opening of the corolla tube, and 5 smaller, inside the flower; the pistils are 3-carpellate, trilocular, with a single ovule in each locule, with trifid stigmas positioned outside the flower when receptive. As for the arrangement in the inflorescence, the female flowers are located in the central region and are always the first to mature; only after the end of anthesis of the last pistillate flower do the male flowers, which are born at the margins of the inflorescence, begin to open, characterizing protogynous flowers. The main differences between the two species consist in the presence of a landing platform in the male flowers of *C. infestus*, resulting from the folding of the apex of the corolla into five lobes, and the dense layer of stinging trichomes surrounding the flowers of *C. urens* (Figure 3).

Figure 3. Inflorescences and flowers of the two studied species of *Cnidoscolus* occurring in Serra do Jatobá, a rocky outcrop in the Caatinga area of Paraíba, Brazil. *C. infestus*: A = Male flowers; B = Female flowers; *C. urens:* C = Male flowers; D = Female flower.



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Data collection

We first distinguished three patches in the area based on the occurrence of the species as follows: NaP_i – Non-associated patch of *C. infestus*; NaP_u – Non-associated patch of *C. urens*; PA_{iu} – Patch of *C. infestus* and *C. urens* in association (Figures 1 and 2). The distance between patches was of 360 m between NaP_u and PA_{iu}, 670 m between NaP_i and NaP_u, and 980 m between NaP_i and PA_{iu}. The patches were distributed along an elevation gradient: NaP_i was located in the lowest altitude, at 540 m, followed by NaP_u at 603 m, and PA_{iu} at the top of Serra do Jatobá, at 710 m.

Phenology and flower production

We marked 25 individuals of the two species in each the three patches, totaling 100 plants (NaP_i – 25 *C. infestus* individuals; NaP_u – 25 *C. urens* individuals; PA_{iu} – 50 individuals, being 25 of *C. infestus* and 25 of *C. urens*) for monthly monitoring of flowering for a period of 18 months (March 2018 to August 2019). We determined the flowering intensity and peak according to Fournier’s (1974) semi-quantitative methodology. Also, in each monthly visit to the plants, we counted the number of flowers and buds, in addition to the number of open male and female flowers to determine the dynamics of flower production and supply of floral resources.

The degree of flowering overlap between the populations of the species pairs was obtained using the percentage overlap index, according to Krebs (1989).

$$Pjk = [\sum_{i=1}^{n}(minimum\ pij, pik\ )]100$$

Where:

Pjk = Percentage of phenophase overlap between populations of species *j* and *k*.

pij = Proportion of resource *i* of the total resources used by species *j* each month.

pik = Proportion of resource *i* of the total resources used by species *k* each month.

n = number of months sampled.

The index was calculated using flowering intensity values, according to the Fournier method (1974).

Floral morphology

Morphometric analyses of the following floral traits were carried out: flower size, tube length and diameter, tube opening diameter, stamen length, anther size, and pistil length. Measurements were taken in the laboratory with the aid of a stereomicroscope and a digital caliper (n = 240; 30 flowers per sexual morph of the species in each patch). Male and female flowers of *C. infestus* and *C. urens* were placed in 15 ml eppendorf tubes and placed in a thermal box for further analysis in the laboratory.

Floral visitors

The registration of floral visitors was carried out through focal observations of the plants. In each condition (associated and non-associated), each species was observed for 25 h, totaling 50 h of observation per species.

At each visit, the time of arrival of the animal in the flower, the number of male and female flowers visited, and the visiting behavior (whether there was contact with the reproductive organs and whether male or female flowers were visited) were recorded. The pollinator visitation frequency was calculated (number of visits divided by the time spent in each species in the patches - 25h). Finally, visitors were classified into effective pollinators, occasional pollinators and thieves based on their visitation behavior and frequency.

Reproductive success

The reproductive success was determined by the number of fruits formed, recorded during the monthly phenological monitoring in 25 individuals of each species per patch. We applied controlled pollination treatments to evaluate the reproductive system: apomixis, hybridization, manual self-pollination, manual cross-pollination, and natural pollination (control). The possibility of hybridization was investigated through interspecific pollinations, placing pollen grains of *C. infestus* on stigmas of *C. urens* and vice versa. In each treatment, 30 flowers from 20 individuals were marked and bagged with 10 x 15 cm organza fabric bags, with the exception of flowers observed for natural pollination, which were left exposed. The success of the treatments was evaluated by the number of fruits formed.

Statistical analyses

Spearman correlation analysis was used to evaluate the relationship between monthly precipitation and flowering intensity of the species. Precipitation data were obtained from the AESA Geoportal. The behavior of the flowering event was evaluated through circular statistics using the software Oriana 4 (see Morellato et al., 2009).

We compared flower production to see whether there were differences in the daily supply of flowers between the two species and between non-associated and associated situations. For that, we used one-way ANOVA followed by Tukey test for pairwise comparisons or the Kruskal-Wallis test followed by the Mann-Whitney test for pairwise comparisons when the variables did not meet the assumptions of normality (Zar, 1999).

To investigate the convergence or divergence of characters in floral morphology, we used one-way ANOVA and the Tukey test for pairwise comparisons of the species in the associated and non-associated situation (Zar, 1999). We compared the following traits: flower size, tube opening length and diameter, anther size, stamen length, and pistil length.

Statistical differences in the fruit set of individuals of the two species in in the associated and non-associated situation were tested using one-way ANOVA and the Tukey test for pairwise comparisons (Zar, 1999). The dataset was normalized by applying the row normalize length method, using the statistical software PAST 4.03.

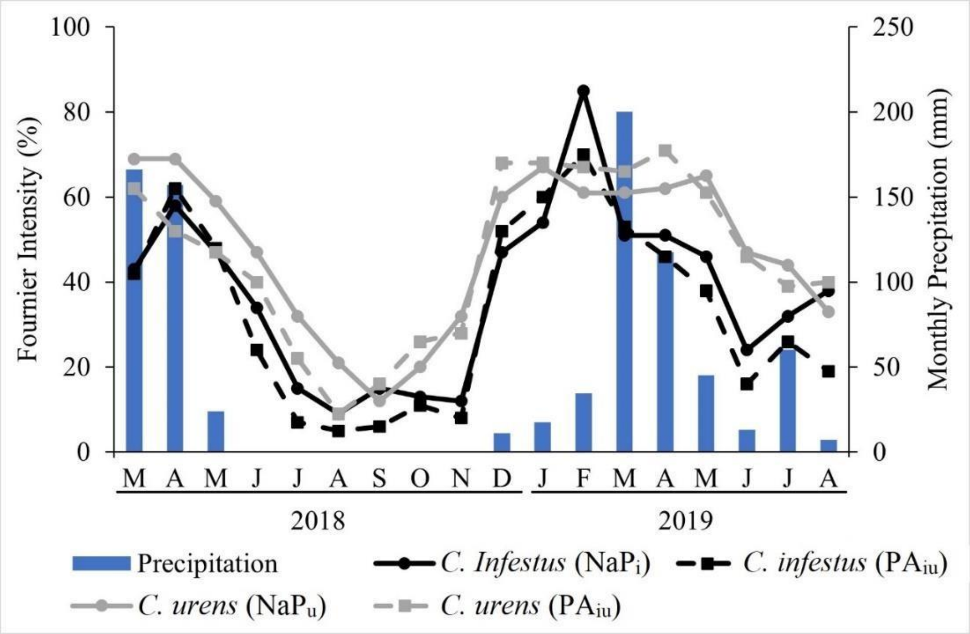
We compared the number of flowers visited by pollinators in each species in the associated and non-associated condition to verify whether facilitation or competition occurred. Comparisons were performed using the Kruskal-Wallis and Mann-Whitney tests (Zar, 1999), also using PAST 4.03.

RESULTS

Phenology and flower production

Flowering occurred throughout the 18 months of the study, with peaks in the rainy season (Figure 4). The two species differed as to the periods of higher flowering activity. *Cnidoscolus infestus* had peaks concentrated in one month in each year (April/2018 and February/2019), regardless of its occurrence in associated or non-associated patches. In turn, *C. urens* showed high flower production for more than one month during the year in the associated patch (March and April/2018; December to April/2019) and the non-associated patch (March to May/2018; January to May/2019) (Figure 4). The degree of flowering overlap between species was high, around 90% in all analyzed pairings (Table 1).

Figure 4. Fournier intensity for flowering of individuals of Cnidoscolus infestus and C. urens, in non-associated (solid lines) and associated (dotted lines) patches located in a caatinga area, in Serra do Jatobá, Paraíba, Brazil. The bars correspond to the precipitation of the respective months and years. NaP$_i$ = Non-associated *C. infestus* Patch; NaP$_u$ = Non-associated *C. urens* Patch; PA$_{iu}$ = Patch with the two associated species.



Source: The author (2023)

Table 1. Flowering overlap percentage index of *Cnidoscolus infestus* and *C. urens* using flowering phenophase intensities between populations under non-associated and associated conditions found in Serra do Jatobá, Serra Branca-PB, Brazil.

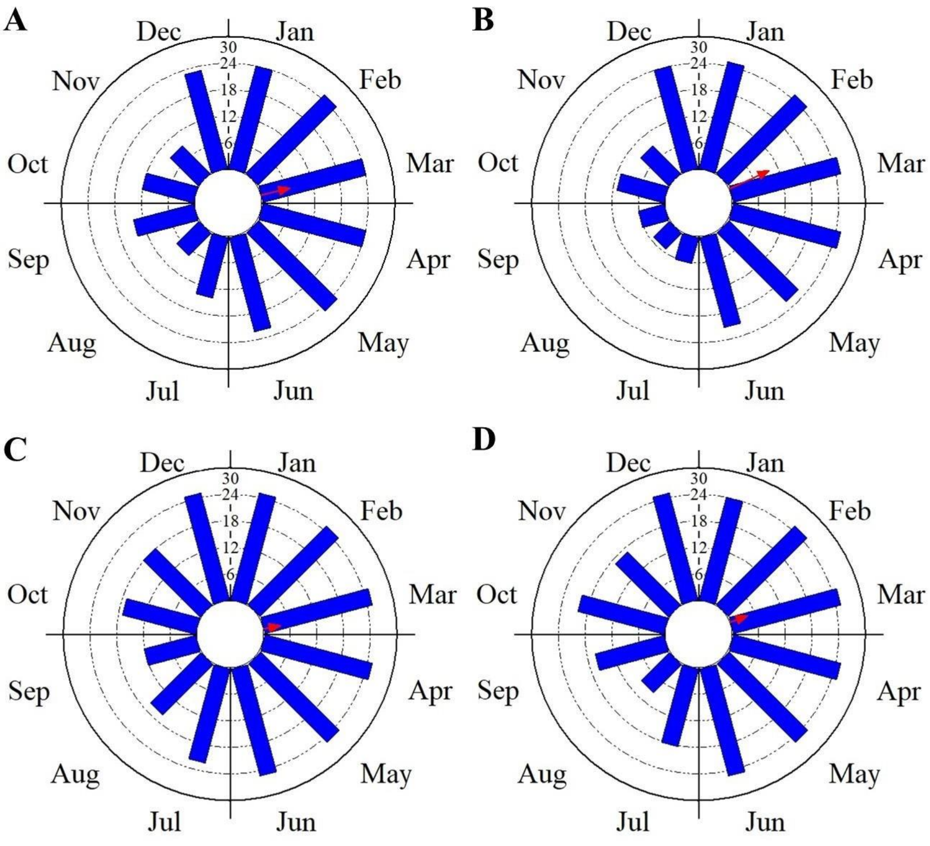
	C. urens NaP$_u$ (non-associated)	*C. infestus* PA$_{iu}$ (associated)
C. infestus NaP$_i$ (non-associated)	90	91

C. urens PA$_{iu}$ (associated)	92	86

Source: The author (2020)

We found a high positive and significant correlation between flowering and the precipitation of the same month in *C. infestus* (PA$_{iu}$ rs = 0.74 and NaP$_{i}$ rs = 0.73) and *C. urens* (PA$_{iu}$ rs = 0.70 and NaP$_{u}$ rs = 0.83). Circular statistics showed low seasonality of the flowering event and average date in March in both the non-associated and associated conditions (Figure 5), with the two species blooming throughout the year, although with lower intensity during the dry period (from June to November, Figure 5).

Figure 5. Circular diagrams with the frequency of flowering individuals of Cnidoscolus infestus and C. urens in non-associated and associated patches located in Serra do Jatobá, Serra Branca-PB, Brazil. A = C. infestus NaP$_{i}$; B = C. infestus PA$_{iu}$; C = C. urens NaP$_{u}$; D = C. urens PA$_{iu}$. NaP$_{i}$ = Non-associated C. infestus Patch; NaP$_{u}$ = Non- associated C. urens Patch; PA$_{iu}$ = Patch with the two associated species.



Source: The author (2023)

Species showed differences in flower production in the non-associated condition (F = 11.8; p < 0.001). When they were associated, there was a tendency to reduce the number of flowers, with significant differences in *C. infestus*. Regarding the proportion of male and female flowers in each species separately, there was a significantly greater number only in the female flowers opening per day in *C. infestus* (H = 13.79; p = 0.003).

Floral morphology

In general, the characteristics of the female flowers varied according to three distinct patterns, namely: I – Phenotypic divergence (when the floral trait differed between associated and non-associated individuals with a tendency towards distinct phenotypes), for example, in the pistil length and flower size; II – Phenotypic convergence (when the floral trait differed between associated and non-associated individuals with a tendency towards similar phenotypes), for example, in the tube length; III – Stability (when the trait did not differ between associated and non-associated individuals), for example, in the tube opening width (Table 2).

In the male flowers, there was a convergent pattern in flower size, tube opening width and anther size. The pattern of stability was observed in tube and stamen length (Table 2).

Table 2. Mean of morphometric attributes measured in 30 staminate and pistillate flowers for each treatment of *Cnidoscolus infestus* and *C. urens*, non-associated and associated, found in Serra do Jatobá, Serra Branca-PB, Brazil. Different letters indicate statistical difference observed through ANOVA and Tukey's test with a significance level of 5% ($p < 0.05$). NaP$_i$ = Non-associated *C. infestus* patch; NaP$_u$ = Non-associated *C. urens* patch; PA$_{iu}$ = Patch with the two associated species.

	C. infestus NaP$_i$	*C. infestus* PA$_{iu}$	*C. urens* NaP$_u$	*C. urens* PA$_{iu}$
Staminate flowers (mm)				
Flower size	12,29a	10,59b	10,42b	10,72b
Tube length	7,69a	8,92a	7,57a	7,74a
Tube opening width	1,96a	1,73b	2,35c	1,9ab
Stamen length	8a	8,14a	8,25a	8,19a
Anther size	1,24a	1,23a	1,45b	1,33a
Pistillate flowers (mm)				
Pistillate flower size	9,39a	8,46bc	8,4b	9,1ac

Tube length	6,23a	5,19b	3,9c	4,59d
Tube opening width	1,57a	1,6a	2,07b	1,98b
Pistil length	5,75ab	5,59a	5,99b	6,58c

Source: The author (2023)

Visitors

Effective pollinators consisted of three species of hummingbirds (*Chlorostilbon lucidus*, *Chrysolampis mosquitus* and *Eupetomena macroura*) and bees, *Bombus* sp. and *Xylocopa* sp. (Figure 6). Among them, only *Chlorostilbon lucidus* visited all patches, especially NaPi, where the frequency reached 40.84 visits.h^{-1} (Table 3). *Cnidoscolus infestus* in both NaP$_i$ and PA$_{iu}$ received visits from hummingbirds and bees, while bees visiting *C. urens* were only observed when the species was associated, in PA$_{iu}$. The number of flowers visited by pollinators differed among patches ($H = 22.41$; $p < 0.0001$), with both species receiving fewer visits when associated (Figure 7). There was also a difference in the frequency of visitation to flowers of the two species in the patch where they were associated (PA$_{iu}$), with a lower number of visits to flowers of *C. urens*.

Figure 6. Some of the pollinators of the Cnidoscolus species studied in Serra do Jatobá, a rocky outcrop located in an area of Caatinga in Paraíba, Brazil. A = Xylocopa sp. in male flower of C. infestus; Note pollen grains on the bee's tongue; B = Male of Chlorostilbon lucidus on male flower of C. urens; C = C. lucidus female after visiting C. urens flower; Note pollen grains adhered to the beak of the hummingbird; D = Female of Chrysolampis mosquitus on flowers of C. urens.

Source: The author (2023)

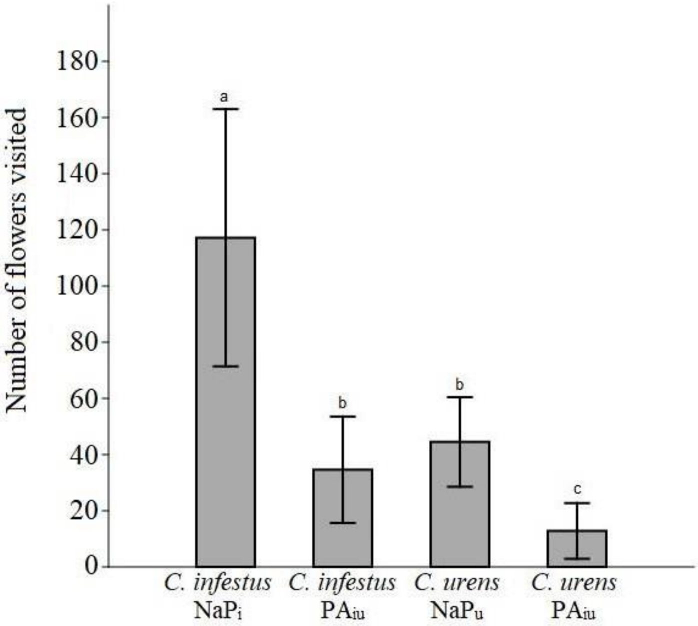
Table 3. List and frequency of floral visitors of the two species of *Cnidoscolus* (*C. infestus*and *C. urens*) in non-associated and associated patches located in Serra do Jatobá, a rocky outcrop in a Caatinga area in the municipality of Serra Branca-PB, Brazil. Frequency of visits. h-1. M = main pollinator; O = occasional pollinator; T = thieves. NaP_i = non-associated *C. infestus* patch; NaP_u = non-associated *C. urens* patch; PA_{iu} = patch with the two associated species.

	C. infestus NaP_i	*C. infestus* PA_{iu}	*C. urens* NaP_u	*C. urens* PA_{iu}
Trochilidae				
Chlorostilbon lucidus Shaw, 1812	40,84 (M)	5,68 (M)	9,12 (M)	3,6 (M)
Chrysolampis mosquitus Linnaeus, 1758	0,28 (O)	0	7,04 (M)	0
Eupetomena macroura Gmelin, 1788	0	0	1,52 (O)	0
Apidae				
Apis mellifera Linnaeus, 1758	8,04 (T)	13,96 (T)	2,08 (T)	3,32 (T)

Bombus sp	4,84 (M)	5,84 (M)	0	0
Centris sp	0,4 (O)	0,96 (O)	0	0
Trigona spinipes Fabricius, 1793	0	11,8 (T)	6,2 (T)	0
Xylocopa sp	0,52 (O)	1,36 (O)	0	0,8 (O)
Lepidoptera				
Pyrgus sp	2,68 (T)	0,2 (T)	0,16 (T)	0,6 (T)
Butterfly sp1	0,2 (T)	0,04 (T)	0,08 (T)	0
Butterfly sp2	0,56 (T)	0,08 (T)	0	0
Vespidae				
Wasp sp1	0	0,04 (T)	4,12 (T)	0

Source: The author (2023)

Figure 7. Number of flowers visited by pollinators of the two species of Cnidoscolus in non-associated and associated patches located in Serra do Jatobá, a rocky outcrop in a Caatinga area in the municipality of Serra Branca-PB, Brazil. Different letters represent statistical differences using the Kruskal-Wallis and Mann-Whitney Test between treatments with a significance level of 5% ($p < 0.05$). NaP$_i$ = non-associated C. infestus patch; NaP$_u$ = unassociated C. urens patch; PA$_{iu}$ = patch of the two associated species.



Source: The author (2023)

Reproductive success

Fruit set was significantly different among treatments (F = 6.99; p = 0.0003). Pairwise comparisons with the Tukey test revealed a reduction in the number of fruits only in *C. infestus* in the associated condition.

The natural pollination treatment yielded the highest reproductive success in *C. infestus* (100%) and *C. urens* (96.6%). The experiments revealed that the species are self-compatible. In the hybridization treatment, two fruits were formed in *C. urens* (N = 30) and only one in *C. infestus* (N = 30). There was no fruit formation in the apomixis test (Table 4).

Table 4. Reproductive system of *Cnidoscolus infestus* and *C. urens* and the success in fruit production in populations located in Serra do Jatobá, a rocky outcrop in an area of Caatinga in the municipality of Serra Branca-PB, Brazil. The numbers in parentheses correspond to the numbers of flowers/fruits.

Treatments	*C. infestus* % Success	*C. urens* % Success
Hand cross pollination	76,6 (30/23)	83,3 (30/25)
Manual self-pollination	50 (30/15)	56,6 (30/17)
Natural pollination	100 (30/30)	96,6 (30/29)
Hybridization	3,3 (30/01)	6,6 (30/02)
Apomixis	0 (30/0)	0 (30/0)

Source: The author (2023)

DISCUSSION

Phenology

The production of flowers throughout the year in the different populations of the two *Cnidoscolus* species indicates a continual flowering pattern, according to the classification of Newstrom et al. (1994). Our results confirm the continual pattern recorded for *C. urens* by Araújo et al. (2012) in Caatinga. Although the availability of data on the flowering pattern of Euphorbiaceae species in Caatinga is limited, other authors have documented continual flowering in species of *Jatropha* L. (Santos et al., 2005). Furthermore, variations in the type of pattern can be found even within the genus *Cnidoscolus*, as in C. *quercifolius* Pohl, and in species of *Croton* L., such as *C. blanchetianus* Bail and *C. heliotropiifolius* Kunth, which show annual flowering patterns (Andrade et al., 2020a; Leite and Machado, 2010; Oliveira et al., 2018). The presence of different flowering patterns in representatives of Euphorbiaceae, which is one of the most important families in terms of number of species in Caatinga (Moro et al., 2014), reinforces the ecological importance of the family, especially in stressful environments such as the semi-arid region of Northeast Brazil where species with continual flowering are rare. Bearing in mind that flowers are fundamental resources for flower visitor guilds, the production of flowers at different times of the year, during the dry and the rainy season, contributes to the maintenance of pollinators in the community.

The correlation between the flowering of the two species and the precipitation of the same month in which flowering was estimated indicates an immediate response to the rains. Many plant species from Caatinga are sensitive to water availability to initiate reproduction (Machado et al., 1997; Quirino and Machado, 2014) and their reproductive events are conditioned by the temporal distribution of rainfall (Andrade et al., 2020b; Quirino and Machado, 2014). However, our results contrast with those of Araújo et al. (2012), who did not observe the effects of precipitation on the floral phenology of *C. urens*, despite their observation of the flowering peak during the rainy season. The reproductive activity of the studied plant species was not concentrated in a single moment of the year and, thus, did not exhibit marked seasonality. The absence of seasonality and long flowering periods can be important factors for the constant supply of resources (Araújo et al., 2012).

The high percentage of flowering overlap among populations resulted from similar phenological patterns exhibited by the two species. The lower overlap index between paired populations when they were associated is explained by the intensity of flowering during the dry season, because the population of *C. infestus* had a strong reduction in flower production during this period. Although the species present the same (continual) phenological pattern, there were differences in the pace of flower offer. While *C. infestus* invested in a massive production of flowers over three months, showing a clear peak in a single month with subsequent reduction, similar to the cornucopia strategy described by Gentry (1974), *C. urens* provided a smaller number of flowers per day during a period of five months, a strategy referred to as steady state flowering by Gentry (1974). The effects of these differences in strategies may even imply the use of different pollinators, since the cornucopia type flowering pattern is associated with the attraction of different groups of potential pollinators, including large and medium-sized bees, birds, among other animals (Gentry, 1974) while the steady state type flowering pattern is more related to pollinators that establish fixed foraging routes or trapliners, often hummingbirds and solitary bees (Gentry, 1974; Janzen, 1971; Tello-Ramos et al., 2015).

The hypothesis of displacement of flowering due to competition for pollinators predicts that plants pollinated by the same visitors are under pressure against phenological overlap and, thus, in this situation, the species are expected to flower sequentially (Devaux and Lande, 2009). Some studies have found results that support this hypothesis (e.g. Aizen and Rovere, 2010; Botes et al., 2008). Our results, however, do not indicate the occurrence of sequential flowering, but the different flowering strategies of the species help in their coexistence, given the particularities of supply of flowers over time. These differences may point to a new way of approaching the traditional hypothesis of phenological displacement, in which species share pollinators and overlap in flowering avoid competition, consequently allowing their coexistence.

Floral morphology

The two species of *Cnidoscolus* share the same set of floral traits, such as tubular, white, odorless flowers supplying nectar and pollen to visitors, with protogynous mating strategy and with the same number, position and size of stamens. In fact, the closer the species are phylogenetically, the more traits they tend to share (Burns and Strauss, 2011). All these common characteristics indicate the use of a similar resource, in this case, pollinators (Bergamo et al., 2017; Machado and Lopes, 2004). This scenario may induce competition, in which selective pressures favor floral isolation or niche shifts, allowing the coexistence of the taxa, since, otherwise, the less competitive species would go extinct (Pfennig and Pfennig, 2009).

The character displacement hypothesis states that sympatric populations of species with overlapping distributions and similar requirements regarding resource use tend to diverge in terms of morphological and/or ecological, behavioral, physiological characters from populations in allopatry (Pfennig and Pfennig, 2009). Our results indicate the occurrence of character displacement, mainly in the morphology of female flowers (Table 2). We found significant differences between species and/or populations in all variables analyzed in female flowers: flower size, tube opening length and diameter, and pistil length. Variations occurred in the traits of the perianth, which are related to the attraction of floral visitors, as well as in the reproductive organ, which is directly related to the fertilization of ovules. Miyake and Inoue (2003) recorded different pistil length values in *Clerodendrum* species (*C. trichotomum* and *C. izuinsulare* - Verbenaceae), with larger pistils observed in co-occurring populations, a result similar to that observed here in *C. urens* also in co-occurrence. Reproductive structures of different sizes can touch the body of pollinators in different places, consequently decreasing the load of heterospecific pollen on conspecific stigmas (Miyake and Inoue, 2003; Muchhala and Potts, 2007), thus avoiding the loss of ovules caused by hybridization (Fishman and Wyatt, 2006).

Phenotypic floral isolation is achieved through character displacement. This process may take place indirectly, when interspecific interactions happen through exploitation, that is, when one species affects the signaling of another and the outcome leads to reproductive costs, or it may occur directly, when interactions between species involve the loss of gametes (Pfennig and Pfennig, 2009). Our results in the hybridization tests showed that fruits are formed in interspecific crosses and, therefore, indicate that there is not yet full reproductive isolation between the two studied *Cnidoscolus* species. However, the spatial separation of the pistils may be acting as a strong pre-pollination reproductive barrier (Baack et al., 2015), likely preventing the natural formation of hybrids. According to Pfenning and Pfenning (2009), as the costs of hybridization increase, the selection against direct interactions between heterospecific pairs increases the likelihood of character displacement. According to the findings of Lowry et al. (2008), pre-zygotic barriers, including pollinator isolation, are more effective against interspecific gene flow. Thus, in general, the character displacement process acts effectively to separate reproductive systems and promote the coexistence of species. Our results corroborate this hypothesis as a mechanism to attenuate competition among species that share pollinators.

Floral visitors and reproductive success

The pollinators of the studied *Cnidoscolus* species were bees and hummingbirds, although the two plant species used them in a slightly different way, as *C. infestus* received visits from both, while *C. urens* depended almost exclusively on hummingbirds. However, the two species are connected by sharing their main pollinator, the hummingbird *C. lucidus*. Bees were recorded only in the patches where *C. infestus* was present and contributed to pollination in a similar way in associated and non-associated populations. It is noteworthy that bees also visited, although less frequently, *C. urens* flowers when this species was associated with *C. infestus*. A striking difference between the staminate flowers of *C. infestus* and *C. urens* is the folding of the petals that form a landing platform in *C. infestus*, often used as a landing base for bees that arrived at the flower, which held on to these petals while collecting the resource. This organization is also thought to increase the visual attractiveness of flowers to bees (Russell et al., 2018). So, even though the interspecific interaction does not consist of facilitation itself, it seems that at least the presence of *C. infestus* can be beneficial to *C. urens* by attracting potential pollinating bees to the local community. The attraction of bees to the flowers of *C. infestus* may be important to ensure pollination when the hummingbird *C. lucidus* is absent, since visits by other bird species to this plant were rare. Both groups of pollinators observed in *C. infestus* flowers may exert a convergent selective pressure on floral traits, given that hummingbirds and long-tongued bees belong to similar functional groups (Fenster et al., 2004). However, further investigations are needed to elucidate whether, in the long term, *C. infestus* tends to converge in the use of both groups of pollinators, or there is actually a process of floral isolation towards bee pollination in co-occurring populations.

The observed reduction in the visitation frequency to the species when occurring in association contradicts the hypothesis of facilitation and points to competition for pollinators. However, competitive effects may be present even when no differences in visitation rates are observed between isolated and co-occurring populations (e.g. Pinto and Schlindwein, 2015). Only when associated with pollen limitation, low visitation rates can negatively impact fruit and seed set (Mitchell et al., 2009). In this situation, ensuring pollinator constancy becomes essential, as the visitor would tend to look for flowers of one species, reducing pollen loss in interspecific movements (Montgomery, 2009). In fact, hummingbirds did not perform interspecific movements when they first visited *C. urens* flowers, but often alternated when the first visit was to *C. infestus*, according to field observations. Pressure due to the low frequency of pollinators in the patch where the species occurred in association may have resulted in an increase in the number of flowers open on the same day, since the supply of flowers increases floral attractiveness (Moeller, 2004) and may lead to a greater number of flowers probed per visit.

Both species had lower fruit set when associated, but the values were significantly lower only in *C. infestus*, providing strong evidence of competition for pollinators (Bell et al., 2005). The results regarding visitation frequency associated with the pollination tests for evaluation of the reproductive system indicate that the species may, in fact, be suffering from pollen limitation. The way these species interfere in the reproduction of each other remains unknown, but there are indicatives of conspecific pollen loss, because of the alternated visits to flowers, and also indicatives of pollinator monopolization, since *C. urens* seems to be preferred by the pollinator.

In summary, our findings point to negative effects of pollinator sharing, since the species showed different flowering strategies, a tendency towards phenotypic distancing in floral morphology, and lower visitation frequency when associated, besides a reduction in the reproductive success of one of them. We verified that these plants have mechanisms to mitigate the competition for pollinators through character displacement, specifically in the length of the pistils in *C. urens*, acting as a barrier against hybridization, and through the use of alternative pollinators in *C. infestus*. It is important to investigate the extent of the effects of competition between these species so as to know whether they can impact *C. infestus* to the point of compromising the survival of a viable population over time or the mitigation mechanisms are capable of allowing the stable coexistence of the species pair.

AKNOWLEDGMENTS

To Ilton Neto, Maria Alinny, Thayana Evangelista, Josefa Carneiro and Ellen Moura, for helping in the field. To Dr. Doralice Barbosa for making her property and facilities in Serra do Jatobá available for the study. Research grants were provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) to ICM (311021/2014-0, 310508/2019-3). To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) for the Master's scholarship to the first author and Finance Code 001.

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4 CONCLUSÕES GERAIS

A floração durante todo o ano das duas espécies de *Cnidoscolus* estudadas fornece recursos importantes para a manutenção dos polinizadores, em especial os beija-flores, na comunidade local. No contexto geral, as flores de *C. infestus* e *C. urens* servem como fonte de alimento para diversos grupos de insetos antófilos na Caatinga.

A competição por polinizadores nem sempre leva ao sequenciamento da floração entre espécies coexistentes que são polinizadas pelos mesmos grupos, e nossos resultados ilustram que diferentes estratégias na disponibilização de flores podem evitar os efeitos da competição. Outro fator que auxilia no impedimento da competição por polinizadores é o deslocamento de caracteres, e nossos resultados indicam que as diferenças no comprimento do pistilo entre as espécies podem estar atuando como isolantes.

A facilitação embora não tenha sido observada entre *C. infestus* e *C. urens*, uma vez que ambas as espécies receberam menor quantidade de visitas quando associadas, também, no entanto, não provocou efeitos negativos significativos no sucesso reprodutivo das populações. Assim, quanto maior a eficiência do polinizador, tanto menor é a limitação de pólen, a facilitação pode não ocorrer e interações neutras podem se estabelecer.

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