UNIVERSIDADE FEDERAL DE LAVRAS

JULIANA TULLER MENDES

TRADE-OFF ENTRE CRESCER, REPRODUZIR E DEFENDER: UMA ANÁLISE EXPERIMENTAL DO BARBATIMÃO E OS INSETOS ASSOCIADOS AOS SEUS FRUTOS

LAVRAS-MG
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Prof. Dr. Lucas Del Bianco Faria
Orientador

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TRADE-OFF BETWEEN GROWTH, REPRODUCTION AND DEFENSE: AN EXPERIMENTAL APPROACH OF BARBATIMÃO AND ITS FRUIT-ASSOCIATED INSECTS

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LAVRAS – MG
2017

Aos meus pais, Júlio e Ana.
Meus irmãos, Ricardo e Leandro,
Meu sobrinho, Pedro,
e a todos meus familiares e amigos
Dedico

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“A mente que se abre a uma nova ideia, jamais voltará ao seu tamanho original.” (Albert Einstein)

RESUMO

A fenologia da planta e o investimento em biomassa vegetativa, reprodução e defesa são influenciados por fatores bióticos e abióticos. A fertilização e a remoção foliar podem alterar a disponibilidade de nutrientes e, em consequencia, alterar a quantidade e qualidade do recurso para os insetos associados à planta. Realizamos um experimento de fertilização do solo e 100% de remoção foliar e testamos as hipóteses: i) a fertilização influencia positivamente a reprodução das plantas enquanto a desfolha influencia negativamente, e a desfolha altera a reposição foliar; ii) a remoção de folhas de *Stryphnodendron adstringens* como fonte de tanino, afeta negativamente a biomassa vegetativa e a reprodução das plantas, e aumenta as concentrações de taninos nos tecidos da planta, e que a fertilização ameniza os efeitos da remoção foliar; e iii) a fertilização influencia positivamente a quantidade e a qualidade do recurso, enquanto que a remoção das folhas influencia positivamente a produção de compostos secundários de defesa, uma vez que a fertilização afeta positivamente os insetos associados aos frutos e a remoção das folhas os afeta negativamente. A biomassa vegetativa foi definida como biomassa foliar total, a reprodução como produção de inflorescências e características e produção de frutos, e defesa como concentração de fenóis totais, taninos hidrolisáveis e de taninos condensados. Proteínas, carboidratos e lipídios dos frutos foram determinados como qualidade do recurso; e número e tamanho de sementes, e biomassa de frutos como quantidade de recurso. Embora todas as plantas tenham florescido, aquelas que sofreram desfolha produziram apenas alguns ou nenhum fruto. A desfolha alterou o tempo de substituição das folhas, já que ocorreu logo após a sua remoção foliar, não de forma sincronizada com as plantas controle. No entanto, a fertilização não afetou a fenologia das plantas, a reprodução e a defesa, mas teve um efeito marginal positivo na biomassa vegetativa. Encontramos grande quantidade de taninos nas folhas de *S. adstringens* produzidas tanto antes como depois da desfolha, sugerindo que as folhas podem servir como uma fonte alternativa sustentável de tanino. Encontramos 26 espécies de insetos associadas aos frutos e sementes de *S. adstringens* e a taxa de predação de sementes variou entre as ordens de insetos, sendo maior para Lepidoptera, seguido de Hymenoptera e Coleoptera. A fertilização não influenciou as características de frutos e sementes ou os insetos associados aos frutos. Defesa química não foi importante para os insetos associados aos frutos, e somente a qualidade do recurso influenciou positivamente a taxa de predação por Hymenoptera. Além disso, a quantidade do recurso aumentou a taxa de predação de sementes por Lepidoptera e Coleoptera, e a abundância e riqueza de insetos totais e abundância de consumidores de sementes. Quanto maior a riqueza e abundancia de insetos consumidores de sementes, maior a riqueza e abundância de parasitoides. Assim, as características do recursos afetam os insetos consumidores de sementes de *S. adstringens* de maneira diferente, mas a maior parte da comunidade de insetos associados aos frutos é determinada pela quantidade ou qualidade do recurso.

Palavras-chave: *Stryphnodendron adstringens*. Fertilização. Remoção foliar. Fenologia. Tanino. Predação de sementes.

ABSTRACT

Plant phenology and plant investment in leaf biomass, reproduction, and defense are influenced by biotic and abiotic effects. Fertilizer application and leaf removal may change nutrient availability, and, in consequence, alter the resource quantity and n for herbivorous insetcs and their parasitoids. A two-way crossed experimental design was performed by fertilizer addition in the soil and 100% of leaf removal and tested the hypotheses that i) fertilization positively influences plant reproduction, and defoliation negatively influences it, and that defoliation alters leaf replacement time; ii) harvesting leaves of *Stryphnodendron adstringens*, as a source of tannin, would negatively affect plant investment in vegetative biomass and reproduction, and increase tannin concentrations in plant's tissues, and that fertilization would ameliorate the effects of leaf harvest; and iii) fertilization positively influences resource quantity and nutritional quality, while leaf removal positively influences chemical compounds production, since the fertilization positively impact fruit-associated insects and leaf removal negatively impact them. Vegetative investment was determined by total leaf biomass, reproduction by inflorescence and fruit production traits, and defense by total phenol, hydrolyzable tannin, and condensed tannin amount. Total protein, carbohydrates and lipids in fruits were determined as resource nutritional quality; and number and size of seeds, and fruit biomass as resource quantity. Although all plants flowered, clipped plants produced only a few or no fruits. Defoliation altered leaf replacement time, since it occurred right after being clipped, assynchronously to control plants. In contrast, fertilization did not impact plant phenology, reproduction and defense, but it had a marginal positive effect on vegetative investment. We found high tannin amounts in leaves of *S. adstringens* produced both prior to and subsequent to clipping, further suggesting that leaves could serve as a sustainable alternative source of tannin. We found 26 insect species associated with *S. adstringens* fruits and seeds and percentage of seed consumed varied among insect orders, which was higher for Lepidoptera, followed by Hymenoptera and Coleoptera. Fertilizer application did not fruit and seed traits or the fruit-associated insects. Defesa química was not important for the fruit-associated insects, and only resource quality positively influenced the rate predation by Hymenoptera. We found that resource quantity increased the percentage of seed consumed by Lepidoptera and by Coleoptera, the total insect abundance and richness and the seed-feeding insect abundance. In addition, the higher the seed-feeding insect richness and abundance, the higher their parasitoid richness and abundance. As such, resource traits affect *S. adstringens* seed-feeding insects differently, but most of the fruit-associated insect community is determined by resource quantity or quality.

Keywords: *Stryphnodendron adstringens*. Fertilization. Leaf removal. Phenology. Tanin. Seed-predation.

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1 INTRODUÇÃO GERAL

Algumas espécies do Cerrado possuem grande valor econômico, sendo exploradas para produção de madeira, alimentação e/ou produção de cosméticos e medicamentos (SILVA et al., 2008; ALMEIDA et al., 2010; CORRÊA et al., 2011). O interesse atual em utilizar esses produtos naturais tem contribuído para a valorização de espécies nativas na indústria brasileira (ALMEIDA et al., 2010).

Muitas espécies do Cerrado são conhecidas por produzirem compostos químicos de interesse farmacológico em grandes quantidades (ROCHA et al., 2011). Por exemplo, as espécies do gênero *Strhyphnodendron* (Fabaceae: Mimosoideae), também conhecidas como barbatimão, têm sido amplamente utilizadas em estudos de interesse das indústrias devido à alta produção desses compostos (JACOBSON et al., 2005; SOUZA et al., 2007; ALMEIDA et al., 2010; CORRÊA et al., 2011).

Diversos estudos têm sido desenvolvidos visando avaliar os níveis de exploração das espécies de barbatimão e têm sugerido a necessidade de planos de manejo para as mesmas (BORGES-FILHO; FELFILI, 2003; MEIRA et al., 2013; FEITOSA; ALBUQUERQUE; MONTEIRO, 2014). Estudos que abordem o conhecimento em sua ecologia podem auxiliar tanto na otimização na coleta do recurso quanto da sua conservação, através do controle da sua sobrexploração.

1.1 Compostos secundários de plantas

Os compostos secundários são produtos do metabolismo vegetal que estão presentes em células especializadas que não são diretamente essenciais para o metabolismo fotossintético, metabolismo respiratório ou o transporte de solutos, mas podem ser necessárias para a sobrevivência de determinadas plantas no ambiente (CHEYNIER et al., 2013). Dentre os compostos secundários produzidos por plantas, os compostos fenólicos se destacam como o tipo de composto mais comumente produzido por plantas (BARBEHENN; CONSTABEL, 2011).

Os compostos fenólicos possuem um grupo funcional hidroxila em um anel aromático, o qual é denominado fenol (MAZID; KHAN; MOHAMMAD, 2011). Eles são bastante diversificados quanto à sua estrutura química, bem como suas propriedades químicas e físicas. Os taninos são um tipo de composto fenólico que apresenta grupos hidroxila que estão parcial, ou totalmente, esterificados e ligados com resíduos de ácido gálico ou seus derivados (GULEWICZ et al., 2014). Eles são comumente classificados como taninos hidrolisáveis e taninos condensados.

Os taninos hidrolisáveis são derivados do ácido gálico, o qual é derivado do ácido chiquímico, e são facilmente hidrolisados por ácidos fracos, bases ou enzimas para formar monômeros (GULEWICZ et al., 2014). Já os taninos condensados são derivados de antocianinas que, juntamente com outros flavonóides, como flavonas, flavonóis e isoflavonas, são o resultado da condensação de compostos derivados de fenilalanina com malonil CoA (GULEWICZ et al., 2014). Além de diferenças moleculares, eles possuem também diferenças no local de armazenamento. Enquanto os taninos hidrolizáveis se concentram na parede celular, os taninos condensados se acumulam no vacúolo das células, frequentemente na epiderme e subepiderme de folhas e frutos (BARBEHENN; CONSTABEL, 2011). A concentração de taninos é uma característica altamente plástica, podendo variar com o genótipo da planta, estágio de desenvolvimento do tecido e condições ambientais (e.g. estresse devido à baixa disponibilidade de nutrientes ou ao sofrimento de injúrias) (BARBEHENN; CONSTABEL, 2011).

Os seres humanos usam os metabólitos secundários como tratamentos contra doenças, como aromatizantes e drogas recreativas. Os taninos também têm grande importância na indústria alimentícia, sendo responsáveis por determinar o gosto e a cor de alguns alimentos, bem como atuar como antioxidantes quando consumidos moderadamente pelos homens (SOUZA et al.. 2007b; GULEWICZ et al., 2014). Em mamíferos, eles causam uma sensação adstringente na boca, a qual é resultado da precipitação de proteínas da saliva e seu consumo em excesso pode ser prejudicial (MAZID; KHAN; MOHAMMAD, 2011). Em maiores concentrações (a partir de 15% da massa seca), esses compostos são responsáveis por causar lesões no intestino de insetos herbívoros, bem como de vertebrados (BARBEHENN & CONSTABEL, 2011). O *fitness,* ou aptidão, dos insetos pode ser afetado também pela quantidade de fenóis, já que são observados consumidores de sementes de tamanho menor em sementes contendo maior concentração desses compostos (KESTRING et al., 2009). Além disso, muitos estudos afirmam que os taninos exercem um efeito negativo sobre a digestão de proteínas (BARBEHENN; CONSTABEL, 2011). Os estudos envolvendo a interação taninos-herbívoros geralmente se referem apenas às folhas e seus consumidores, mas muitas vezes a planta também apresenta esses compostos nas sementes, os quais se encontram associados a flavonóides com função de evitar dessecação (LEPINIEC et al., 2006).

1.2 Interações tróficas e insetos associados a frutos

As relações tróficas são interações antagônicas entre organismos que se caracteriza pelo ato do consumo em partes ou total de um organismo por outro. Fundamentadas no pressuposto de alta complexidade das comunidades naturais, é sugerido que as diferentes espécies deveriam ser agregadas em níveis tróficos discretos – i.e., produtores primários, herbívoros, predadores primários e predadores de topo (POLIS et al., 2000). As plantas representam a base energética das cadeias tróficas, atuando como produtores primários. As plantas representam a maior biomassa do planeta e são amplamente consumidas pelos herbívoros. A herbivoria constitui uma interação entre plantas e diferentes organismos com importantes repercussões ecológicas e evolutivas (COLEY; BARONE, 1996).

Para escapar das injúrias causadas por herbívoros, as plantas desenvolveram estratégias de defesa baseadas na presença de compostos químicos, barreiras mecânicas ou associações biológicas (CORNELISSEN; FERNANDES, 2001). Além das defesas, a quantidade e qualidade do alimento utilizado também está relacionada à fisiologia, ecologia e comportamento dos insetos, tornando-se fundamentais nas relações tróficas entre insetos e plantas (AWMACK; LEATHER, 2002). As variações nas condições bióticas e/ou abióticas locais afetam a qualidade e quantidade de recursos oferecidos pela planta hospedeira (DANKS, 2002). Além disso, mudanças nas características físicas e químicas das plantas, tal como os tipos e quantidades de defesa produzidas contra herbívoros, muitas vezes estão associadas aos níveis de dano sofridos ao longo de toda sua vida (BARTON; KORICHEVA, 2010).

Um grupo bastante impactante para a vida das plantas são os insetos consumidores de sementes. Muitas vezes, a reprodução das plantas pode ser intensamente afetada, positiva ou negativamente, pelo consumo de sementes por esses animais (SOUTHGATE, 1979; TOMAZ; KESTRING; ROSSI, 2007; FOX et al., 2012; TULLER et al., 2015). Os frutos e sementes são órgãos vegetais bastante nutritivos e, por isso, atraem uma ampla variedade de consumidores (WOODWELL; WHITTAKER; HOUGHTON, 1975). Os insetos das ordens Coleoptera, Lepidoptera, Hymenoptera, Diptera e Hemiptera destacam-se como os principais grupos de consumidores de sementes (JANZEN, 1971). Os coleópteros da família Chrysomelidae, subfamília Bruchinae, são organismos exclusivamente consumidores de sementes em sua fase larval e, em grande parte das vezes, estão associados a espécies de leguminosas

(SOUTHGATE, 1979, FOX et al., 2012). Na fase adulta, sua alimentação consiste de pólen, néctar, ou, em alguns casos, eles não se alimentam nesta fase (SOUTHGATE, 1979). As interações entre bruquíneos e suas plantas hospedeiras podem ser determinadas pela fenologia da planta e pela morfologia e química dos frutos e sementes, o que os torna bastante específicos quando aos seus hospedeiros (JANZEN, 1969; SOUTHGATE, 1979).

Os insetos da ordem Lepidoptera consomem não só as sementes, mas também a polpa dos frutos de leguminosas (PENTEADO-DIAS; NASCIMENTO; DIAS, 2008; MORALES-SILVA; ZAMPIERON, 2016). Assim como a predação de sementes por bruquíneos, os lepidópteros afetam negativamente a germinação das sementes, o que diminui o *fitness* das suas plantas hospedeiras (PINTO et al., 2013).

Dentre os insetos da ordem Hymenoptera, os do gênero *Allorhogas* sp. (Braconidae), descrito há cerca de 30 anos (MACÊDO; MONTEIRO, 1989), têm se destacado por sua ampla ocorrência em sementes de leguminosas (MACÊDO; MONTEIRO, 1989; TULLER et al., 2015, MORALES-SILVA; ZAMPIERON, 2016). Esses insetos possuem um comportamento semelhante a insetos galhadores, causando uma hipertrofia dos tecidos das sementes e deformando as sementes atacadas por eles (MACÊDO; MONTEIRO, 1989, TULLER et al., 2015).

Como a larva dos insetos consumidores de sementes se encontram em um lugar de difícil acesso a predadores, os seus principais inimigos naturais são os insetos parasitoides. Esses insetos penetram frutos e sementes com o seu ovipositor e colocam seus ovos no interior ou na superfície de ovos e/ou larvas do seu hospedeiro e são conhecidos como endo e ectoparasitoides, respectivamente. Geralmente, os endoparasitoides são caracterizados como idiobiontes – i.e. matam o seu hospedeiro assim que depositam o seu ovo – enquanto os ectoparasitoides muitas vezes são considerados coinobiontes – i.e. permitem o desenvolvimento do seu hospedeiro enquanto se alimentam dele até o parasitoide quase ter atingido a fase adulta (BLACKBURN, 1991). Assim, como os seus hospedeiros, os parasitoides são bastante restritos quanto à sua gama de hospedeiros devido à complexidade exigida para a exploração do seu recurso (STRAND; OBRYCKI, 1996). A permanência de consumidores de sementes e parasitoides durante toda a fase larval dentro de frutos facilita a coleta de grande parte da comunidade de insetos associados a frutos. Isso viabiliza o estudo de redes tróficas com esses organismos, já que combina relativamente baixo esforço amostral e alta eficácia na captura da comunidade (TULLER et al., 2015).

1.3 Efeito de cascata trófica, Hipótese do Trade-off, Hipótese do Balanço Carbono-Nutrientes e Hipótese da Defesa Ótima

Um dos padrões mais comuns das comunidades é a ocorrência de efeitos de cascata trófica (ECT), o qual é o efeito indireto que uma espécie exerce sobre outros de níveis tróficos não adjacentes ao seu, capaz de alterar a abundância, riqueza e composição de espécies ou a interação entre elas (PAINE, 1980). Os ECT do tipo topo-base (*top-down*) ocorrem quando espécies de níveis tróficos superiores controlam as de níveis tróficos inferiores; já os ECT do tipo base-topo (*bottom-up*) ocorrem quando espécies de níveis tróficos inferiores controlam as de níveis tróficos superiores (PACE et al., 1999; TERBORGH; HOLT; ESTES, 2010).

Ainda que haja uma grande quantidade de organismos consumidores de plantas, elas ainda representam a maior porção de biomassa verde do planeta. Duas hipóteses foram sugeridas para explicar “porque o mundo é verde” (HAIRSTON; SMITH; SLOBDINK, 1960) e ainda hoje são discutidas (WILKINSON; SHERRATT, 2016). A primeira sugere que os EFC do tipo topo-base controlam as populações de herbívoros a partir da ação de seus inimigos naturais, o que permite a maior proliferação das plantas (HAIRSTON; SMITH; SLOBDINK, 1960). Já a segunda hipótese se baseia nos ECT do tipo base-topo e prediz que isso ocorre devido à produção de compostos secundários de defesa que as torna impalatáveis para muitos herbívoros (MURDOCH et al., 1966).

Em solos mais ricos em nutrientes, as plantas se reproduzem com maior intensidade produzindo frutos e sementes com maior biomassa e maior número de sementes por fruto (MENDES, 2013). Em consequência, há uma maior abundância de consumidores de sementes, bem como de seus parasitoides, o que indica a ocorrência de um efeito de cascata trófica *bottom-up* via nutrientes do solo (MENDES, 2013). Relacionados com o maior número de sementes produzidas por fruto, os frutos de maior biomassa possuem um maior número de *loci* e, em consequência, maior quantidade de sementes como recurso disponível para os insetos consumidores de sementes. Já as sementes de maior biomassa proporcionam maior quantidade de recurso disponível para o desenvolvimento dos insetos (Janzen, 1969; YANG; FUSHING; HORNG, 2006), o que propicia adultos maiores (BONAL; MUÑOZ, 2009; KESTRING et al., 2009, BONAL et al., 2012) e, em consequência, o maior *fitness* dos mesmos (COPE; FOX, 2003).

Já plantas sob intenso dano foliar podem atrasar a produção de frutos e ainda produzí-los em menor número, podendo causar ainda uma maior taxa de predação de sementes

(KERSCH-BECKER; LEWINSOHN, 2012), o que se deve ao menor número de frutos e sementes disponíveis. Além disso, plantas impactadas pelo dano foliar apresentam maior taxa de predação de sementes em solo mais pobre devido ao estresse intenso da falta de nutrientes e dano somados (KERSCH-BECKER; LEWINSOHN, 2012). Por outro lado, plantas sem dano foliar são mais atacadas quando em solo com adição de nutrientes (KERSCH-BECKER; LEWINSOHN, 2012).

O balanço carbono/nutrientes na planta determina fortemente a sua palatabilidade para herbívoros (BRYANT; CHAPIN III; KLEIN, 1983). Segundo a Hipótese do Balanço Carbono-Nutrientes (HBCN), plantas em solo rico em nutrientes irão investir em defesas à base de N enquanto plantas em solo pobre irão produzir defesas à base de C, tal como os fenóis e taninos (BRYANT; CHAPIN III; KLEIN, 1983). Além disso, plantas em solos com nutrientes limitados irão apresentar taxa de crescimento menor para compensar essa deficiência (BRYANT; CHAPIN III; KLEIN, 1983). Então, como N é mais limitante do que a luz e C, em solos pobres em N as plantas tendem a investir em compostos de defesa à base de C, tais como taninos ou terpenos, já que a fotossíntese diminui e há um acúmulo de C na planta (MATTSON-JR, 1980). Para estudos que abordam a HBCN, Koricheva (2002) sugere a avaliação dos metabólitos secundários taninos (à base de C) e ligninas (à base de N), já que estes estão ligados diretamente à via do ácido chiquímico. Esta via converte precursores de hidratos de carbono simples derivados da glicólise e pentoses-fosfato para os aminoácidos aromáticos nos organismos, interferindo diretamente na produção de proteína (HERMANN; WEAVER, 1999).

Apesar de não estarem envolvidos no metabolismo primário das plantas, a produção de compostos secundários de defesa é dispendiosa para as plantas (HERMS; MATTSON, 1992). Devido a esse custo, muitas plantas desenvolveram a estratégia de produção de compostos de defesa apenas em caso de ocorrência de algum estresse, também conhecidos como compostos de defesa induzidos (STOCK; LEROUX; VAN DER HEYDEN, 1993; KARBAN; BALDWIN, 1997). Essa habilidade de produzir compostos induzidos permite que plantas invistam pouco ou nenhum recurso em defesas antes do estresse. Como consequência, elas podem utilizar esses recursos para outras funções metabólicas, tal como crescimento e reprodução (NEILSON et al., 2013).

Devido a limitações na disponibilidade de recursos no ambiente, as plantas são induzidas a fazer um balanço para investir em crescimento, reprodução e defesa, visando maximizar o seu *fitness*. Uma estratégia bastante comum frente às restrições na disponibilidade de recursos é a ocorrência de *trade-offs*, ou seja, a planta passa a investir mais em uma ou mais funções em detrimento de outras (HERMS; MATSON, 1992; BRIGGS; SCHULTZ 1999; WEINER et al., 2009). Além disso, as plantas também podem realizar *trade-offs* dentro de uma mesma função. Por exemplo, elas podem produzir mais de um determinado tipo de defesa em detrimento de outro (COLEY, 1983; KORICHEVA et al., 2004; DOSTÁLEK et al., 2016). Existe também uma diferenciação na concentração de compostos de defesa entre os órgãos das plantas (GODSCHALX et al., 2016). A Hipótese da Defesa Ótima prediz que os órgãos melhor defendidos serão aqueles com maior valor para o *fitness* da planta e/ou aqueles mais vulneráveis à herbivoria, otimizando assim o investimento de recursos em defesa contra herbívoros e patógenos (MCKEY, 1974; GODSCHALX et al., 2016).

1.4 O barbatimão e sua importância

Stryphnodendron adstringens (Mart.) Coville (Fabaceae: Mimosoideae) é popularmente conhecida como barbatimão, barbatimão-verdadeiro, barba-de-timão, barbatimão-vermelho, casca-da-virgindade, iba-timão, chorãozinho-roxo, dentre outros nomes. Essa planta é uma leguminosa que esta distribuída amplamente na porção central do Brasil, ocorrendo desde o Pará, passando pelo planalto central brasileiro, Minas Gerais e São Paulo (FELFILI et al., 1999). Ela pertencente ao bioma Cerrado e é comumente encontrada em formações savânicas primárias e secundárias (FELFILI et al., 1999).

Os indivíduos desta espécie ocorrem em elevadas densidades e podem atingir até 5 m de altura e 30 cm de diâmetro no nível do solo (FELFILI et al., 1999). É descrita como uma espécie perenifólia, com pico de floração, produção de folhas novas e queda de folhas entre os meses de julho e novembro (FELFILI et al., 1999). As suas inflorescências têm número variável de pequenas flores de cor marrom, hermafroditas, as quais são visitadas principalmente por abelhas (ORTIZ et al., 2003). Os frutos são produzidos em baixa quantidade e o estágio de maturação dura praticamente o ano todo (ORTIZ et al., 2003).

A grande concentração de taninos nos tecidos do barbatimão tem sido empregada em diversas áreas, como na produção de sabonete antisséptico (SOUZA et al., 2007a) e adesivos comerciais (ALMEIDA et al., 2010), além da utilização de sua madeira de cerne vermelha para a construção civil (LORENZI, 1992). Há também sua ampla utilização como um item da medicina popular dos povos do Cerrado brasileiro (CORRÊA et al., 2012). O extrato de barbatimão já se mostrou efetivo no tratamento de cáries, diarreias, infecções urinárias e cicatrização de feridas (AUDI et al., 2004; LOPES et al., 2005; ISHIDA et al., 2006; OLIVEIRA et al., 2007; SOUZA et al., 2007b; SANTOS et al. 2009; FIORI et al., 2013).

Apesar da grande quantidade de informação com relação à importância da planta na área farmacológica, pouco se sabe sobre as interações dos insetos consumidores de sementes e seus parasitoides. Em um estudo preliminar foi observada a espécie *Acanthoscelides gregorioi* (Pic) (Chrysomelidae: Bruchinae) consumindo as sementes do Barbatimão (SARI; RIBEIRO-COSTA, 2012). Outros estudos também observaram que o impacto que consumidores de sementes possuem sobre a viabilidade das sementes de *S. adstringens* está entre 12 e 50% de sementes viáveis (CORRÊA et al., 2012). Trabalhos sobre interações ecológicas entre essa espécie de planta e seus consumidores, polinizadores e/ou dispersores constituem um campo de enorme importância para uma real interpretação de suas informações biológicas, taxonomia e ecologia.

O objetivo geral desse estudo foi avaliar a resposta da planta *S. adstringens* e da rede de insetos associados aos seus frutos à remoção foliar severa e à fertilização. Para este fim, no primeiro capítulo acompanhamos a fenologia das plantas ao longo de 14 meses após o início dos experimentos de remoção foliar e fertilização. No segundo capítulo, testamos a ocorrência de *trade-offs* entre crescer, reproduzir e defender frente às diferenças na disponibilidade de recursos originadas pelos tratamentos. Além disso, analisamos quais os órgãos da planta e sob quais circunstâncias havia uma maior concentração de taninos. Por fim, no terceiro capítulo, avaliamos a ocorrência de um efeito de cascata trófica originado pelas características de frutos e sementes até os parasitoides de consumidores de frutos e sementes de *S. adstringens* submetidas à remoção foliar severa e à fertilização.

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SEGUNDA PARTE

ARTIGOS

ARTIGO 1

PHENOLOGICAL CHANGES IN RESPONSE TO DEFOLIATION AND FERTILIZATION IN A NEOTROPICAL SAVANNA TREE

Phenological changes in response to defoliation and fertilization in a Neotropical savanna tree

Abstract

Plant phenology is influenced by both biotic and abiotic factors, which determine the resource availability and the conditions for each phase to happen. We manipulated the resource availability for the medicinal plant *Stryphnodendron adstringes* and evaluated its phenological responses to it. We increased the resource availability by applying fertilizer and decreased it, by removal of 100% of the leaves by clipping and, posteriorly, by leaf-cutter ant attack. We hypothesized that fertilization positively influences plant reproduction, and defoliation negatively influences it, and that defoliation alters leaf replacement time. Although all plants flowered at least once along the study, clipped plants produced only a few or no fruits. In addition, defoliation altered leaf replacement time, since it occurred right after being clipped, and also one more time, but not synchronously as with the unclipped plants. In contrast, fertilization did not impact plant phenology, as control and fertilized plants exhibited the same pattern of reproduction and leaf replacement. Plants in habitats with high leaf damage levels are subject to adaptations under intense stress. The plant *S. adstrigens* alters their phenology, flowering following defoliation addressing emergency reproduction, but fruit production was very low due to resource limitations, which was not satisfactorily supplied by fertilization.

Keywords: S*tryphnodendron adstringens*; barbatimão; leaf removal; NPK; phenology; stress-induced flowering.

Running title: Plant manipulation and phenological change

Introduction

Leaf replacement, flowering, fruit production and seed development, dispersal and germination typically have a sequence that favor their occurrence under the best conditions (Ehrlén 2015). Leaf damage and fertilization may alter resource availability and, consequently, the occurrence time or the quantity of resource invested in each phase. In response to leaf damage, plants may present under-compensation, equal-compensation or over-compensation in leaf biomass production, and fertilizer application may favor higher fitness following damage (Maschinski & Whitham 1989). For example, fertilization increases resource availability and also flower and fruit production (Sperens 1997). On the other hand, some plants anticipate or delay flower and fruit production, or do not produce fruits due to resource restriction after herbivore attack (Freeman et al 2003, Kersch-Becker & Lewinsohn 2012, Wang et al. 2016). Changes in flowering time may represent plant tolerance to leaf damage, anticipation to which is related to more tolerant plants and a delay, to less tolerant plants (Barton 2013). Changing flowering onset time is not advantageous for pollinator limited plants (Bierzychudek 1981), since those flowers produced earlier or later may have a lower pollinization rate because they occur asynchronously to higher pollinator abundances; however, seeds originated from flowers produced at a later time, for example, undergo lower seed-predation rates (Ehrlén et al. 2015).

Stryphnodendron adstringens (Mart.) Coville, also known as *barbatimão*, is a species widely distributed in Cerrado (Brazilian savanna), and its stem bark is commonly used by rural populations against several diseases (Corrêa et al. 2012). This species produces racemose andromonoecious inflorescences that are visited by bees, wasps, flies, butterflies, and beetles, the first two groups representing more than 90% of all visits (Ortiz et al. 2003). *Stryphnodendron adstringens* fruit production is low and highly taxing on the plant (Ortiz et al. 2003). Its inflorescences are produced from July to November and its fruit development occurrs throughout the year (Felfili et al. 1999). Moreover, senescent and resprouting leaves occur at the same time as fruit ripening, from July to November (Felfili et al. 1999).

Considering that plant phenology is highly dependent on resource availability, and that fertilization increases plant resource availability and leaf damage decreases it, the aim of this study was to evaluate how resource manipulation influences *S. adstringens* phenology. We hypothesized that fertilization positively influences plant flower and fruit production, as more resources are available, and defoliation should negatively influence phenology as resources are depleted. Clipped plants should not replace leaves at the same level as unclipped plants, as they already spent resources replacing leaves right after undergoing intense defoliation.

Materials and Methods

The studied sites were in Itumirim, Minas Gerais state, Brazil (Site 1: 21°14'9.82"S 44°49'43.29"W; Site 2: 21°13'41.98"S 44°51'0.43"W). The region is characterized by mild summers and dry winters, the annual average temperature is 19.4°C, and the annual average rainfall is 1530 mm (Sparovek et al. 2007). The original vegetation of this region is Atlantic Forest seasonal forest (Veloso et al. 1991) in transition with the Cerrado biome. It is a very altered region, mainly by coffee plantations and pastures.

In April 2014, we selected two sites, 2.4 km apart, containing reproductive individuals of *S. adstringens* to conduct our experiments. We selected 80 plants 3-4 m high and with similar crown size and architecture and removed all inflorescences and infrutescences from them. The coordinates of the plants are in the Table 1SM of the Supplementary Material. Then, in each site, we randomly assigned ten individuals to the following treatments: control (Co); application of NPK fertilizer (F); 100% of leaves removed by clipping (C); application of NPK fertilizer and 100% of leaves removed by clipping (CF) (Figure 1). Another treatment was included after ants severely attacked five of the CF plants, which was application of NPK fertilizer + 100% of leaves removed by clipping followed by 100% of leaves removed by leaf-cutter ants immediately after three months (CFA) (Figure 1). This intense leaf removal by leaf-cutter ants shows that high levels of leaf removal is common for *S. adstringens* in the studied areas.

We clipped all leaves from plants assigned to the treatments C, CF and CFA in April 2014. Moreover, from April 2014 to February 2015, we added 100 g of NPK (10-10-10) fertilizer on the soil around each plant of treatments F, CF, and CFA on alternative months. In total, 600 g of NPK fertilizer were applied to each plant. We applied fertilizer into three holes around the plants to avoid loss through surface runoff.

From May 2014 to June 2015, the sites were visited monthly, and the following reproductive organs per plant were counted: i) closed inflorescences ii) opened inflorescences, iii) infrutescences, and iii) developed fruits. We also observed the occurrence of leaf replacement, such as senescent or resprouting leaves, monthly.

We performed GLM to monthly compare the phenological reproductive phases (number of closed inflorescences, opened inflorescences, infrutescences, and developed fruits) of control plants (Co) and plants of the treatments fertilized (F), clipped (C), clipped + fertilized (CF), and clipped + fertilized + attacked by leaf cutter ants (CFA). Then, we conducted contrast analyses to compare the treatments. All analyses were performed using the software R (R Development Core Team 2016).

Results

All plants produced inflorescences at least once along the study. Closed inflorescences occurred from May to November 2014, and again in January and February 2015 (Figure 2a). Closed inflorescences occurred mainly in 2014 August and September in unclipped plants - Co and F - , in May and June 2014 in clipped plants - C, CF, and CFA - , and also in September 2014 in the plants clipped and posteriorly attacked by leaf-cutter ants - CFA - (Figure 2a). Opened inflorescences occurred from July to December 2014, and in February 2015 (Figure 2b). Unclipped plants - Co and F - had higher number of opened inflorescences in September and October 2014, but clipped plants - C, CF, and CFA - had only a few of opened inflorescences that occurred from July to December 2014 (Figure 2b). In summary, as we hypothesized, inflorescences were highly influenced by damage occurrence that anticipated their production in harvested plants.

Infrutescences were observed from October 2014 to March 2015 (Figure 2c). Most of the infrutescences developed from November 2014 to January 2015, and clipped plants produced only a few or no infrutescences. Despite this, CFA plants produced slightly more infrutescences than the other clipped plant treatments. Developed fruits occurred in August 2014, and from November 2014 to June 2015 (Figure 2d). Most of the developed fruits were observed from February to June 2015, which were almost all from unclipped plants. Unclipped plants - Co and F plants - produced more than 90% of all sampled fruits, while CFA plants produced around 8%, and C and CF plants together only produced less than 2% of the total. As well as infrutescences, clipped plants produced only a few or no fruits. We partially corroborated our hypothesis that fertilization will positively influence plant flower and fruit production and defoliation will negatively influence it, since only CFA plants, among clipped-fertilized plants, produced more fruits than only clipped plants, and CF plants produced the same quantity of fruits as C plants and F and Co plants produced the same quantity of fruits.

Clipped plants not only replaced leaves right after being harvested by clipping and by ants, but also they replaced all leaves once more, as required by plants in natural conditions such as Co plants. Despite this, while Co and F plants replaced leaves from August to November 2014 (Figure 3), clipped plants were very variable and their leaf replacement occurred between July 2014 and April 2015 (Figure 3). The Table 1 presents the mean, and the standard error of all reproductive events of each treatment and control monthly and the statistical results of the comparisons among treatments monthly.

Discussion

We partially corroborated our hypothesis that fertilization positively impacted plant reproduction and leaf replacement, and defoliation negatively impacted these, since fertilization did not affect them, but defoliation negatively influenced plant reproduction and altered their flowering and leaf replacing time. Some plant species present stress-induced flowering in response to different kinds of stress, such as low nutrient availability, high-intensity light, drought and leaf damage (Wada & Takeno 2010). Although the stressed plants produced flowers as an emergency response of reproduction, *Stryphnodendron adstringens* fruit production was negatively impacted following 100% defoliation, as predicted by our hypothesis. Indeed, many plants do not produce fruits due to resource restriction after being damaged, showing that reproduction is costly for plants (Freeman et al 2003, Kersch-Becker & Lewinsohn 2012, Wang et al., 2016). Ortiz and collaborators (2003) conducted a pollination experiment with *S. adstringens* and observed that although pollinated, many flowers did not develop fruits because of maternal resource limitations.

In contradiction to our hypothesis, fertilization neither impacted *S. adstringens* reproduction nor leaf replacement. *Rhizobium* and its nodules may be responsible for nutrient gathering in *S. adstringens* (Lemos 2009) and fertilizer application might inhibit this kind of association (Streeter 1985, Puaiatti & Sodek 1999). Thus, it is possible that fertilization decreased nodule activity during our study and the plants might have similar nutrient availability under fertilized and non-fertilized conditions, not impacting plant phenology.

Defoliation altered leaf replacement time, since it occurred right after being clipped, and also one more time, but not synchronously with unclipped plants. Leaf replacement is critical for defoliated plants to maintain photosynthetic activity and the production of metabolites to accomplish their functions (Hetherington et al. 1998). In addition, leaf resprouting right after the damage stressed the plants, altering their physiology and, in consequence, their leaf turnover occurred at a different time than in unclipped plants.

Plants in habitats with high leaf damage levels are subject to adaptations under intense stress. *Stryphnodendron adstrigens* alter their phenology following severe leaf damage and change the leaf turnover dynamics and the flowering time addressing an emergency leaf replacement and reproduction; however, it does not necessarily indicate that the plant will produce more fruits than unclipped plants because the leaf replacement requires much energy as well as fruit production and flowers might not become fruits if the plant is under resource limitations. In addition, the fertilization may not represent higher nutrient availability for *S. adstringens*, since they present associations with *Rizhobium* species and it may be inhibited under high nutrient availability (Streeter 1985, Puaiatti & Sodek 1999).

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FIGURES

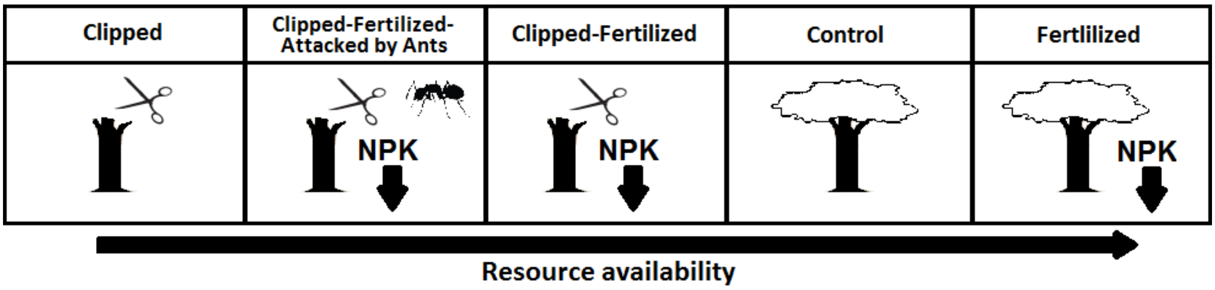
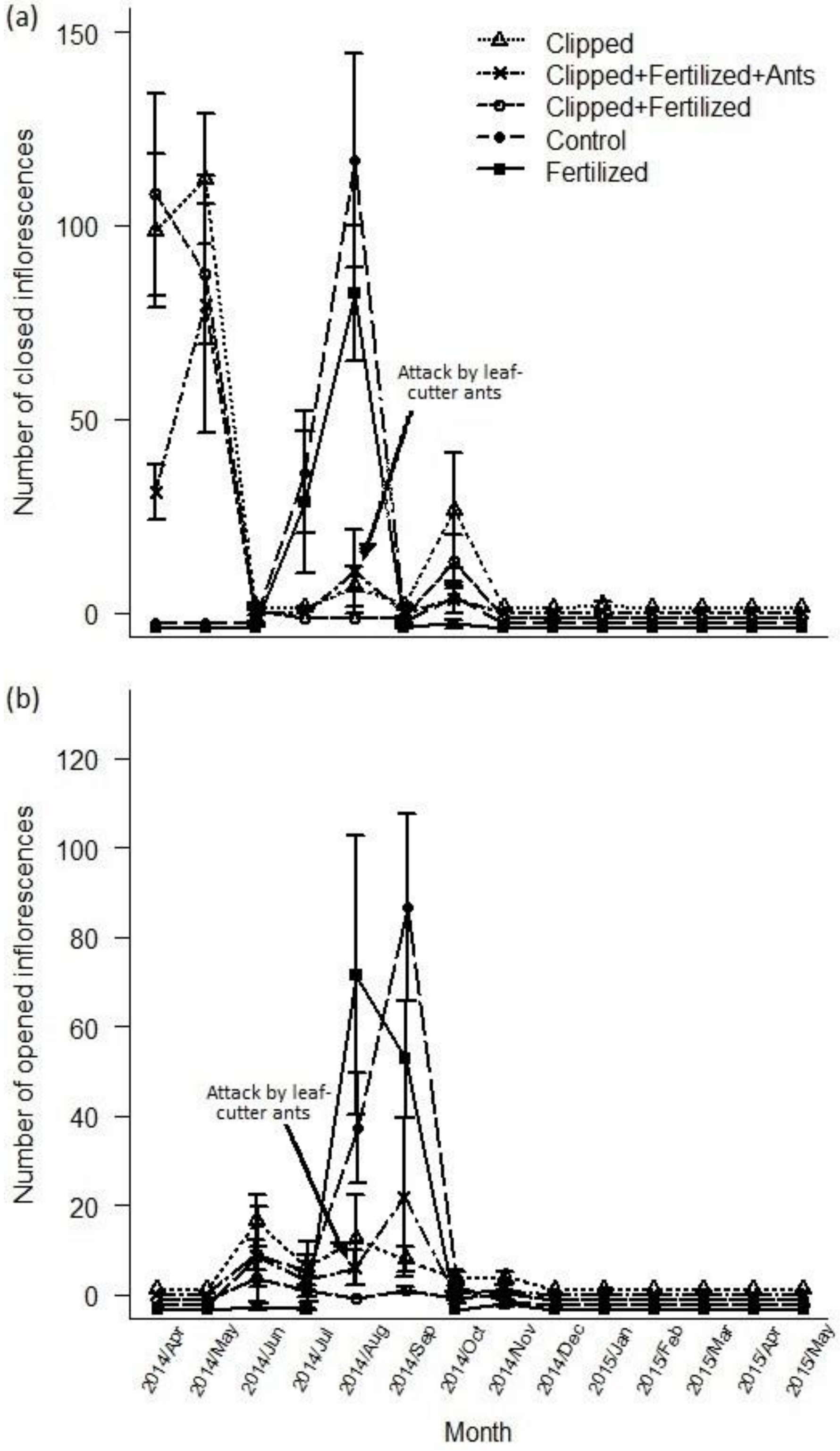


Figure 1. Treatments realized with *Stryphnodendron adstringens* plants in two areas in Itumirim, Southest of Brazil. From the lowest to the highest resource availability, the treatments were: Clipped (100% of all leaves removed by clipping), Clipped-Fertilized-Attacked by Ants (100% of leaves removed by clipping, application of NPK fertilizer to the soil and 100% of leaves posteriorly removed by leaf-cutter ants), Clipped-Fertilized (100% of leaves removed by clipping and application of NPK fertilizer to the soil), Control and Fertilized (application of NPK fertilizer to the soil).


(a)
Number of closed inflorescences
150
100
50
0
Clipped
Clipped+Fertilized+Ants
Clipped+Fertilized
Control
Fertilized
Attack by leaf-cutter ants
(b)
Number of opened inflorescences
120
100
80
60
40
20
0
Attack by leaf-cutter ants
2014/Apr
2014/May
2014/Jun
2014/Jul
2014/Aug
2014/Sep
2014/Oct
2014/Nov
2014/Dec
2015/Jan
2015/Feb
2015/Mar
2015/Apr
2015/May
Month

Continue...

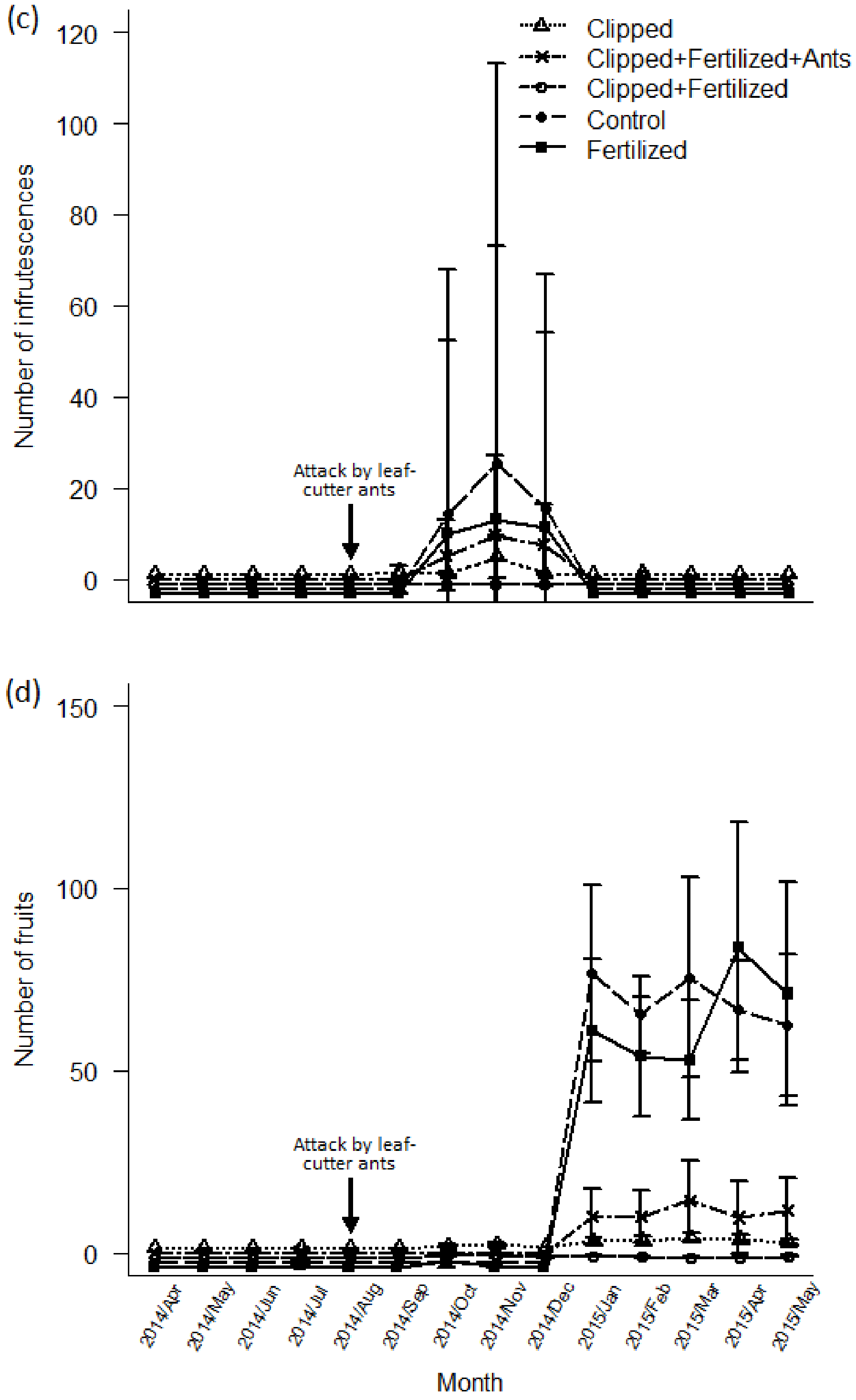


Figure 2. Monthly observation of (a) closed inflorescences, (b) opened inflorescences, (c) infrutescences, and (d) developed fruits from *Stryphnodendron adstringens* of the treatments Clipped, Clipped-Fertilized-Attacked by leaf-cutter ants, Clipped-Fertilized, Control, and Fertilized from May 2014 to June 2015 in Itumirim, Minas Gerais, Brazil.

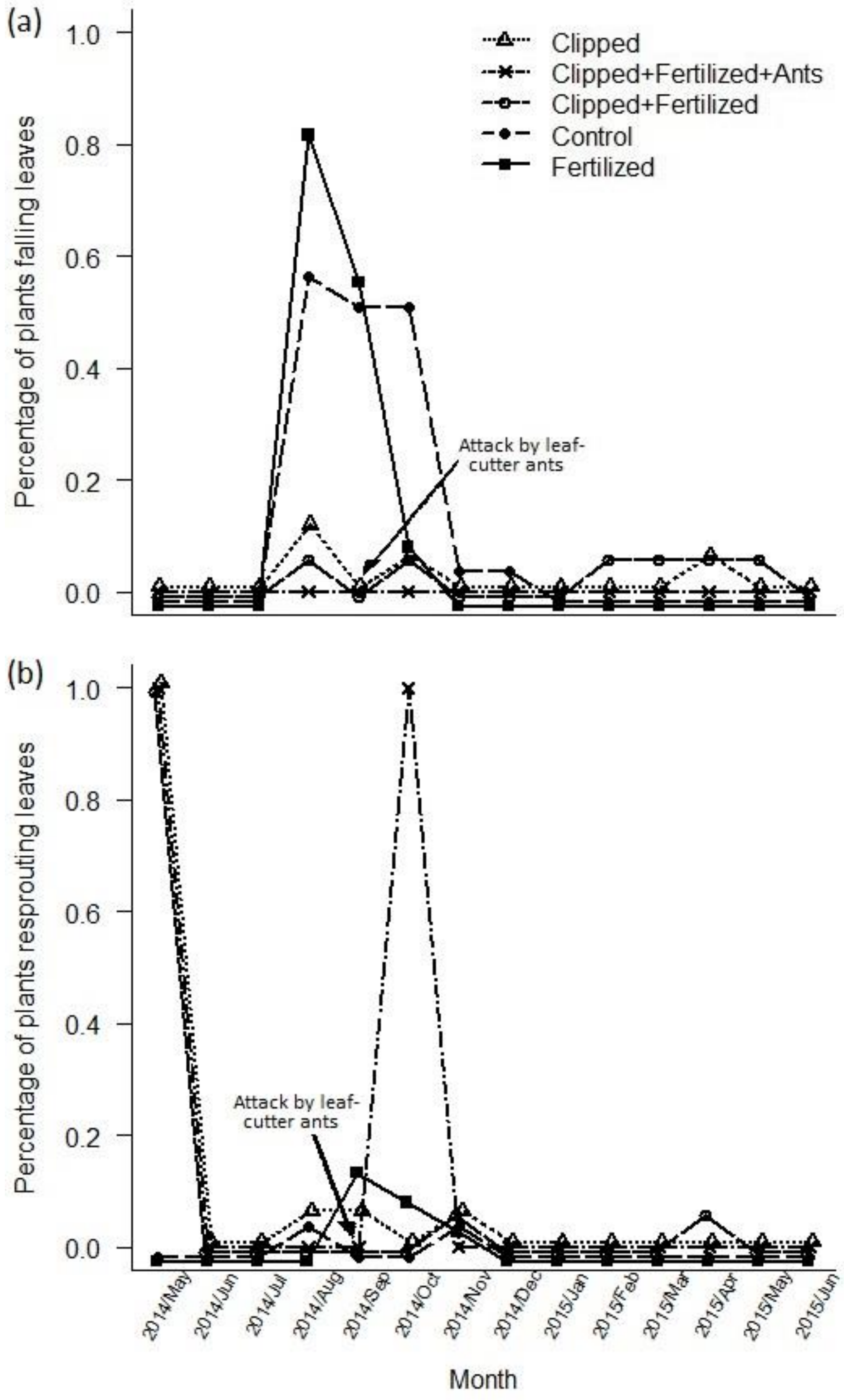


Figure 3. Monthly observation of the percentage of plants replacing leaves, which is represented by (a) falling leaves, and (b) leaf resprouting occurrence in *Stryphnodendron adstringens* from the treatments Clipped, Clipped-Fertilized-Attacked by leaf-cutter ants, Clipped-Fertilized, Control, and Fertilized from May 2014 to June 2015 in Itumirim, Minas Gerais, Brazil.

TABLE

Table 1. Monthly observation of *Stryphnodendron adstringens* reproductive events of Control plants (Co), and plants from the treatments Fertilized (F), Clipped (C), Clipped-Fertilized (CF), and Clipped-Fertilized-Attacked by ants (CFA) from May 2014 to June 2015 in Itumirim, Minas Gerais, Brazil. The table presents the average and the error pattern of each variable per month. Different letters represent values statistically different, considering $p \leq 0.05$.

Event	Treatment	Month													
		2014/May	2014/Jun	2014/Jul	2014/Aug	2014/Sep	2014/Oct	2014/Nov	2014/Dec	2015/Jan	2015/Feb	2015/Mar	2015/Apr	2015/May	2015/Jun
Number of closed inflorescences	Co	0 *a*	0 *a*	0.11 ± 0.07 *a*	38.84 ± 15.66 *a*	119.32 ± 27.57 *a*	0.11 ± 0.11 *a*	6.68 ± 4.12 *a*	0 *a*	0.16 ± 0.16 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*
	F	0 *a*	0 *a*	0.21 ± 0.12 *a*	32.31 ± 18.28 *a*	86.39 ± 17.42 *a*	0.16 ± 0.12 *a*	1.11 ± 1.11 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*
	C	97.33 ± 19.87 *b*	110.72 ± 16.89 *b*	0.50 ± 0.19 *a*	0.06 ± 0.06 *b*	5.56 ± 5.16 *bc*	0.67 ± 0.50 *a*	25.17 ± 14.79 *a*	0 *a*	0 *a*	0.83 ± 0.83 *a*	0 *a*	0 *a*	0 *a*	0 *a*
	CF	109.40 ± 26.07 *b*	88.87 ± 18.13 *b*	2.13 ± 1.51 *a*	0 *b*	0 *b*	0.13 ± 0.13 *a*	14.53 ± 6.88 *a*	0 *a*	0 *a*	0.33 ± 0.33 *a*	0 *a*	0 *a*	0 *a*	0 *a*
	CFA	31.20 ± 7.07 *c*	79.60 ± 33.23 *b*	0 *a*	0 *b*	10.80 ± 10.80 *bc*	0 *a*	3.60 ± 3.60 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*
Number of opened inflorescences	Co	0 *a*	0 *a*	10.95 ± 10.84 *a*	7.32 ± 6.68 *a*	39.47 ± 12.29 *a*	88.79 ± 20.97 *a*	3.84 ± 3.57 *a*	0.63 ± 0.53 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*
	F	0 *a*	0 *a*	0.79 ± 0.79 *a*	0.11 ± 0.07 *a*	74.83 ± 31.31 *a*	56.05 ± 13.21 *ac*	0 *a*	1.37 ± 0.59 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*
	C	0 *a*	0 *a*	15.33 ± 5.72 *a*	5.17 ± 2.38 *a*	11.22 ± 10.07 *b*	6.78 ± 2.81 *b*	2.67 ± 1.60 *a*	2.83 ± 1.32 *a*	0 *a*	0.06 ± 0.06 *a*	0 *a*	0 *a*	0 *a*	0 *a*
	CF	0 *a*	0 *a*	4.73 ± 1.87 *a*	2.00 ± 1.30 *a*	0.20 ± 0.11 *b*	2.67 ± 0.83 *b*	0.27 ± 0.27 *a*	1.47 ± 0.63 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*
	CFA	0 *a*	0 *a*	8.80 ± 3.31 *a*	3.40 ± 2.23 *a*	6.00 ± 3.89 *b*	21.80 ± 17.66 *bc*	0.40 ± 0.40 *a*	1.00 ± 1.00 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*

Continue...

Event	Treatment	Month													
		2014/May	2014/Jun	2014/Jul	2014/Aug	2014/Sep	2014/Oct	2014/Nov	2014/Dec	2015/Jan	2015/Feb	2015/Mar	2015/Apr	2015/May	2015/Jun
Number of infrutescences	Co	*0 a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	16.34 ± 53.47 *a*	27.52 ± 87.68 *a*	17.52 ± 51.37 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*
	F	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0.07 ± 0.11 *a*	13.13 ± 42.26 *abc*	16.20 ± 59.95 *a*	14.54 ± 42.74 *a*	0.10 ± 0.12 *a*	0.14 ± 0.21 *a*	0 *a*	0 *a*	0 *a*
	C	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0.59 ± 1.33 *a*	0.18 ± 0.44 *b*	3.79 ± 4.72 b	0.11 ± 0.11 *a*	0.33 ± 0.33 *a*	0.33 ± 0.33 *a*	0 *a*	0 *a*	0 *a*
	CF	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0.33 ± 0.33 *a*	0 *b*	0 *ab*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*
	CFA	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	5.14 ± 7.80 *c*	9.61 ± 17.60 *ab*	7.59 ± 9.00 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*
Number of developed fruits	Co	0 *a*	0 *a*	0 *a*	0.26 ± 0.26 *a*	0 *a*	0 *a*	0.05 ± 0.05 *a*	0 *a*	0 *a*	79.42 ± 24.21 *a*	67.84 ± 10.46 *a*	78.05 ± 27.34 *a*	69.26 ± 13.59 *a*	65.16 ± 19.49 *a*
	F	0 *a*	0 *a*	0 *a*	0.47 ± 0.42 *a*	0 *a*	0 *a*	1.63 ± 1.63 *a*	0.11 ± 0.11 *a*	0 *a*	64.89 ± 19.76 *ad*	57.79 ± 16.35 *a*	56.74 ± 16.40 *ad*	87.68 ± 34.34 *a*	75.00 ± 30.73 *ad*
	C	0 *a*	0 *a*	0 *a*	0.06 ± 0.06 *a*	0 *a*	0 *a*	0.61 ± 0.44 *a*	1.00 ± 0.58 *a*	0.28 ± 0.16 *a*	1.94 ± 1.08 *bc*	2.11 ± 1.33 *bc*	2.78 ± 1.40 *b*	2.44 ± 1.33 *b*	1.67 ± 0.90 *bc*
	CF	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0.66 ± 0.46 *a*	0.93 ± 0.32 *a*	0.20 ± 0.14 *a*	0.47 ± 0.22 *c*	0.13 ± 0.09 *b*	0.07 ± 0.07 *c*	0.07 ± 0.07 *c*	0.38 ± 0.21 *b*
	CFA	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	0 *a*	10.00 ± 7.38 *bd*	10.00 ± 7.20 *c*	14.40 ± 10.87 *d*	9.80 ± 9.80 *bc*	11.6 ± 8.97 *cd*

SUPPLEMENTARY MATERIAL

Table 1SM. Coordinates of *Stryphnodendron adstringens* individuals assigned to the experiments realized in Itumirim, Minas Gerais, Brazil.

Site	Plant code	Treatment	Elevation (m)	Localization
A1	C01	Control	920	S 21°14'10.1" W 44°49'47.2"
	C02	Control	906	S 21°14'10.9" W 44°49'46.2"
	C03	Control	912	S 21°14'11.2" W 44°49'44.8"
	C04	Control	922	S 21°14'13.1" W 44°49'44.0"
	C05	Control	931	S 21°14'13.2" W 44°49'41.3"
	C06	Control	912	S 21°14'11.1" W 44°49'43.8"
	C07	Control	929	S 21°14'11.0" W 44°49'41.3"
	C08	Control	927	S 21°14'11.9" W 44°49'36.1"
	C09	Control	931	S 21°14'13.8" W 44°49'34.1"
	C10	Control	935	S 21°14'13.7" W 44°49'38.1"
	F01	Fertilized	908	S 21°14'10.7" W 44°49'46.1"
	F02	Fertilized	917	S 21°14'10.5" W 44°49'45.3"
	F03	Fertilized	898	S 21°14'11.2" W 44°49'45.3"
	F04	Fertilized	913	S 21°14'12.2" W 44°49'44.6"
	F05	Fertilized	926	S 21°14'12.4" W 44°49'41.2"
	F06	Fertilized	920	S 21°14'11.5" W 44°49'43.8"
	F07	Fertilized	924	S 21°14'10.5" W 44°49'38.5"
	F08	Fertilized	933	S 21°14'11.9" W 44°49'35.0"
	F09	Fertilized	943	S 21°14'14.4" W 44°49'34.9"
	F10	Fertilized	943	S 21°14'14.5" W 44°49'38.2"
	P01	Clipped	901	S 21°14'10.5" W 44°49'46.7"
	P02	Clipped	907	S 21°14'10.4" W 44°49'46.4"
	P03	Clipped	916	S 21°14'11.6" W 44°49'44.4"
	P04	Clipped	924	S 21°14'13.2" W 44°49'42.6"
	P05	Clipped	925	S 21°14'12.5" W 44°49'41.0"
	P06	Clipped	931	S 21°14'11.6" W 44°49'41.9"
	P07	Clipped	922	S 21°14'11.3" W 44°49'37.7"
	P08	Clipped	934	S 21°14'12.2" W 44°49'34.5"
	P09	Clipped	938	S 21°14'14.2" W 44°49'35.5"
	P10	Clipped	938	S 21°14'14.5" W 44°49'39.2"
	PF01**	Clipped-Fertilized	899	S 21°14'10.8" W 44°49'47.1"
	PF02**	Clipped-Fertilized	908	S 21°14'11.1" W 44°49'46.1"
	PF03	Clipped-Fertilized	905	S 21°14'11.3" W 44°49'45.8"

Continue...

Site	Plant code	Treatment	Elevation (m)	Localization
A1	PF04	Clipped-Fertilized	925	S 21°14'12.6" W 44°49'43.6"
	PF05	Clipped-Fertilized	928	S 21°14'12.5" W 44°49'41.2"
	PF06	Clipped-Fertilized	909	S 21°14'10.8" W 44°49'46.1"
	PF07	Clipped-Fertilized	924	S 21°14'10.1" W 44°49'41.3"
	PF08	Clipped-Fertilized	926	S 21°14'10.9" W 44°49'36.3"
	PF09	Clipped-Fertilized	941	S 21°14'12.8" W 44°49'34.4"
	PF10	Clipped-Fertilized	938	S 21°14'14.3" W 44°49'37.4"
A2	C01	Control	945	S 21°13'40.4" W 44°51'05.6"
	C02	Control	934	S 21°13'41.6" W 44°51'05.2"
	C03	Control	928	S 21°13'43.2" W 44°51'04.0"
	C04*	Control	923	S 21°13'43.9" W 44°51'04.6"
	C05	Control	932	S 21°13'46.2" W 44°51'05.7"
	C06	Control	929	S 21°13'46.0" W 44°51'08.6"
	C07	Control	906	S 21°13'48.8" W 44°51'08.7"
	C08	Control	918	S 21°13'49.5" W 44°51'08.4"
	C09	Control	915	S 21°13'50.0" W 44°51'08.4"
	C10	Control	907	S 21°13'50.3" W 44°51'07.3"
	F01	Fertilized	939	S 21°13'41.3" W 44°51'05.1"
	F02	Fertilized	931	S 21°13'42.2" W 44°51'04.0"
	F03	Fertilized	932	S 21°13'42.7" W 44°51'04.5"
	F04	Fertilized	931	S 21°13'44.8" W 44°51'05.3"
	F05	Fertilized	930	S 21°13'45.8" W 44°51'08.0"
	F06	Fertilized	932	S 21°13'45.0" W 44°51'08.0"
	F07	Fertilized	918	S 21°13'47.2" W 44°51'08.1"
	F08	Fertilized	924	S 21°13'49.1" W 44°51'09.0"
	F09	Fertilized	919	S 21°13'49.4" W 44°51'08.4"
	F10**	Fertilized	918	S 21°13'49.3" W 44°51'08.6"
	P01	Clipped	922	S 21°13'42.4" W 44°51'04.8"
	P02	Clipped	989	S 21°13'41.3" W 44°51'04.8"
	P03	Clipped	950	S 21°13'42.8" W 44°51'04.3"
	P04*	Clipped	927	S 21°13'43.6" W 44°51'04.0"
	P05*	Clipped	924	S 21°13'45.4" W 44°51'05.7"
	P06	Clipped	912	S 21°13'49.3" W 44°51'05.2"
	P07	Clipped	929	S 21°13'41.6" W 44°51'00.6"
	P08	Clipped	929	S 21°13'41.4" W 44°50'59.2"
	P09	Clipped	926	S 21°13'42.0" W 44°50'58.0"
	P10	Clipped	926	S 21°13'42.3" W 44°50'58.1"

Continue...

Site	Plant code	Treatment	Elevation (m)	Localization
A2	PF01	Clipped-Fertilized	941	S 21°13'40.7" W 44°51'05.0"
	PF02*	Clipped-Fertilized-Attacked by ants	937	S 21°13'41.8" W 44°51'04.6"
	PF03*	Clipped-Fertilized-Attacked by ants	929	S 21°13'43.4" W 44°51'03.3"
	PF04*	Clipped-Fertilized-Attacked by ants	918	S 21°13'44.2" W 44°51'05.5"
	PF05	Clipped-Fertilized	923	S 21°13'46.4" W 44°51'07.4"
	PF06*	Clipped-Fertilized-Attacked by ants	907	S 21°13'50.3" W 44°51'08.2"
	PF07	Clipped-Fertilized	907	S 21°13'50.0" W 44°51'06.9"
	PF08*	Clipped-Fertilized-Attacked by ants	914	S 21°13'48.8" W 44°51'05.9"
	PF09	Clipped-Fertilized	906	S 21°13'49.2" W 44°51'04.9"
	PF10	Clipped-Fertilized	903	S 21°13'49.6" W 44°51'05.0"

* Plants attacked by ants
** Dead plants

In April 2014, we collected four soil samples from the Site 1 and four from the Site 2 in Itumirim, Minas Gerais, Brazil, to perform micronutrient, macronutrient, organic matter, and granulometry analyses. We collected five soil subsamples at 20 cm using an auger and mixed them to collect only one sample from each of the four different point in the sites. The eight samples were stored into plastic bags and were sent to the Laboratório de Análises de Solo at Universidade Federal de Lavras to perform the analyses. The results of soil analyses are in the Table 2SM.

Table 2SM. Soil analysis for macronutrients, micronutrients, organic matter, and granulometry of the two sites which were realized the experiments with *Stryphnodendron adstringens* individuals in Itumirim, Minas Gerais, Brazil.

	Site 1				Site 2			
	1	2	3	4	1	2	3	4
Al	0.50	0.80	0.60	0.60	0.70	0.60	0.60	0.70
B	0.08	0.06	0.06	0.08	0.33	0.08	0.13	0.13
Ca	0.10	0.10	0.10	0.10	3.90	0.20	0.10	0.10
Cu	1.40	1.98	1.73	1.38	1.11	1.14	1.44	1.26
Fe	35.11	43.96	35.46	39.45	40.44	37.22	34.38	42.16
K	28.00	34.00	28.00	28.00	44.00	38.00	38.00	42.00
Mg	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mn	2.77	2.30	0.78	0.60	1.79	1.71	1.55	2.19
P	1.13	1.13	1.42	0.84	0.56	0.56	0.56	0.56
S	5.87	5.12	5.49	5.12	2.63	3.32	4.03	7.03
Zn	0.24	0.34	0.22	0.26	0.85	0.50	0.29	0.50
pH	5.30	5.20	5.20	5.10	5.30	5.20	5.20	5.20
H + Al	4.52	5.64	5.05	5.05	6.30	5.64	5.64	5.64
SB	0.27	0.29	0.27	0.27	4.11	0.40	0.30	0.31
t	0.77	1.09	0.87	0.87	4.81	1.00	0.90	1.01
T	4.79	5.93	5.32	5.32	10.41	6.04	5.94	5.95
v%	5.67	4.84	5.11	5.11	39.51	6.58	5.01	5.17
m%	64.94	73.39	68.97	68.97	14.55	60.00	66.67	69.31
P_{rem}	6.29	5.01	6.29	5.95	5.01	5.16	4.87	5.63
Organic matter	3.00	3.56	2.74	3.00	3.14	3.14	3.28	3.14
Clay	0.57	0.60	0.49	0.57	0.56	0.55	0.57	0.53
Silt	0.19	0.19	0.30	0.22	0.30	0.34	0.27	0.34
Sand	0.24	0.21	0.21	0.21	0.14	0.11	0.16	0.13

Methods: Water pH, KCl, and CaCl$_2$ – Relation 1:2,5; P-Na-K-Fe-Zn-Mn-Cu – Mehlich extractor 1; Ca-Mg-Al-KCl 1 mol/L extractor; H + Al – SMP extractor; B – Hot water extractor; S – Extractor of monocalcium phosphate in acetic acid; Organic matter – oxydation Na$_2$Cr$_2$O$_7$ 4 N + H$_2$SO$_4$ 10 N. **H+Al**: potential acidity; **SB**: sum of bases (Ca + Mg + K + Na); **t**: effective cation-exchange capacity; **T**: cation-exchange capacity at pH 7; **v%**: bases saturaction index; **m%**: aluminum saturation index; **P_{rem}**: Remanescent phosphorus.

ARTIGO 2

TRADE-OFFS BETWEEN GROWTH, REPRODUCTION AND DEFENSE IN WILD POPULATIONS OF A NEOTROPICAL SAVANNA TREE

Trade-offs between growth, reproduction and defense in wild populations of a Neotropical savanna tree

Summary.

1.Plant investment in vegative biomass, reproduction, and defense varies with resource availability, which is determined by both abiotic and biotic factors.

2.We evaluated the effect of resource loss following leaf removal, and resource increase, through soil nutrient addition, on vegetative biomass, reproduction and defense of the medicinal plant *Stryphnodendron adstringens*. The traditional method of exploiting this tree species is to remove the bark as a source of medicinal tannin. Harvesting bark, however, often kills the tree. Harvesting leaves, which might serve as an alternative source of tannin, may have a less detrimental effect on the tree. In a two-way crossed experimental design, we either clipped 100% of leaves or applied NPK fertilizer to the soil. We tested the hypothesis that harvesting leaves of *S. adstringens*, as a source of tannin, would negatively affect plant vegetative investment but not as severely as harvesting bark, and that fertilization would ameliorate the effects of leaf harvest. Vegetative investment was determined by total leaf biomass, reproduction by inflorescence and fruit production traits, and defense by total phenol, hydrolyzable tannin, and condensed tannin amounts.

3.Fertilization had a marginal positive effect on total leaf biomass. Defoliation had no effect on subsequent leaf production, and most importantly, no plants died as a result of defoliation. We found high tannin amounts in leaves of *S. adstringens* produced both prior to and subsequent to clipping, further suggesting that leaves could serve as a sustainable alternative source of tannin. After clipping, plants invested more in tannin production and less in reproduction.

4.S*ynthesis and Applications*. Our results suggest that leaf harvest may be more sustainable than harvesting of bark in *S. adstringens*. We suggest the need for further investigation of the medicinal properties of leaf tannins to formulate a sustainable management plan for the exploitation of this plant species.

Key-words: *Stryphnodendron adstringens*; barbatimão; tannins; phenolics; secondary compounds; leaf removal; fertilization

Running title: Trade-offs in a medicinal plant species

Introduction

Plants allocate resources to maximize their fitness in the face of varying abiotic (e.g., nutrient availability) and biotic (e.g., leaf removal) limitations. Limited resources may result in conflicting demands for such resources, with the result that plants may not be able to invest simultaneously in vegetative biomass, reproduction and defense (Herms & Matson 1992; Briggs & Schultz 1999; Weiner *et al.* 2009). According to the 'trade-off hypothesis', plants may respond to resource limitations by making 'choices', that is, by investing in one or two functions instead of all, or investing more in one kind of anti-herbivore defense than another (Coley 1983; Koricheva, Nykänen & Gianoli 2004; Dostálek *et al.* 2016).

The amount of damage experienced by a plant is often determined by biotic and abiotic conditions that affect the quality and quantity of plant defense (Danks 2002). In turn, biotic and abiotic conditions also influence plant responses to leaf damage (Stowe *et al.* 2000). The 'continuum of plant response to herbivory hypothesis' predicts that herbivory may be detrimental, neutral, or beneficial based on the plant's ability to replace tissue consumed by herbivores under different environmental conditions (Maschinski & Whitham 1989; Stowe *et al.* 2000). For example, damaged plants may delay fruit production or produce fewer fruits compared to non-damaged plants (Kersch-Becker & Lewinsohn 2012; Wang *et al.* 2016). However, fertilization might positively affect the production of flowers, fruits and seeds (Sperens 1997).

Plants have evolved several strategies to avoid herbivore attack, such as associations with ants, physical barriers, and production of secondary chemical compounds (Cornelissen & Fernandes 2001; War *et al.* 2012). Phenolics are the secondary chemical compounds most commonly produced by plants, and often are involved in defense against damages (Lattanzio, Lattanzio & Cardinali 2006; Boudet 2007). In high concentrations, phenolics can injure herbivore guts (Barbehenn & Constabel 2011). Generally they have a negative effect on insect fitness (Kestring *et al.* 2009).

Tannins are the type of phenolic compounds most commonly produced by plants (Barbehenn & Constabel 2011), coming in either hydrolyzable or condensed form (Dai & Mumper 2010). Tannin production varies with the plant's genotype, development stage, and environmental conditions (e.g. stress in response to nutrient availability or injuries) (Barbehenn & Constabel 2011; Rubert-Nason *et al.* 2015). In addition, the production of tannins and other defensive compounds may vary among plant organs (Godschalx *et al.* 2016). The 'optimal defense hypothesis' suggests that the highest defended plant organs are those that represent the highest fitness value, considering energetic costs and/or vulnerability to damage (McKey 1974;

Godschalx *et al.* 2016).

Stryphnodendron adstringens (Mart.) Coville (Fabaceae: Mimosoideae), known locally as barbatimão, is a tree species very common in the Cerrado biome (Brazilian Savanna) that has high tannin levels in its tissues (Felfili & Silva-Jr 1993). Tannins represent an important source of antioxidants in human diet (Boudet 2007; Dai & Mumper 2010). For this reason, barbatimão is an important medicinal plant for rural populations in Brazil. There are several pharmacological studies of the use of this species as a medicine against numerous diseases (Oliveira *et al.* 2007; Corrêa *et al.* 2011). Rural populations sometimes overexploit the bark of *S. adstringens* as a source of tannin, often removing more than 50% of the bark, which can result in tree death (Borges-Filho & Felfili 2003; Soldati & Albuquerque 2012). Some studies have suggested sustainable management strategies for this species, supporting the active management of populations of *S. adstringens* (Borges-Filho & Felfili 2003; Feitosa, Albuquerque & Monteiro 2014). Some authors have developed exploitation plans for plants that provide medicine to improve medicine production while conserving the species (Hussein *et al*. 2006; Kindscher *et al*. 2014). However, there are no experimental studies that have deliberately manipulated tissue harvest levels of *S. adstringens* to determine the impact of that harvest on plant vegetative biomass and survivorship, reproduction, and defense.

The Cerrado biome is characterized by low soil fertility (Miatto, Wright & Batalha 2016) and high levels of leaf damage (Meloni, Lopes & Varanda 2012). Fertilization might be used to mitigate the effects of leaf harvest. Thus, a sustainable harvest plan might link fertilization with leaf harvest. In a crossed experimental design, we either completely defoliated (harvested leaves) plants or not, crossing this treatment with the addition of fertilizer to the soil of experimental plants or not. The results would indicate which plant treatment and which plant organ produces more tannins, and which plant treatment results in the lowest impact on survivorship, vegetative biomass investment, reproduction, and defense against future damages.

We evaluated the impact of leaf harvest in the context of plant allocation and defense theory. Considering a gradient from lowest to highest resource availability across four treatments (low to high: Clipped (C), Clipped-Fertilized (CF), Control (Co), and Fertilized (F)) (Fig. 1), we tested the following hypotheses: i) the 'trade-off hypothesis': all plants will invest in leaf production over reproduction to maintain photosynthetic activity. Furthermore, higher resource availability will increase investment in reproduction, while leaf removal will increase defense investment, suggesting a trade-off between reproduction and defense; ii) the 'continuum of plant response to herbivory hypothesis': resource availability will determine plant investment in reproduction and defense, CF plants are less resource limited than C plants, resulting in the lowest leaf replacement by C plants, but a neutral or positive impact on CF plants; and iii) the 'optimal defense hypothesis': the highest defended tissue will be the leaves, the most important tissue to produce photosynthetic metabolites. Overall, we hypothesized that harvesting leaves of *S. adstringens*, as a source of medicinal tannin, would negatively affect plant vegetative biomass but not as much as the harvest of bark, and that fertilization would ameliorate harvest effects.

Materials and methods

Study area

The study sites were in Itumirim, in the state of Minas Gerais, Brazil (Site 1: 21°14'9.82"S 44°49'43.29"W; Site 2: 21°13'41.98"S, 44°51'0.43"W). This region is characterized by mild summers and dry winters, with an annual average temperature of 19.4°C, and an annual average rainfall of 1,530 mm (Sparovek, VanLier & Dourado-Neto 2007). The original vegetation of this region was seasonal forest of the Atlantic Forest biome (Veloso, Rangel-Filho & Lima 1991) in transition with the Cerrado biome. Most of the native forest is very fragmented by human activity, such as coffee crops, pastures and human settlements.

Sampling design and field experiment set up

In April 2014, we selected two sites, 2.4 km apart, marking 80 reproductive individuals of *S. adstringens* 3-4 m high and with similar crown architecture and size. At each site, ten individuals each were randomly assigned to the following treatments: control (Co); application of NPK fertilizer to the soil (F); 100% of leaves removed by clipping (C); application of NPK fertilizer to the soil and 100% of leaves removed by clipping (CF).

In April 2014, we clipped all leaves from plants assigned to treatments C and CF. From April 2014 to February 2015, on alternative months, we applied 100 g of NPK (10-10-10) fertilizer to the soil around plants assigned to the treatments F and CF. In total, 600 g of NPK fertilizer was added to each plant. The fertilizer was applied to three holes around each plant to avoid loss through surface runoff.

Plant vegetative investment

We dried and weighed the leaf biomass from clipped plants to estimate the investment in vegetative biomass up until the beginning of the experiments. In May 2014, one month after we clipped the plants, we counted the number of leaves produced by each clipped plant to estimate the immediate response to clipping. At the end of the experiment, in June 2015, we clipped all leaves from all plants to measure investment in vegetative biomass by each plant. We dried all leaves at 60ºC for 72 h, and then weighed them.

Plant reproductive investment

In April 2014, we collected all fruits from all plants before setting up the field experiments. We counted and dried all fruits at 40ºC for 48 h and weighed them to determine plant's reproductive investment before the treatments. In June 2015, 14 months after the field experiment started, we collected all fruits again, counted, and determined the total biomass per plant. In addition, we visited the study area monthly, from May 2014 to June 2015, to count the number of inflorescences. We designated the maximum number of inflorescences observed at the field as the total number of inflorescences produced by each individual subsequent to the initiation of the treatments. Fruit set was calculated as the number of fruits produced per inflorescence. The impact of the treatments on fruit production was estimated as the difference between the number of fruits produced before and after treatments.

Secondary compound analyses of leaves, fruits, and seeds

We estimated defense investment by evaluating the total phenolic content (Petrakis *et al.* 2011), and the total concentration of condensed tannins and hydrolyzable tannins (War *et al.* 2012) of leaves, fruits and seeds. We randomly collected four leaves from each plant to determine leaf tannin content. For fruit and seed analysis, we opened a maximum of 75 fruits per plant, classifying the seeds as apparently healthy or injured by insects. We submitted apparently healthy seeds to x-ray to verify which seeds contained insect larvae within. We dried and milled leaves, undamaged seeds and the fruits for total phenolics, condensed tannin and hydrolyzable tannin analyses.

We purified tannins from leaves, fruits and seeds using Sephadex LH-20 assay to produce three bulk extractions, one for each plant organ (Forkner, Marquis & Lill 2004). We used purified tannin powder to build one standard curve for each plant organ and chemical analysis. Standard curves were built using known concentrations and their related absorbance values obtained from a spectrophotometer (Forkner, Marquis & Lill 2004). We only considered $r^2 \geq 0.95$ for standard curves. Finally, we prepared each individual sample extract with acetone and L-ascorbic acid solution to conduct the three defensive compound analyses. Total phenol concentration of each sample was estimated using Folin-Denis assay (Swain & Hillis 1959), condensed tannins by a 95% butanol: 5%HCl assay (Bate-Smith 1977), and hydrolyzable tannins by potassium iodate assay (Schultz & Baldwin 1982).

Data analyses

We performed linear regression to evaluate the influence of initial leaf biomass of clipped plants on number of leaves produced one month after clipping, and a general linear model with Gaussian error distribution to evaluate the influence of treatments on leaf biomass 14 months after clipping. We also performed principal component analysis (PCA) to evaluate reproduction, number of inflorescences, fruit set, and number and biomass of fruits, and plant tissue defense traits combining total phenols, hydrolyzable tannins, and condensed tannins. We used the first component, which captured the largest amount of variance among traits, as the response variable, and performed analysis of variance (ANOVA) to compare variations of reproduction and defense traits between treatments, and then performed post-hoc analysis using Tukey contrasts.

We also used GLM, followed by contrast analysis, to compare the chemical concentrations among the three plant tissues. Moreover, we performed Shapiro-Wilk test for normality and Pearson correlation analysis for each pair of chemical compounds to test the correlation between chemical compounds and the different plant tissues and phenotypic trade-offs between chemical compound production by each plant tissue. It was not possible to evaluate fruit and seed chemical compounds among treatments, because most clipped plants did not produce sufficient fruit and seed material. All analyses were conducted using R (R Development Core Team 2016).

One plant from the F treatment and two from the CF died during our experiment period. Also, eight plants were excluded from our analyses because they suffered almost complete leaf area loss to leaf cutter ants. However, this observation demonstrates that severe damage by herbivores, as we performed on our experimentation, may occur naturally in this habitat. The excluded plants were five CF, two C, and one Co, and all were from the set 2 (A2).

Results

Leaf biomass production following treatment application varied highly among plants, and marginally so among treatments. F plants had the highest biomass of the four treatments ($p = 0.074$, $F = 2.42$, Fig. 2). Larger plants (i.e., those that had more leaves prior to the experiment) produced greater leaf biomass subsequent to treatments ($p < 0.001$, $F = 44.71$, Fig. 3), but final leaf biomass did not differ between C and CF treatments ($p = 0.359$, $F = 0.87$).

All reproductive traits grouped on a single axis and reproduction eigenvalues showed a positive correlation between the number of inflorescences, fruit set, and number and biomass of fruits (total variance of PC1: 58.2%, Fig 4a). ANOVA showed that clipping had a negative impact on reproductive traits ($p < 0.001$, $F = 7.84$, Fig. 4b). Defensive chemical compounds also grouped on only one axis. Hydrolyzable and condensed tannins had positive values in the PC1 axis, while total phenols had negative ones (total variance of PC1: 61.49%, Fig. 4c). In contrast to reproduction eigenvalues, defense eigenvalues showed that clipping increased production of hydrolyzable and condensed tannins, but decreased total phenolic investment in plant tissues ($p < 0.01$, $F = 8.62$, Fig. 4d).

Concentration of secondary compounds also varied among leaves, fruits and seeds of *S. adstringens*. Seeds produced more total phenols, followed by fruits and leaves ($p < 0.001$, $F = 28.52$, Fig. 5a); leaves produced more hydrolyzable tannins than fruits and seeds ($p < 0.001$, $F = 27.57$, Fig. 5b); and had the highest condensed tannin concentration, followed by leaves and seeds ($p < 0.001$, $F = 120.43$, Fig. 5c).

There was no correlation between the concentration of chemical compounds when comparing across tissue types, that is leaves, fruits and seeds (Table 1SM – Supplementary material). In contrast, we observed a number of significant correlations within tissue types (Table 1). There was a positive correlation between hydrolyzable and condensed tannins in leaves and fruits (Table 1, Figs 6a & 6b). We further observed trade-offs between hydrolyzable and condensed tannin amounts and total phenols in leaves, and between hydrolyzable tannin and condensed tannin amounts in fruits (Table 1, Figs 7a-c).

Discussion

This is the first study to quantify the impact of experimental harvest and management on *Stryphnodendron adstringen*s. We hypothesized that leaf harvest, as a means of extracting tannins, would negatively affect tree vegetative biomass but that fertilization would ameliorate this impact. Contradicting our hypothesis, leaf harvest did not affect leaf production but reduced fruit production. In contrast to the negative effect of bark harvest reported in previous studies (Borges-Filho & Felfili 2003; Soldati & Albuquerque 2012), survivorship was not decreased by leaf harvest. Fertilization had a marginally positive effect on leaf production but only for the non-clipped treatment, and did not impact reproduction. Plants that had high leaf biomass initially had high leaf production subsequent to defoliation. This result suggests that there is an influence of local environmental conditions and/or plant genotype on vegetative biomass and reproduction. The clipping treatment induced a trade-off in plant response, such that reproduction decreased but tannin concentration increased in plant tissues. The 'continuum of plant response to herbivory hypothesis' was not corroborated, since plants of both clipped treatments produced similar total leaf biomass, independent of the fertilization treatment. We observed that leaves, fruits and seeds exhibited different levels of total phenols, hydrolyzable tannins and condensed tannins. As predicted by the 'optimal defense hypothesis', leaves were the highest defended tissue. In addition, we observed both positive and negative correlations (the latter indicating trade-offs) in the production of secondary compounds in leaves, fruits and seeds.

This species showed a trade-off between reproduction, on the one hand, and vegetative biomass and defense on the other. Plants invested fewer stored resources in reproduction and more in defense following defoliation. Severe damage requires allocation of resources to replace lost leaf tissue (Kersch-Becker & Lewinsohn 2012, Huot *et al.* 2014). Maintenance of leaf production in the face of defoliation apparently resulted in reduced reproduction in this study. Higher levels of defense in newly produced leaves may have further compounded the tradeoff. Inducible defense is an important strategy, considering that damage varies in space and time (Koricheva 2002; Dostálek *et al.* 2016). Phenolics often have a defensive function against herbivores (Lattanzio, Lattanzio & Cardinali 2006; Boudet 2007; Barbehenn & Constabel 2011). Our results are consistent with the hypothesis that damage induces higher secondary compound production to reduce the likelihood of subsequent leaf damage (Lattanzio, Lattanzio & Cardinali 2006). In addition, investing in defensive compounds following herbivore attack instead of reproduction may be more profitable for long-lived trees, like *S. adstringens*, because they may have many reproductive episodes.

After clipping, we observed that condensed tannin and hydrolyzable tannin concentrations increased, while total phenolics decreased. Thus, levels of condensed and hydrolyzable tannins showed a positive correlation. In contrast, we observed a trade-off between condensed tannins and total phenols, and between hydrolyzable tannin and total phenol production in plant tissues. Producing multiple kinds of defense helps the plant avoid damage by a variety of herbivorous insect species but may result in trade-offs among different kinds of defenses (Koricheva, Nykänen & Gianoli 2004), as observed in this study.

Contradicting the 'continuum of plant response to herbivory hypothesis', damaged plants with higher versus lower resource availability (i.e. CF and C plants) did not differ in leaf production following defoliation. Fertilization only affected plant vegetative biomass, but did not affect reproduction or defense. *S. adstringens* has associations with *Rhizobium* and nodule formation (Lemos 2009), and high nutrient availability might inhibit this association (Streeter 1985; Puaiatti & Sodek 1999). In this way, plants might have similar nutrient availability under fertilized and non-fertilized conditions, resulting in approximately equal investment in vegetative biomass, reproduction, and defense. It is important to note that this plant species showed a high capability to replace its leaves after severe damage. Whether they could do so following multiple defoliations awaits further experimentation. Maintenance of leaf area in the face of herbivore attack is critical for maintenance of functions (Hetherington, Smillie & Davies 1998).

Leaf removal also affected plant defense, as clipped plants reduced total phenol production and increased hydrolyzable and condensed tannin production in plant tissues. Individuals of *S. adstringens* differentially invest in the defense of leaves, fruits and seeds. Leaves and fruits have around 30% of their biomass composed of condensed tannins, while seeds have less than 10%. Leaves also have the highest hydrolyzable tannin amount of all tissues, but the lowest total phenol amount; fruits have low hydrolyzable tannins and intermediate total phenol amounts; seeds have a high total phenol amount, but very low quantities of the other two. Leaves should be highly defended tissues due to their great importance for photosynthesis (Huot *et al.* 2014). Therefore, we partially agree with the 'optimal defense hypothesis', because leaves had the highest production of condensed tannins and hydrolyzable tannins, but they did not have more total phenols. Studies about differences in the investment of secondary compounds production help to determine which tissue would be more profitable for commercial exploitation (Elfalleh *et al.* 2012). In the case of *S. adstringens*, high leaf tannin concentrations found in leaves suggests a commercial use for them in place of bark for medicine production.

Currently, popular medicine only uses the bark of *Stryphnodendron adstringens*, causing the death of overexploited plants (Borges-Filho & Felfili 2003; Soldati & Albuquerque 2012). We observed that leaves of *S. adstringens* have a similar tannin concentration to bark (Lima, Martins & Souza-Júnior 1998), and complete defoliation neither kills trees nor reduces their vegetative biomass. These results together suggest that leaves could replace bark as the exploited resource without the concomitant negative impact on tree survivorship. How plants might respond to multiple defoliations is yet to be determined. Nor do we know the impact of leaf and bark harvest on plant population viability via reduced reproduction. Although there are several studies about the use of *S. adstringens* bark for medicinal purposes, only a single study has focused on leaves (Santos-Filho, Ferreira & Gouvêa 2011). Thus, we suggest that pharmacological studies should focus on the use of leaves of *S. adstringens* as a source of medicine as this may ultimately lead to a more sustainable exploitation of this plant species.

Authors' contributions statement: JT, RJM and LDBF conceived the ideas and designed the methodology; All the authors collected the data; JT and ABM analysed the data; JT wrote the manuscript. All authors contributed critically to manuscript drafts and gave final approval for submission for publication.

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FIGURES

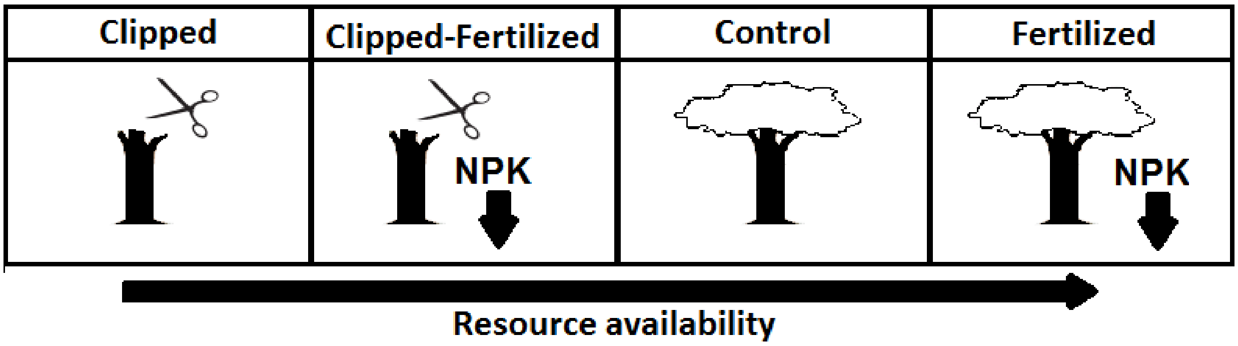

Figure 1. Treatments realized with *Stryphnodendron adstringens* plants in two areas in Itumirim, Southest of Brazil. From the lowest to the highest resource availability, the treatments were: Clipped (100% of all leaves removed by clipping), Clipped-Fertilized (100% of leaves removed by clipping and application of NPK fertilizer to the soil), Control and Fertilized (application of NPK fertilizer to the soil).

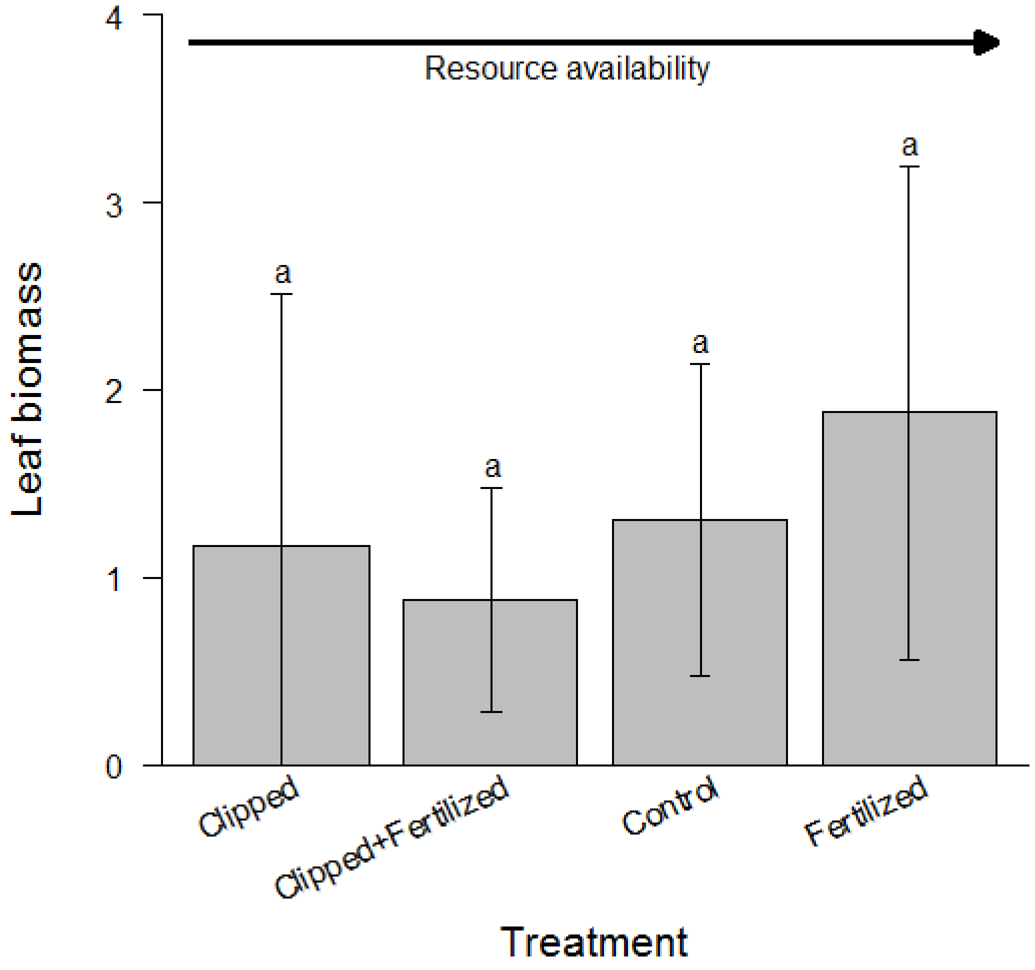

Figure 2. Leaf biomass (g) produced by *Stryphnodendron adstringens* from the treatments (from the lowest to the highest resource availability) Clipped (C), Clipped-Fertilized (CF), Control (Co) and Fertilized (F) treatments (p = 0.074, F = 2.42).

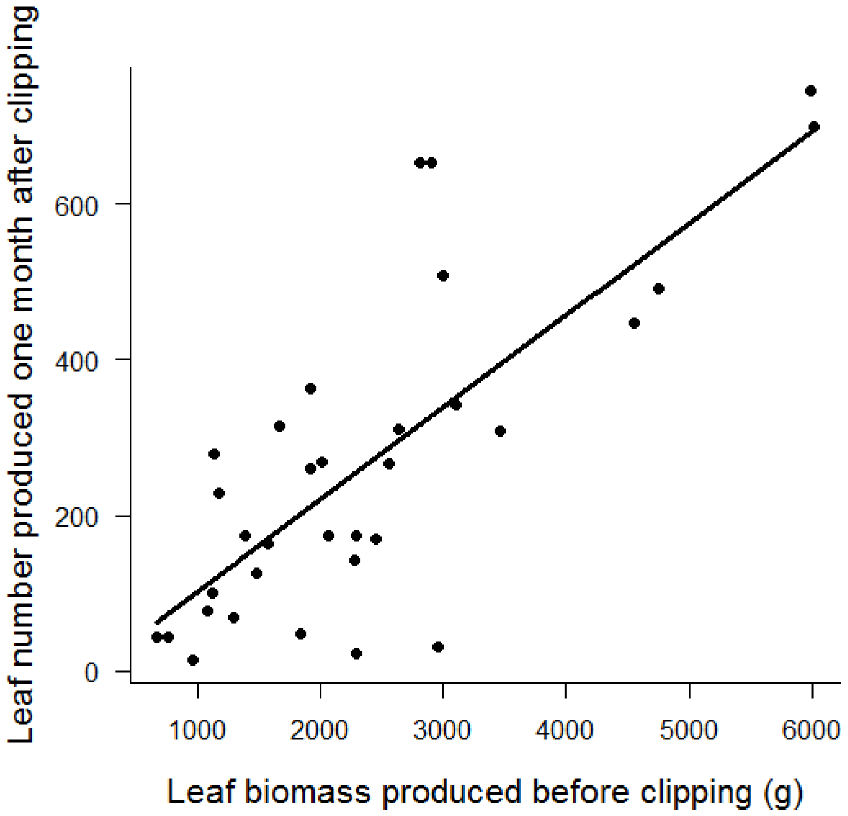

Figure 3. Leaf biomass (g) produced by *Stryphnodendron adstringens* from Clipped and Clipped-Fertilized treatments before clipping and number of leaves produced by them one month after clipping (p < 0.01, F= 44.71, y = -16.92 + 0.12 * x).

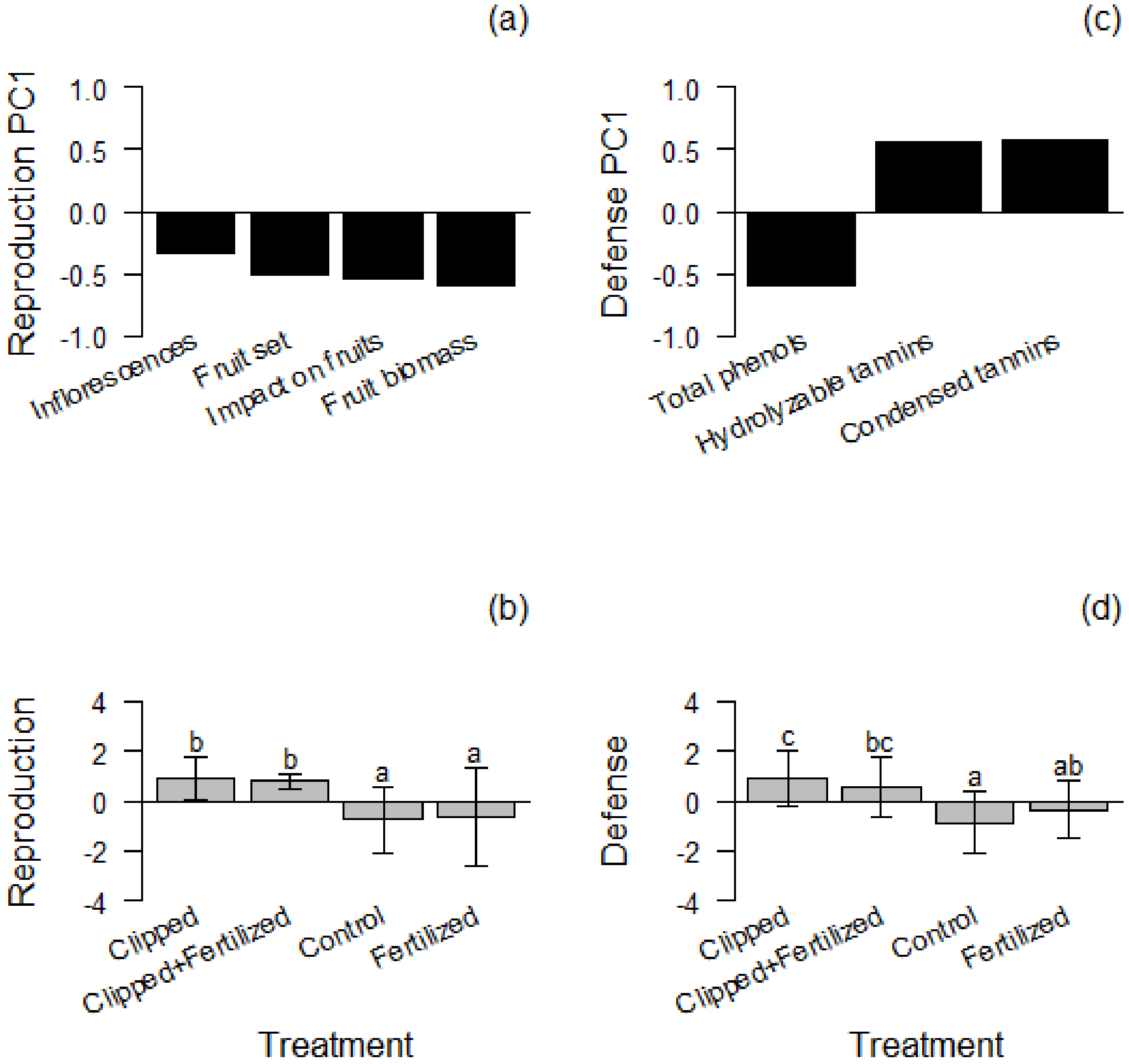

Figure 4. PCA eigenvalues and the tendency of each variable within them and ANOVA built with PCA eigen values of reproduction (a, b) and defense traits (c, d) of the plant *Stryphnodendron adstringens* (ANOVA - Reproduction: $p < 0.001$, $F = 7.84$; Defense: $p < 0.01$, $F = 8.62$). Different letters represent significant differences and similar letters no difference between treatments, considering significant values at $p \leq 0.05$.

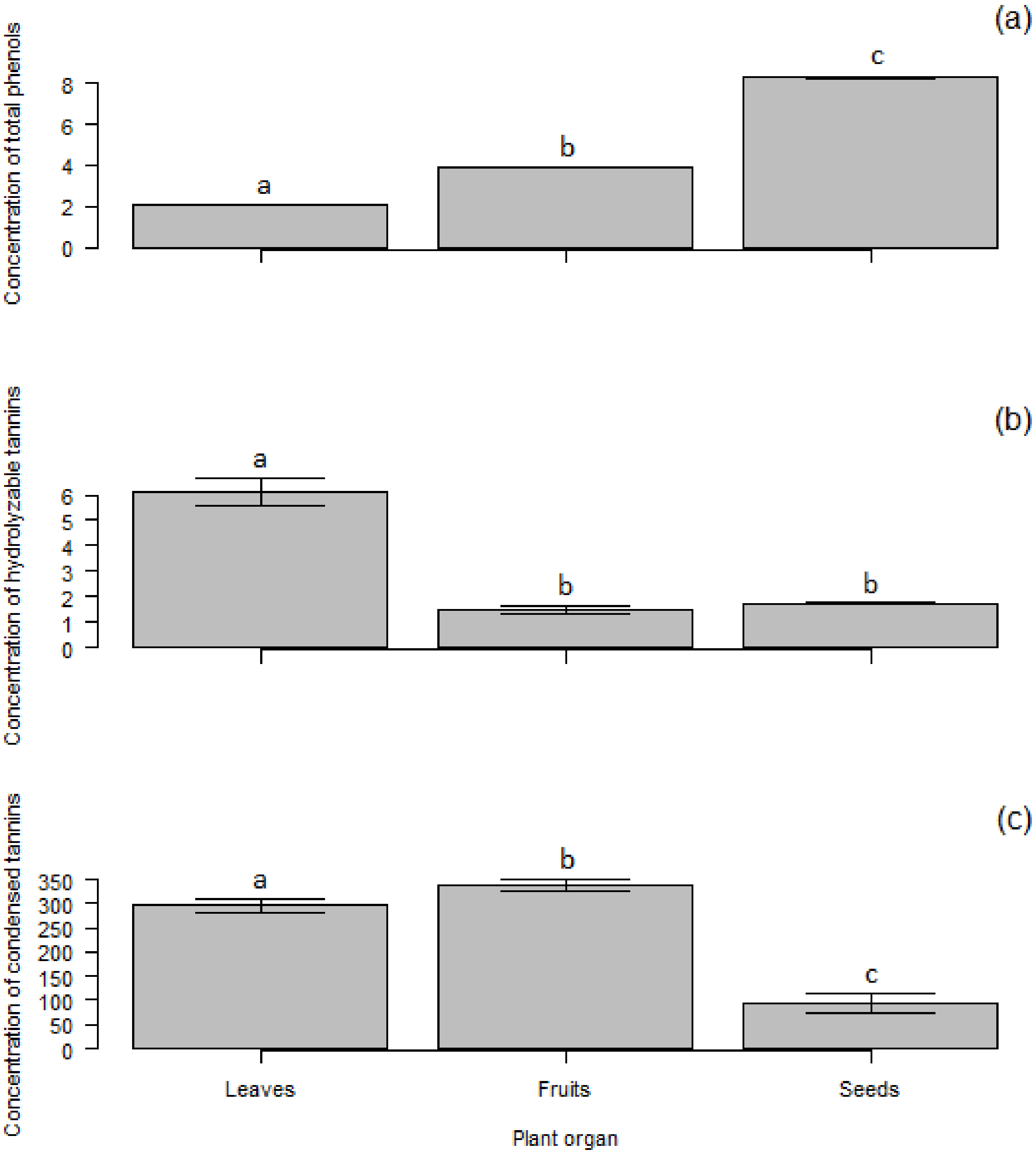

Figure 5. Concentration (mg/g of plant tissue) of (a) total phenols ($p < 0.001$, $F = 120.43$), (b) hydrolyzable tannins ($p < 0.001$, $F = 27.57$) and (c) condensed tannins ($p < 0.001$, $F = 28.52$) on leaves, fruits and seeds of *Stryphnodendron adstringens*. Different letters represent significant differences and similar letters no difference between treatments, considering significant values as $p \leq 0.05$.

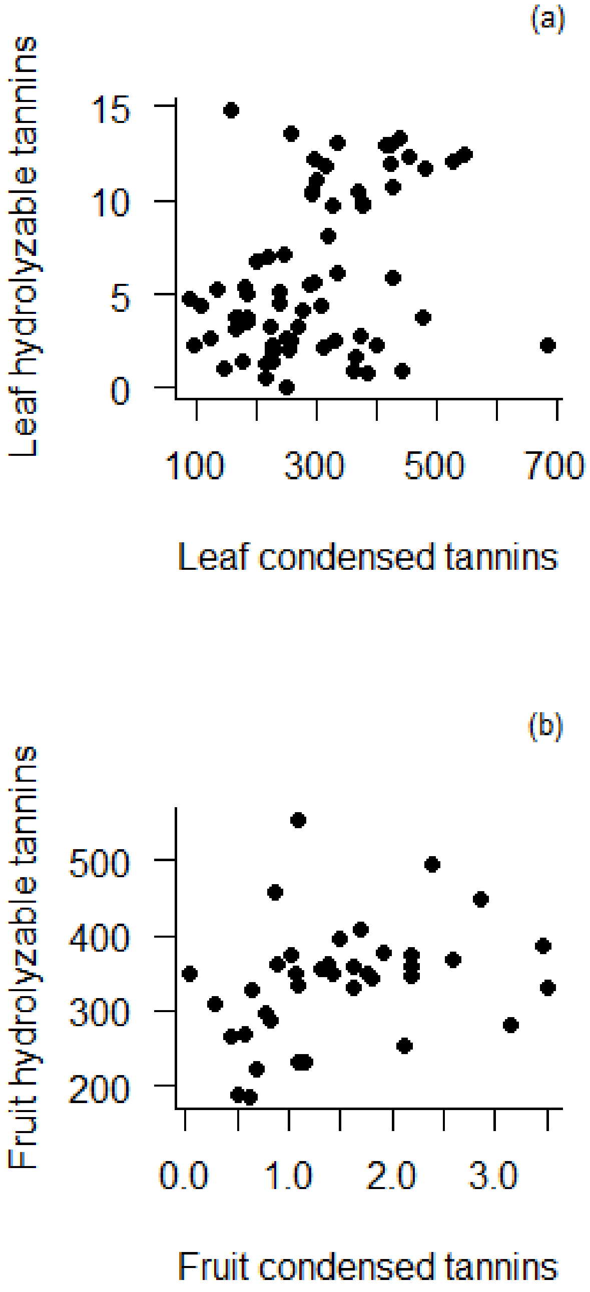

Figure 6. Positive correlations between amount (mg/g of plant tissue) of (a) hydrolyzable and condensed tannins on leaves ($p = 0.005$, $r = 0.33$), and (b) on fruits of *Stryphnodendron adstringens* ($p = 0.046$, $r = 0.33$).

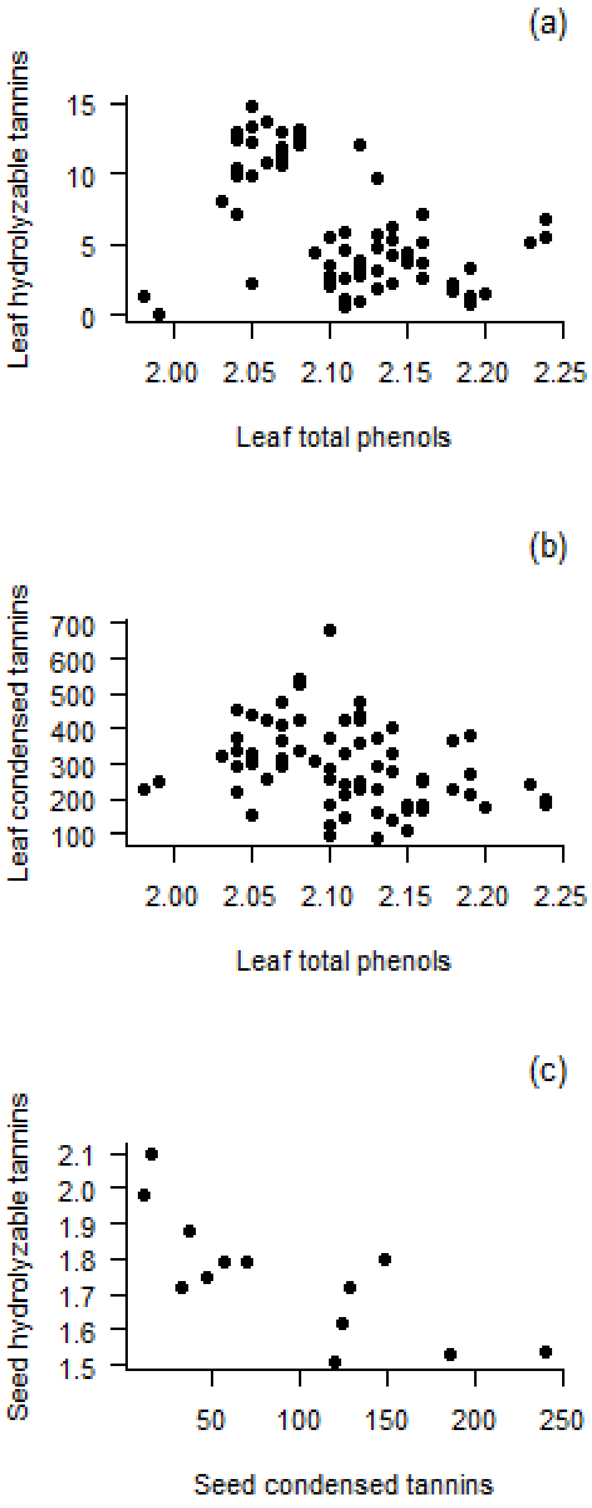

Figure 7. Negative correlations between amount (mg/g of plant tissue) of (a) total phenols and hydrolyzable and condensed tannins on leaves ($p < 0.001$, $r = -0.44$), (b) total phenols and condensed tannins on leaves ($p = 0.017$, $r = -0.29$), and (c) hydrolyzable and condensed tannins on seeds of *Stryphnodendron adstringens* ($p = 0.002$, $r = -0.77$).

TABLE

Table 1. Correlations in chemical secondary compounds (mg/g of plant tissue) within the same tissue type of *Stryphnodendron adstringens*.

Plant organ	Variables		r	p
Leaf	Leaf total phenols	Leaf hydrolyzable tannins	-0.44	<0.001*
	Leaf total phenols	Leaf condensed tannins	-0.29	0.017*
	Leaf hydrolyzable tannins	Leaf condensed tannins	0.33	0.005*
Fruit	Fruit total phenols	Fruit hydrolyzable tannins	-0.02	0.892
	Fruit total phenols	Fruit condensed tannins	<0.01	0.991
	Fruit hydrolyzable tannins	Fruit condensed tannins	0.33	0.046*
Seed	Seed total phenols	Seed hydrolyzable tannins	-0.20	0.510
	Seed total phenols	Seed condensed tannins	0.37	0.218
	Seed hydrolyzable tannins	Seed condensed tannins	-0.77	0.002*

*represents statistically significant results, considering $p \leq 0.05$

SUPPLEMENTARY MATERIAL

Table 1SM. Correlations in chemical secondary compounds (mg/g of plant tissue) between tissue types of *Stryphnodendron adstringens*.

Chemical compound	Variables		*r*	*p*
Total phenols	Leaf total phenols	Fruit total phenols	0.09	0.604
	Leaf total phenols	Seed total phenols	-0.21	0.498
	Fruit total phenols	Seed total phenols	0.11	0.722
Hydrolyzable tannins	Leaf hydrolyzable tannins	Fruit hydrolyzable tannins	0.23	0.169
	Leaf hydrolyzable tannins	Seed hydrolyzable tannins	-0.09	0.733
	Fruit hydrolyzable tannins	Seed hydrolyzable tannins	-0.12	0.698
Condensed tannins	Leaf condensed tannins	Fruit condensed tannins	-0.16	0.326
	Leaf condensed tannins	Seed condensed tannins	0.21	0.497
	Fruit condensed tannins	Seed condensed tannins	0.11	0.722

ARTIGO 3

WHAT MATTERS FOR FRUIT-ASSOCIATED INSECTS: CHEMICAL DEFENSES, NUTRITIONAL QUALITY OR QUANTITY OF RESOURCE?

What matters for fruit-associated insects: chemical defenses, nutritional quality or quantity of resource?

Abstract

Traits of seeds and fruits, such as defensive compounds and resource quantity and nutritional quality, can determine insect preference and performance for fruits. Also, fertilizer application and leaf removal may change nutrient availability of the host plant, altering its resource traits. In this study, a crossed-design experiment was performed to evaluate the influence of fertilizer application and leaf clipping on *Stryphnodendron adstringens* seed and fruit traits and on a fruit-associated insect community. Total phenols, hydrolyzable and condensed tannins were categorized as defense traits (Def); total protein, carbohydrates and lipids as resource nutritional quality (Nut); and number and size of seeds, and fruit biomass as resource quantity (Qtt). We found 26 insect species associated with *S. adstringens* fruits and seeds and percentage of seed consumed varied among insect orders, which was higher for Lepidoptera, followed by Hymenoptera and Coleoptera. Fertilizer application did not directly affect fruit and seed traits – Def, Nut, and Qtt – or indirectly affect the insect community associated with them – percentage of seed consumed by Lepidoptera (P_{Le}), Coleoptera (P_{Co}), and Hymenoptera (P_{Hy}), and insect abundance (A_T) and richness (S_T). In general, Def was not important for any insect order or insect abundance and richness, and our results indicate that only Nut positively influenced P_{Hy}. We found that Qtt increased P_{Le}, P_{Co}, A_T and S_T and also positively impacted seed-feeding insect abundance (A_{SF}), which lead to an increase in parasitoid abundance (A_p). In addition, the higher the seed-feeding insect richness (S_{SF}), the higher their parasitoid richness (S_T). As such, resource traits affect *S. adstringens* seed-feeding insects differently, but most of the fruit-associated insect community is determined by Qtt and Nut.

Keywords: seed-feeding insects; seed-predation; Bruchinae; *Allorhogas*; parasitoid; tannin; protein; lipid; carbohydrate.

Running title: Fruit-associated insects and their resource traits

Introduction

Seeds and fruits are rich in nutrients that are attractive to herbivorous insects. Seed-feeding insects often reduce seed viability partially or wholly by consuming parts of or the seed in entirety (Southgate 1979, Sari 2007, Tomaz et al. 2007, Tuller et al. 2015). As a result, investment in defensive compounds in seeds has evolved to reduce seed predation (Janzen 1971, Sallabanks & Courtney 1992). These defensive compounds in combination with the nutrient content of the tissues they protect determine the preference and performance of their consumers (Sallabanks & Courtney 1992). Even though they share resources, different species of seed-feeding insects consuming the seeds of the same host plant may respond to fruit and seed traits differently (Maia et al. 2016). There are no studies about how defensive compounds and nutritional quality and quantity of resources might impact different orders of seed-feeding insects feeding on seeds of the same host plant species.

Phenols and tannins are secondary chemical compounds commonly produced by plants that may act as defenses against herbivorous insects (Barbehenn & Constabel 2011). Herbivorous insect fitness may also be influenced by resource nutritional quality, i.e., concentration of lipids, carbohydrates, and proteins, which determines the nutritive value of seeds for consumers (Joern & Behmer 1997, Stiling & Moon 2005, Lazar et al. 2014). For example, while phenol and tannin concentrations increase development time of seed-feeding insects, increasing protein and carbohydrate concentrations decrease development time (Lazar et al. 2014). Another determining factor for herbivorous insects is resource quantity, i.e., the size and number of seeds. The more available a resource is, the higher the energy intake by consumers (Janzen 1969). In this way, larger seeds are preferred by seed-feeding insects, which positively affects their biomass (Souza & Fagundes 2016, Maia et al. 2016).

According to the Carbon/Nutrient Balance Hypothesis, (CNBH), C/N balance is controlled in part by resource availability, which determines secondary metabolite production and plant palatability for herbivorous insects (Bryant et al. 1983). In low N soils, for example, plants have low N concentrations and high C concentrations and may invest less in protein synthesis (N-based) and more in tannin production (C-based) (Mattson-Jr 1980, Herrmann & Weaver 1999). Thus, plant manipulations (i.e., fertilizer addition) may influence defensive traits, nutritional quality and quantity of plant tissues by altering the plant metabolism. A bottom-up trophic cascade may result, affecting the entire herbivorous insect community (Forkner & Hunter 2000, Wimp et al. 2010): soil nutritional quality directly affects plant traits, indirectly causing changes in the abundance and performance of herbivores as well as of higher trophic level species (Harvey et al. 2003; Bukovinszky et al. 2008; Kos et al. 2012).

Stryphnodendron adstringens Coville (Mart.) (Fabaceae: Mimosoideae), known as barbatimão, is an important medicinal plant widely distributed in the Brazilian savanna (Cerrado) (Felfili et al. 1999). Brazilian rural populations use extracts from its bark to treat many diseases due to its high tannin concentration. There are several studies about the efficacy of this species as medicine (Ishida et al. 2006, Albuquerque et al. 2007, Oliveira et al. 2007, Santos et al. 2009, Santos-Filho et al. 2011). In addition to stem bark commercialization, *S. adstringens* seeds are available on the internet for US $ 15.00/100g (around 250 seeds) (ArboCenter 2017). Nonviability of seeds may reach 80% per plant due to seed predation by insects (Sari 2007). However, studies of *S. adstringens* seeds and their fruit-associated insect community are restricted to the bionomic characteristics and the impact of *Acanthoscelides gregorioi* (Chrysomelidae: Bruchinae) on *S. adstringens* seed germination, as well as the description and natural history of insect community associated with fruits of *S. adstringens* (Sari 2007, Morales-Silva & Zampieron 2016).

The goal of this study was to evaluate which fruit and seed traits impact seed-predation by different insect orders. Also, we investigated the impact of fertilizer on the abundance and richness of insects associated with *S. adstringens* fruits and seeds. We tested the following hypotheses: i) fertilization decreases plant investment in defensive traits but increases investment in nutritional quality and quantity; ii) defensive traits negatively impact the *S. adstringens* fruit-associated insect community, while the nutritional quality and the resource quantity positively impact them; iii) fertilization has an indirect positive impact on the *S. adstringens* fruit-associated insect community; iv) a bottom-up effect of fertilization on fruit-associated insects community is expected, starting from the soil towards the parasitoid level.

Materials and Methods

Study area

We selected two study sites in Itumirim, in the state of Minas Gerais, Brazil (Site 1: 21°14'9.82"S 44°49'43.29"W; Site 2: 21°13'41.98"S 44°51'0.43"W). This region is characterized by mild summers and dry winters, the annual average temperature is 19.4°C, and the annual average rainfall is 1,530 mm (Sparovek et al. 2007). The vegetation is Seasonal Forest of the Atlantic Forest biome (Veloso et al. 1991) in transition with the Brazilian savanna (Cerrado) biome.

Field experiment setup

In April 2014, we selected two areas containing individuals of *S. adstringens* to set up our experiments. We selected 40 reproductive individuals 3-4 m high and with similar crown architecture and size in each area, totaling 80 plants. Ten individuals of each area were randomly assigned to be manipulated according to a crossed-design experiment as follows: application of NPK fertilizer to the soil and 100% of the leaves removed by clipping (CF); application of NPK fertilizer to the soil (F); 100% of the leaves removed by clipping (C); and, control (Co). Please see Tuller et al. (chapter 2) for more details.

Resource availability (quantity) and insects associated with fruits and seeds

In June 2015, at the end of the experiment, we collected all fruits from all 80 plants in the experiment. In the lab, we randomly selected a maximum of 75 fruits from each plant. Each fruit was individually labeled and wrapped in voile tied with a cotton string. The fruits were stored for two months to allow for insect emergence.

At the end of two months, we opened all fruits and counted and categorized the seeds in the following groups: (i) aborted, (ii) apparently healthy, and (iii) attacked by Lepidoptera, (iv) attacked by Coleoptera, or (v) attacked by Hymenoptera. Percentage of seed predation by Lepidoptera (P_{Le}), Coleoptera (P_{Co}), and Hymenoptera spp. (P_{Hy}) was calculated as:

$$\frac{\textit{Number of seeds attacked by determinated order}}{\textit{All attacked seeds + All healthy seeds}}$$

Apparently healthy seeds were submitted to x-ray to verify the presence of dead insect larvae within them. All seeds containing larvae were excluded from the healthy seed category. We then dried, weighed and milled all fruits and healthy seeds to do defensive compounds and nutrient analyses.

All seed-feeding insects obtained from seeds and fruits were stored in Eppendorff tubes containing 70%. The Bruchinae species were identified by Dr. C. Ribeiro-Costa, the Hymenoptera species by T. Morales and the Lepidoptera species were separated by morpho-species. We calculated the total number of insects per fruit (A_T) and their species richness (S_T), the abundance (A_{SF}) and richness (S_{SF}) of seed feeding insects only per fruit, and the abundance (A_p) and richness (S_T) of parasitoids per fruit.

Nutritional quality and defensive traits of fruits and seeds

Nutritional quality was characterized by the carbohydrate, protein, and lipid concentration of fruits. We determined the carbohydrate, protein, and lipid concentration using the Somogyi-Nelson assay (Nelson 1944), the Kjeldahl assay (Kjeldahl 1883), and the Soxhlet assay (Isac-García et al. 2016), respectively.

We conducted tannin extractions of fruits and seeds using the Sephadex LH-20 assay (Forkner et al. 2004) and purified tannin powder was used to build one standard curve for each plant organ and for the chemical analysis. We then prepared each sample individually using an acetone:L-ascorbic solution for each chemical analysis. Total phenols of each sample were estimated by the Folin-Denis assay (Swain & Hillis 1959), condensed tannins by the 95% butanol: 5% HCl assay (Bate-Smith 1977), and hydrolyzable tannins by the potassium iodate assay (Schultz & Baldwin 1982).

Data analyses

We excluded all clipped plants from our analyses because only a few plants of those treatments produced fruits, and those that did produced only a few fruits. We tested the normality of all variables using Mardia's multivariate normality test (MMNT). Three principal component analyses (PCA) were conducted, one for each group of plant traits. The PCA was used because it captures most of the variance of variables on only one axis, known as PC1, allowing to summarize large datasets (Oksanen 2016). In the PCA of quantity of nutritional resource available (Qtt), the variables of seed number and size, and fruit biomass of *S. adstringens* were used; PCA of resource nutritional quality (Nut) was determined by the of protein, lipid and carbohydrate concentration of *S. adstringens* fruits; and the total phenols, via hydrolyzable tannins and condensed tannins of *S. adstringens* fruits were used to determine the PCA of fruit defensive compounds (Def). The seed-feeding insects were grouped by order and we constructed a path analysis diagram organized in three levels: i) soil (fertilized/unfertilized - NPK), ii) fruit and seed traits (PC1 of Qtt, Nut, and Def), and iii) percentage of seed consumed per insect order (P_{Le}, P_{Co}, and P_{Hy}), and total fruit-associated insect abundance and richness (A_T, and S_T). We also constructed a path analysis diagram with four levels: i) soil, ii) fruit and seed traits, iii) seed-feeding abundance and richness (A_{SF}, and S_{SF}), iv) parasitoid abundance and richness (A_p, S_T). All analyses were conducted using the software *R* (R Development Core Team 2016) and the packages *vegan* (Oksanen et al. 2016), *MVN* (Korkmaz et al. 2014) and *lavaan* (Rosseel 2012) were used to perform PCA, MMNT, and Path Analysis, respectively.

Results

We evaluated 1,173 fruits, which represented 51.04% of the total fruits produced by the plants. Seed-feeding insects found in *S. adstringens* fruits and seeds belonged to the orders Blattodea, Coleoptera, Diptera, Hymenoptera, and Lepidoptera (Table 1). We found 26 species associated to S. adstringens fruits and the most abundant species were *Acanthocelides gregorioi*

(Coleoptera, Chrysomelidae: Bruchinae), *Cydia tonosticha* (Lepidoptera: Tortricidae), Lepidoptera sp.1, sp. 2, sp. 3 and sp. 4, and *Allorhogas* sp. 1 (Hymenoptera: Braconidae). Lepidopteran larvae attacked 39.05% ± 16.44% (SD), Hymenoptera 7.44% ± 6.21%, and Coleoptera 7.26% ± 6.04% of all available seeds, a 52.52% predation levels by insects of all orders.

We grouped fruit and seed traits into three PC1 axes: Def (total variance of PC1: 99.99%), Nut (total variance of PC1: 93.47%) and Qtt (total variance of PC1: 89.59%) (Figure 1). Def PC1 showed that total phenols, and hydrolyzable and condensed tannins of fruits had the same tendency, which is the increase in the production of fruit chemical defenses as a high as the PC1 axis value (Figure 1a). However, the Nut PC1 traits of carbohydrate and protein showed an opposite tendency to that of lipid concentration in the Nut PC1 axis (Figure 1b). Similar to Nut PC1, all Qtt PC1 traits, seed number and size and fruit biomass showed the same tendency presenting positive eigenvalues (Figure 1c).

According to our first hypothesis, NPK negatively influences defense and positively influences Nut and Qtt; however, NPK did not affect any seed and fruit traits (Table 1, Figure 2). We suggested in our second hypothesis that Def negatively influences the insect community associated with *S. adstringens* fruits and seeds, while resource Nut and Qtt positively influences it. Def did not impact percentage of seed consumed of any insect order (Table 1, Figure 2). Nut marginally and positively influenced the P_{Hy}. This result indicates that P_{Hy} was positively correlated with carbohydrate and protein content and negatively correlated with lipid content of fruits and seeds (Figure 1b). Finally, Qtt positively impacted both P_{Le} and P_{Co} since P_{Le} and P_{Co} were positively influenced by seed number and size, and by fruit biomass (Figure 1c). Similar to P_{Le} and P_{Co}, the abundance (A_T) and richness (S_T) of all insects associated with *S. adstringens* fruits and seeds were positively influenced by Qtt (Table 1, Figure 2). Therefore, we partially corroborate our second hypothesis since Nut only impacted P_{Hy}; and Qtt impacted P_{Le}, P_{Co}, A_T and S_T. However, we did not corroborate our third hypothesis, since NPK showed no indirect effect on the percentage of seed consumed of any insect order or on the insect abundance and richness. Finally, we partially corroborate our fourth hypothesis, since fertilizer addition had no direct or indirect impact in any variable, but Qtt positively influenced the seed-feeder abundance, which led to an impact on their parasitoid abundance (Figure 3, Table 1). Thus, the food web showed a bottom-up trophic effect, starting from the fruit and seed traits (i.e. Qtt) towards the parasitoid abundance. Although the higher the seed-feeders richness, the higher their parasitoid richness, any seed and fruit trait influenced the seed-feeder richness (Figure 3, Table 1). Please, see Table 1 - Supplementary material for more details about all the indirect effects.

Discussion

We found 26 insect species associated with *S. adstringens* fruits and seeds. The percentage of seed consumed varied among insect orders, the highest rate presented by Lepidoptera, followed by Hymenoptera and Coleoptera. NPK did not directly affect *S. adstringens* fruit and seed traits – composed of chemical defense (Def), nutritional quality (Nut), and quantity of resource (Qtt) – or indirectly affected the insect community associated with them – percentage of seeds consumed by Lepidoptera (P_{Le}), percentage of seeds consumed by Coleoptera (P_{Co}), percentage of seeds consumed by Hymenoptera (P_{Hy}), mean insect abundance (A_T), mean insect richness (S_T). In general, chemical defense was not important for any insect order as well as total insect abundance and richness, while nutritional quality positively influenced percentage of seeds consumed by Hymenoptera, and quantity of resource increased the percentage of seeds consumed by Lepidoptera and by Coleoptera, and the abundance and richness of fruit-associated insects. Moreover, there was a bottom-up trophic cascade of resource quantity influencing the seed-feeder abundance, which led to an impact in their parasitoid abundance. Also, the higher the seed-feeder richness, the higher their parasitoid richness, even ~~al~~though no seed and fruit trait influenced the seed-feeder richness.

We reported the greater part of the insect species associated with *S. adstringens* fruits and seeds already shown by Sari (2007) and Morales-Silva and Zampieron (2016) in their studies about the description of *S. adstringens* insect community who found 35 and 27 species, respectively. Most of those species registered by Sari (2007) are rare and were reported only once, despite the large number of fruits collected for the study – 2,146 fruits in total. Lepidoptera species showed the highest percentage of seed consumed, almost seven times higher than Coleoptera and Hymenoptera seed-feeder species. Even though the percentage of seed consumed by Lepidoptera was the highest, it was not the most abundant order. The percentage of seed consumed by Lepidoptera spp. may be much higher than by Coleoptera spp. because they eat more than one seed in the same fruit (Penteado-Dias et al. 2008), while the Coleoptera species feeds only in one seed (Southgate 1979) per fruit.

We did not corroborate our first and third hypotheses because NPK did not change fruit and seed traits or indirectly influence the fruit-associated insect community. It is possible that nutrient addition inhibited *Rhizobium* nodule activities in *S. adstringens* plants, and the fertilizer application did not show an effective increase in nutrient availability and also did not influence seed and fruit traits (Tuller et al. - Chapter II).

The second hypothesis was partially corroborated, since Def did not influence, but Nut and Qtt positively influenced the *S. adstringens* fruit-associated insect community. Despite the high Def compound concentrations, it did not influence the insect community associated with *S. adstringens* fruits and seeds because phenols and tannins are more effective against generalist insects (Barbehenn & Constabel 2011). Nevertheless, seed-feeding insects are specialized in only a few host plant species, or genera (Janzen 1980), which may explain why Def did not impact their preferences. Qtt positively influenced the percentage of seed consumed by Lepidoptera and Coleoptera insect orders. Seed-feeding beetles feed on only one seed along their entire larval phase (Southgate 1979), while Lepidoptera larvae feed on seeds and fruit pulp from only one fruit (Penteado-Dias et al. 2008). In consequence, female oviposition preference for fruits with enough nutritional resource availability is essential for seed-feeder success during their larval phase. For example, the greater the seed biomass, the greater the seed-feeding beetle size and consumption, and it may also reflect the allocation of resources, by the female for, reproduction (Maia et al. 2016) or fitness (Cope & Fox 2003). In the same way, the higher the seed number and fruit biomass, the greater the resource availability for seed-feeding insect exploitation, as well as abundance and richness of fruit-associated insects (Kersch-Becker & Lewinsohn 2012). Also, the majority of the total *S. adstringens* fruit-associated insect abundance was represented by Lepidoptera and Coleoptera orders, and it is expected that the majority of the parasitoid species should be linked to them. It might be the reason why the total abundance and richness of fruit-associated insect species were positively influenced by Qtt, such as the percentage of seed consumed by Lepidoptera and Coleoptera orders.

However, Nut positively influenced the percentage of seed consumed by Hymenoptera. The species of the *Allorhogas* genus cause deformities in developing seeds and present behavior similar to that of gall-inducing insects (Macêdo & Monteiro 1989, Tuller et al. 2015). Some gall-inducing insects may act as a sink of the host plant nutritional resources for their own benefit, modifying plant resource allocation (Hartley 1998, Marini-Filho & Fernandes 2012). Thus, the higher percentage of seed predation in higher nutritional quality fruits may be not related to the *Allorhogas* sp. 1 female oviposition preference for these fruits, but to a resource allocation induction to these fruits during the immature insect development, resulting in fruit with higher nutritional quality. The sink behavior also explains why Qtt traits are not important for this species, since they may get enough nutritional resources in other ways. In addition, non-preference for bigger seeds and fruits with a high number of seeds decreases *Allorhogas* sp. niche overlap with Lepidoptera and Coleoptera, decreasing the competition between them.

Our results partially corroborate the fourth hypothesis, since there was a bottom-up trophic cascade effect, not starting from the soil level (i. e. NPK) but starting from one level higher, which is from the fruit and seed nutritional traits (i.e. Qtt) to the seed-feeder and their parasitoids. In the same way highlighted in our third hypothesis, the majority of the seed-feeder abundance was represented by Lepidoptera and Coleoptera species, which explains the positive influence of Qtt on the seed-feeder abundance. Also, the seed-feeder richness positively influenced their parasitoid richness. Due to the fact that parasitoid species are generally specialists (Strand & Obrycki 1996), the higher the seed-feeder richness, the higher the host range availability and, consequently, the higher the parasitoid richness.

In conclusion, different resource traits influence the community of herbivorous insects in different ways. Even though resource quantity influences the abundance and richness of the entire fruit-associated insect community, the studies must focus on approaching the percentage of seed consumed by different seed-feeding groups separately. In addition, future studies are necessary to investigate how resource availability may influence the fitness of different insect orders or guilds since it influences most of them. Also, we suggest that further studies should evaluate if *Allorhogas* sp., acting as a nutrient sink, influences the female oviposition preference and the performance of other seed-feeding insect species.

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FIGURES

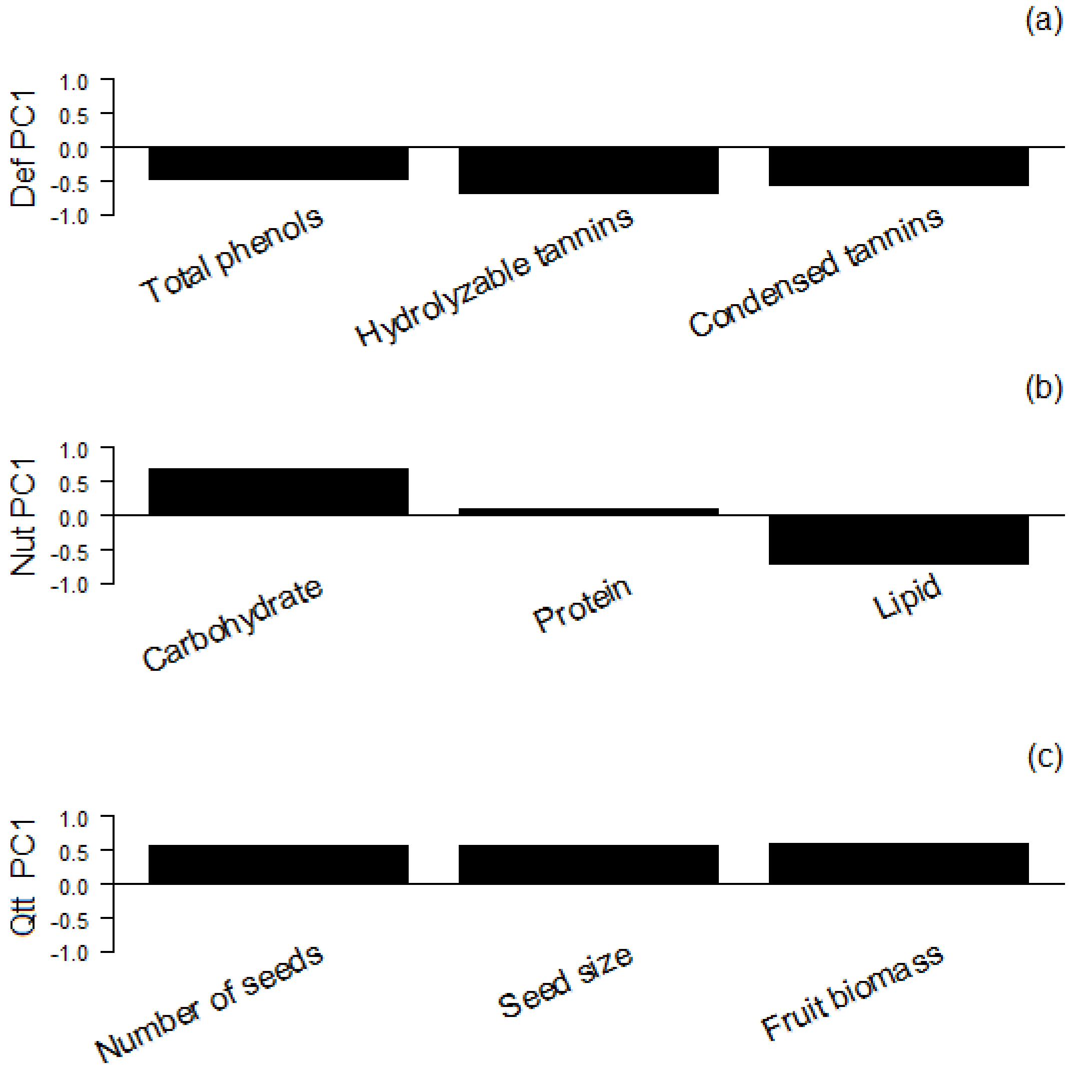

Figure 1 PCA eigenvalues and the tendency of each variable within (a) chemical defense trait eigen (Def PC1), (b) resource nutritional quality trait eigen (Nut PC1), and (c) resource quantity trait eigen (Qtt PC1) of the plant *Stryphnodendron adstringens*.

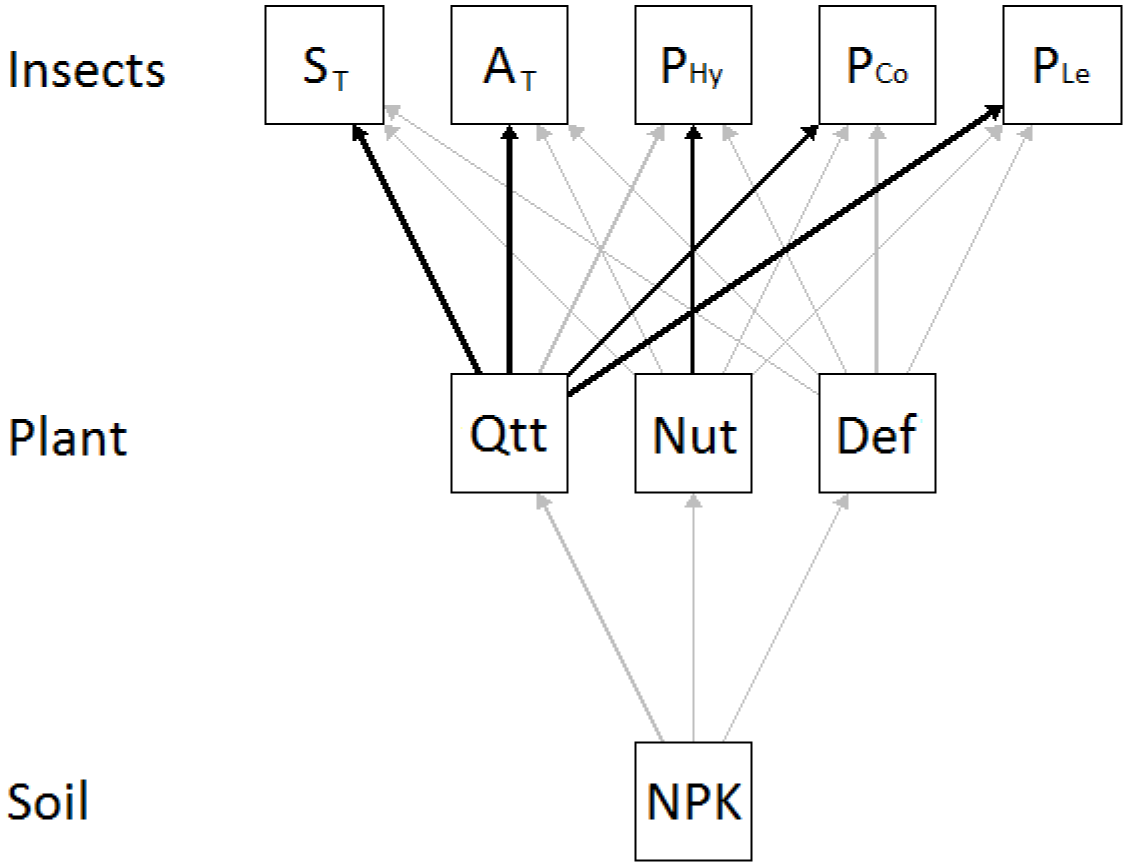

Figure 2 Diagram of path analysis showing the links of direct effects of soil (NPK) on plant traits (Qtt, Nut, and Def), and of plant traits on seed-feeding insects (P_{Le}, P_{Co}, P_{Hy}, A_T, and S_T) associated with fruits and seeds of *Stryphnodendron adstringens*. Black arrows represent statistically significant values and gray arrows non significant values, considering significant values as $p \leq 0.05$. The arrows' thickness vary from lower to higher effect intensity (estimate value). NPK: Fertilizant addition; Def: fruit chemical defense traits; Nut: fruit nutritional quality traits; Qtt: resource availability traits; P_{Le}: predation rate by Lepidoptera; P_{Co}: predation rate by Coleoptera; P_{Hy}: predation rate by Hymenoptera, A_T: mean abundance of insects associated with the fruits and seeds; S_T: mean richness of insects associated with the fruits and seeds.

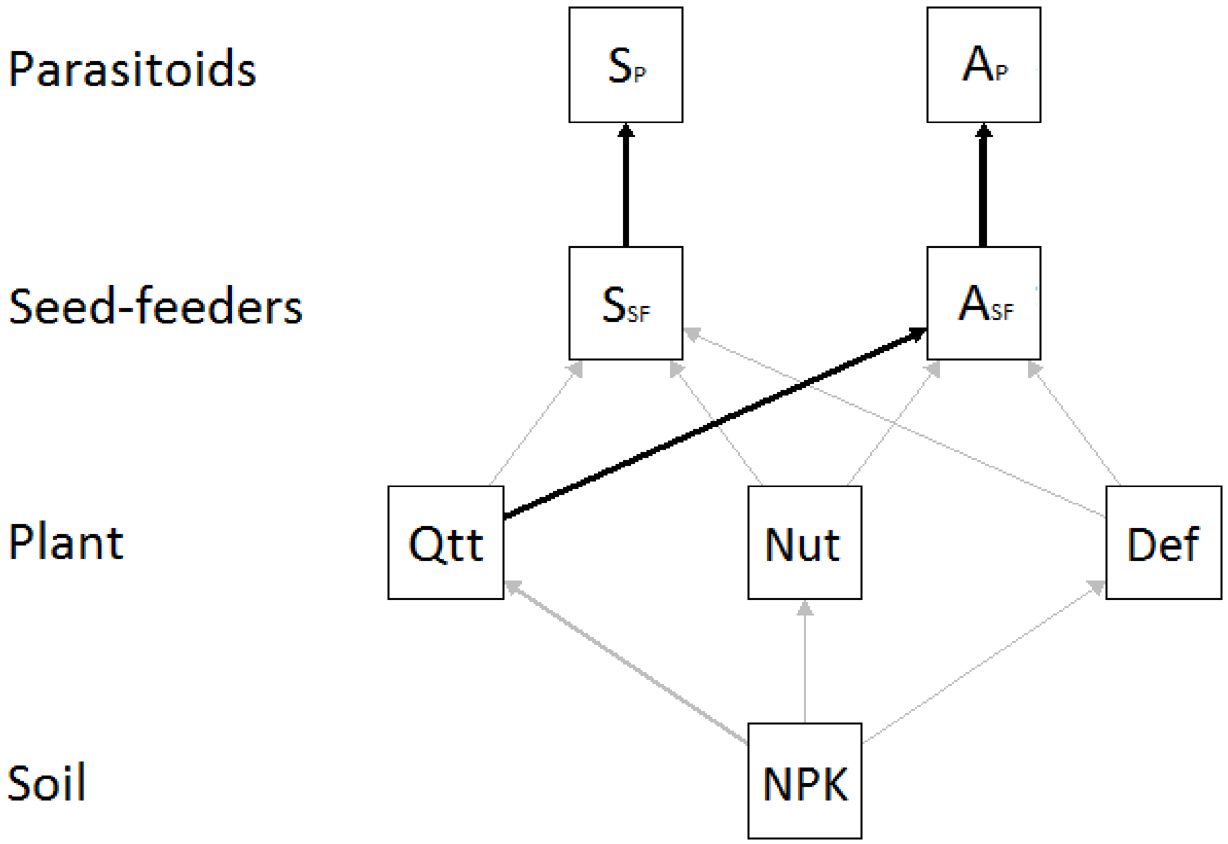

Figure 3 Diagram of path analysis showing the links of direct effects of soil (NPK) on plant traits (Qtt, Nut, and Def), plant traits on seed-feeding insects (S_{SF} and A_{SF}), and seed-feeding insects on their parasitoids (S_T and A_p) associated with fruits and seeds of *Stryphnodendron adstringens*. Black arrows represent statistically significant values and gray arrows non significant values, considering significant values as $p \leq 0.05$. The arrows' thickness vary from lower to higher effect intensity (path estimated value). NPK: Fertilizant addition; Def: fruit chemical defense traits; Nut: fruit nutritional quality traits; Qtt: resource availability traits; S_{SF}: mean richness of seed-feeding insects; A_{SF}: mean abundance of seed-feeding insects; S_T: mean richness of seed-feeder's parasitoid insects; A_p: mean abundance of seed-feeding's parasitoid insects.

TABLES

Table 1 Abundance and guild of the insects associated with fruits of *Stryphnodendron adstringens* in the region of Lavras, Minas Gerais, Brazil.

Order	Family	Species	Guild	Abundance		
				C	F	Total
Blattodea		Blattodea sp. 1	-	2	2	4
Coleoptera	Chrysomelidae (Bruchinae)	*Acanthoscelides gregorioi* (Pic)	Seed-feeder	287	141	428
	Dermestidae	Dermestidae sp. 2	Seed-feeder	2	0	2
	Scolytidae	Scolytinae sp. 1	Seed-feeder	3	0	3
Diptera	Sciaridae	Sciaridae sp. 1	Seed-feeder	63	40	103
Hymenoptera	Bethylidae	*Goniozus* sp. 1	Parasitoid	16	0	16
	Braconidae	*Allorhogas* sp. 1	Seed-feeder	9	30	39
	Braconidae	*Apanteles* sp. 1	Parasitoid	3	6	9
	Braconidae	Braconidae sp. 1	Parasitoid	1	0	1
	Braconidae	*Chelonus* (*Microchelonus*) sp. 1	Parasitoid	1	4	5
	Braconidae	*Pseudophanerotoma (P.) alvarengai* Zettel, 1990	Parasitoid	76	56	132
	Eulophidae	*Horismenus* sp. 1	Parasitoid	20	20	40
	Eupelmidae	*Eupelmus* sp. 1	Parasitoid	1	0	1
	Eurytomidae	*Eurytoma* sp. 1	Parasitoid	11	5	16
	Formicidae	Formicidae sp. 1	-	6	0	6
	Formicidae	Formicidae sp. 2	-	0	2	2
	Ichneumonidae	Pimplinae sp. 1	Parasitoid	1	0	1
	Ichneumonidae	Pimplinae sp. 2	Parasitoid	3	1	4
	Ichneumonidae	Pimplinae sp. 3	Parasitoid	0	1	1
	Torymidae	*Torymus* sp. 1	Parasitoid	19	27	46
	Torymidae	*Torymus* sp. 2	Parasitoid	1	0	1
Lepidoptera	Tortricidae	*Cydia tonosticha* (Meyrick, 1922)	Seed-feeder	210	116	326
		Lepidoptera sp. 1	Seed-feeder	14	14	28
		Lepidoptera sp. 2	Seed-feeder	10	13	23
		Lepidoptera sp. 3	Seed-feeder	0	2	2
		Lepidoptera sp. 4	Seed-feeder	3	0	3
Total				762	480	1,242

Table 2. Values of standardized estimate and *p* of the relationships presented on the path diagrams of the figures 2 and 3.

Variable 1	Variable 2	Estimated effect	*p*
FER	Def	-0.090	0.633
	Nut	0.098	0.602
	Qtt	-0.275	0.130
Def	P_{Le}	-0.105	0.513
	P_{Co}	0.249	0.127
	P_{Hy}	-0.127	0.451
	A_T	-0.054	0.749
	S_T	-0.095	0.560
	A_{SF}	-0.115	0.510
	S_{SF}	-0.160	0.385
Nut	P_{Le}	-0.141	0.379
	P_{Co}	0.206	0.207
	P_{Hy}	0.327	0.053*
	A_T	-0.054	0.745
	S_T	0.048	0.769
	A_{SF}	0.121	0.488
	S_{SF}	0.075	0.683
Qtt	P_{Le}	0.501	0.002*
	P_{Co}	0.390	0.017*
	P_{Hy}	-0.268	0.113
	A_T	0.432	0.011*
	S_T	0.495	0.002*
	A_{SF}	0.354	0.043*
	S_{SF}	0.149	0.419
A_{SF}	A_p	0.422	<0.001*
S_{SF}	S_T	0.462	<0.001*

*significant values considering $p \leq 0.05$. NPK: Fertilizant addition; Def: fruit chemical defense traits; Nut: fruit nutritional quality traits; Qtt: resource availability traits; S_{SF}: mean abundance of seed-feeding insects; A_{SF}: mean richness of seed-feeding insects; S_T: mean richness of seed-feeder's parasitoid insects; A_p: mean abundance of seed-feeding's parasitoid insects.

SUPPLEMENTARY MATERIAL

Table 1SM. Values of standardized estimate and *p* of the indirect interactions presented on the path diagram of the Figure 2 of the main file.

Path way	Variables	Estimate	*p*
Def	NPK → P_{Le}	0.009	0.700
	NPK → $_{Pco}$	-0.022	0.649
	NPK → P_{Hy}	0.011	0.687
	NPK → A_T	0.005	0.790
	NPK → S_T	0.009	0.712
Nut	NPK → P_{Le}	-0.014	0.654
	NPK → P_{Co}	0.020	0.630
	NPK → P_{Hy}	0.032	0.614
	NPK → A_T	0.005	0.783
	NPK → S_T	0.005	0.798
Qtt	NPK → P_{Le}	-0.138	0.173
	NPK → P_{Co}	-0.107	0.201
	NPK → P_{Hy}	0.074	0.273
	NPK → A_T	-0.119	0.193
	NPK → S_T	-0.136	0.176

NPK: Fertilizant addition; Def: fruit chemical defense traits; Nut: fruit nutritional quality traits; Qtt: resource availability traits; S_{SF}: mean abundance of seed-feeding insects; A_{SF}: mean richness of seed-feeding insects; S_T: mean richness of seed-feeder's parasitoid insects; A_p: mean abundance of seed-feeding's parasitoid insects.

Table 2SM. Values of standardized estimate and *p* of the indirect interactions presented on the path diagram of the Figure 3 of the main file.

Path way	Variables	Estimate	*p*
Def	NPK → S_{SF}	0.014	0.676
	NPK → S_T	-0.067	0.398
	NPK → A_{SF}	0.010	0.699
	NPK → A_p	-0.053	0.516
Nut	NPK → S_{SF}	0.007	0.748
	NPK → S_T	0.032	0.685
	NPK → A_{SF}	0.012	0.677
	NPK → A_p	0.056	0.494
Qtt	NPK → S_{SF}	-0.041	0.476
	NPK → S_T	0.063	0.430
	NPK → A_{SF}	-0.097	0.225
	NPK → A_p	0.163	0.070

NPK: Fertilizant addition; Def: fruit chemical defense traits; Nut: fruit nutritional quality traits; Qtt: resource availability traits; S_{SF}: mean abundance of seed-feeding insects; A_{SF}: mean richness of seed-feeding insects; S_T: mean richness of seed-feeder's parasitoid insects; A_p: mean abundance of seed-feeding's parasitoid insects.

CONSIDERAÇÕES FINAIS

Plantas em habitas com altos níveis de herbivoria, como o Cerrado, estão sujeitas a adaptações devido ao estresse sofrido pelo ataque. Em resposta à remoção total de suas folhas, a planta Stryphnodendron adstrigens é induzida a alterar sua fenologia e a realizarem trade-offs. Devido à restrição de recursos frente à herbivoria intensa e a necessidade de reposição das folhas, as plantas passam a investir maior energia na produção de taninos para se defender contra danos posteriores e reduzem drasticamente seu investimento em reprodução. De fato, S. adstringens produzem frutos que requerem grande quantidade de energia para serem produzidos e, apesar de produzirem inflorescências, as plantas não chegam a produzir frutos.

Dentre folhas, frutos e sementes, a folha é o único órgão que apresenta taninos em concentração semelhante às cascas do tronco. Ressaltamos também que, ao contrário da retirada excessiva das cascas de troncos das plantas de S. adstringens que causam sua morte, a retirada das folhas apenas direcionam o recurso para a produção de mais folhas e com uma concentração de taninos semelhante às cascas. Portanto, este estudo sugere que em uma área onde haja a intensa exploração de taninos oriundos de S. adstringens, a retirada das folhas da planta seja uma maneira menos agressiva para a utilização dessa matéria-prima comercialmente. Também se faz necessária a realização de estudos que testem a eficácia da utilização das folhas de S. adstringes como matéria-prima para a produção de medicamentos.

A adição de fertilizantes ao solo exerceu pouca influência sobre a planta, aumentando a biomassa de folhas produzidas apenas de maneira branda. A utilização de fertilizantes não seria muito indicado devido à planta apresentar associações com micro-organismos que poderiam ser inibidas com essa prática.

Quanto aos insetos associados aos frutos de S. adstringens, pudemos chegar à conclusão de que a separação desses organismos em guildas limita a determinação de quais fatores determinam as características do recurso preferidas pelos insetos. Observamos também que os insetos do gênero Allorhogas (Braconidae), os quais são descritos como galhadores de sementes, podem estar desviando recursos da plantas para o o fruto e/ou semente da qual se alimenta.

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