



**TAMIRES CAMILA TALAMONTE DE OLIVEIRA**

**THE ROLE OF PARASITOIDS IN THE INTEGRATION AND  
STRUCTURING OF TROPHIC NETWORKS**

**LAVRAS-MG**

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Entomologia, área de concentração em Biologia e Ecologia de Insetos, para a obtenção do título de Doutora.

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Orientador  
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**O PAPEL DOS PARASITOIDES NA INTEGRAÇÃO E ESTRUTURAÇÃO DE  
REDES TRÓFICAS**

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*Em homenagem à minha mãe Elsa Margit Talamonte pelo amor e dedicação  
em minha criação e por todas as palavras incentivadoras  
durante o árduo processo de formação acadêmica*

**DEDICO**

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## RESUMO

A maior parte de toda biodiversidade global de insetos é composta por complexas redes de interações tri-tróficas, formadas por parasitoides, seus herbívoros hospedeiros e as plantas ao qual esses insetos vivem e se alimentam. Nos últimos anos houve um elevado aumento nas taxas de extinção e invasão nos ecossistemas, de forma que estamos perdendo espécies que nem mesmo chegamos a conhecer. Desta forma, estudos de comunidades, especialmente envolvendo a descrição das espécies e suas as interações contribuem para um maior entendimento das causas e consequências que a perda ou adição de uma espécie podem ocasionar para todo ecossistema. Em especial o entendimento das interações parasitoides-hospedeiro pode acarretar em conhecimento de uso prático no controle biológico e redução de uso de agroquímicos. No primeiro artigo nós caracterizamos a comunidade de insetos herbívoros e seus parasitoides associados à três espécies de Fabaceae: *Senna multijulga*, *Inga vera* e *Leucaena leucocephala*, com 5,353 indivíduos, e 77 espécies. Nós descobrimos que a ordem Hymenoptera, um grupo dominado por vespas parasitoides, foi a mais diversa em todos os sistemas estudados (n= 48). Além disso, a comunidade de insetos associadas à estas plantas parecem ser altamente específicas em seus hospedeiros (plantas e herbívoros hospedeiros), uma vez que apenas quatro espécies (três parasitoides e um herbívoro) conectaram diferentes plantas. Descobrimos que o número de semente promoveu um efeito de cascata trófica sobre a abundancia e riqueza de herbívoros e parasitoides assim como os grupos de insetos mais abundantes sobre a abundância e riqueza de parasitoides. No segundo artigo nós descrevemos a rede trófica de *L. leucocephala*, e relacionamos as métricas quantitativas, padrão de predação e comportamento de oviposição de *Acantoscélides macrophthalmus* com o tamanho do recurso. Os resultados do artigo mostram uma comunidade de 17 espécies, compostos por 6 herbívoros, 9 parasitoides e 1 hiperparasitoide. O traço do recurso biomassa de semente mostrou forte influência sobre os insetos e a complexidade da rede trófica. Nós observamos que frutos com sementes mais pesadas tinham maior abundância, riqueza, conectividade e número de interações. Além disso, *A. macrophthalmus* ovipositou mais ovos em frutos com sementes mais pesadas. E no geral sementes predadas por Colepteras, principalmente representada por essa espécie eram quatro vezes mais pesadas que sementes de outras categorias, inclusive as não predadas. Por fim, nosso estudo enfatiza a importância de incluir parasitoides em investigações de comunidade, pois eles são muito diversos e tem um papel importante em serviços nos ecossistemas.

**Palavras-chave:** Interações tri-tróficas. Biodiversidade. Entomofauna. Predação de sementes.



## ABSTRACT

Most of the global biodiversity of insects is composed of complex networks of tri-trophic interactions formed by parasitoids, their herbivorous hosts, and the plants on which they live and feed. In recent years, there has been a high increase in the taxa of extinction and invasion of ecosystems, in such a way that we are losing species that we did not even know about. In this way, studies of communities, especially involving the description of the species and their interactions, contribute to a better understanding of the causes and consequences that the loss or addition of a species can cause for all ecosystems. In particular, understanding the interactions of host-parasitoids can carry knowledge of practical use in the biological control of insects and the reduction of the use of agrochemicals. In the first article, we characterized communities of herbivorous insects and their parasitoids associated with three species of Fabaceae: *Senna multijulga*, *Inga vera*, and *Leucaena leucocephala*, with 5,353 individuals and 77 species. We found that the Hymenoptera order, a group dominated by parasitoid wasps, was more diverse in all systems studied ( $n = 48$ ). Furthermore, the insect communities associated with these plants seem to be highly specific to their hosts (plant and herbivore hosts), as only four (three parasitoids and one herbivore) species connect different plants. We discovered that the number of seeds promoted a trophic cascade on the abundance and richness of herbivores and parasitoids as well as the most abundant insect groups on the abundance and richness of parasitoids. In the second article, we described the trophic network of *L. leucocephala*, and we relate the quantitative metrics, the pattern of predation, and oviposition behavior of *Acantoscelides macrophthalmus* to the size of the resource. The results of the article show a community of 17 species, composed of 6 herbivores, 9 parasitoids, and 1 hyperparasitoid. The seed biomass trait showed a strong influence on the insects and the complexity of the food web. We observed that fruits with heavier seeds have greater abundance, richness, connectivity, and number of interactions. Furthermore, *A. macrophthalmus* laid more eggs in fruits with heavier seeds. In general, seeds predated by Coleoptera, mainly represented by *A. macrophthalmus*, were four times heavier than seeds from other categories, including those not predated. Finally, our study emphasizes the importance of including parasitoids in community research because they are very diverse and have an important role in ecosystem services.

**Key-words:** Tri-trophic interactions. Biodiversity. Entomofauna. Seed predation.

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## **PRIMEIRA PARTE**

## 1. INTRODUÇÃO GERAL

Diante do elevado aumento nas taxas de extinção e invasão de espécies nos últimos anos, tem se tornado cada vez mais crucial o entendimento das causas e consequências da perda de biodiversidade, bem como a maneira pelo qual a adição ou remoção das espécies em um determinado ambiente podem influenciar tanto na estabilidade como nas funcionalidades aos quais os sistemas biológicos estão sustentados (MCCANN, 2000).

Comunidades naturais são compostas por diversas espécies que estão conectadas através de interações ecológicas formando complexas redes tróficas (POLIS; STRONG, 1996). Essas interações ecológicas podem ocorrer de forma direta ou indireta (SCHMITZ; SUTTLE, 2001). Os efeitos diretos são resultados da interação física entre duas espécies e de fácil interpretação (ex: predação e parasitismo) e os efeitos indiretos necessitam de uma terceira espécie intermediária para que ocorram e diferentemente do anterior não são tão óbvias assim (WOOTTON, 1994). Os efeitos indiretos podem ser observados na natureza através dos efeitos de cascata trófica, descrito como o efeito em que uma espécie tem sobre outras de níveis tróficos não adjacentes ao seu (POLIS et al., 2000). Tal efeito pode influenciar na abundância, riqueza e composição de espécies ou nas interações entre elas (PAINE, 1980).

Além disso, as interações ecológicas podem também ser caracterizadas pela força com que elas ocorrem na natureza, sendo definida como a mudança que a abundância de uma espécie causa na outra (PAINE, 1980). De fato, existe um número muito maior de forças fracas nos ecossistemas do que forças fortes (PAINE, 1992; WOOTTON, 1997) e estudos teóricos tem demonstrado que essa distribuição de forças de interações tem um efeito estabilizador nos sistemas biológicos (EMMERSON; YEARSLEY, 2004). Por isso, estimar forças de interação são de suma importância para o entendimento dos padrões ecológicos entre as espécies.

A estabilidade em redes tróficas tem sido alvo de muitas discussões teóricas e empíricas fazendo com que muitas hipóteses tenham sido levantadas nos últimos anos. Entre elas podemos destacar: a hipótese da diversidade-estabilidade (ELTON, 1958) que foi inicialmente levantada por Charles Elton que afirmava que “Comunidades mais simples são perturbadas mais facilmente do que comunidades ricas e são também mais susceptíveis a oscilações e invasões”. E a hipótese estrutura-estabilidade (YODZIS, 1981) que afirma que a maneira como as espécies estão estruturadas (interações, força de interações, níveis tróficos) causaria estabilidade. E mais recentemente, foi elaborada a teoria de que os consumidores de topo em uma rede trófica teriam

um papel muito importante na estabilização dos sistemas biológicos (MCCANN; RASMUSSEN; UMBANHOWAR, 2005).

Segundo essa teoria, os consumidores de topo ligam as redes tróficas no espaço e essa estrutura espacial combinada com uma resposta rápida no comportamento de forrageio podem influenciar fortemente na dinâmica das redes tróficas (MCCANN; RASMUSSEN; UMBANHOWAR, 2005; MCCANN.; ROONEY, 2009). De fato, em um estudo realizado no Canadá foi observado que os parasitoides têm um comportamento previsível em função da abundância do seu principal hospedeiro controlando-os e gerando um efeito de cascata trófica na diversidade da rede em estudo (EVELEIGH et al., 2007). Além disso, nesse mesmo trabalho foi evidenciado que ambientes mais heterogêneos causavam um aumento na diversidade de parasitoides e hiperparasitoides, mostrando a importância desses consumidores na determinação da presença da estabilidade ou a ausência da mesma.

Levando-se em consideração a potencialidade da teoria citada anteriormente no entendimento dos mecanismos que geram a estabilidade, bem como a escassez de trabalhos empíricos de redes tróficas que visam entender a relação hospedeiro-parasitoide (ROUGERIE et al., 2011) o uso de uma rede trófica do tipo *source web* (COHEN; BRIAND; NEWMAN, 1990) parece ser a mais adequada, uma vez que através dela busca-se compreender os processos envolvidos nas interações entre um recurso fonte e os organismos que interagem com ele (herbívoros e parasitoides) (PIMM; LAWTON; COHEN, 1991). Deste modo, sistemas que possuem insetos associados a frutos e sementes, em especial da família Fabaceae, são sistemas interessantes para estudar interações do tipo hospedeiro-parasitoide, por serem de fácil amostragem e ricos em espécies e interações (TULLER et al., 2015). Para o desenvolvimento dessa tese nós selecionamos as seguintes espécies de plantas pertencentes a família Fabaceae: *Senna multijulga* (Rich.) H. S. Irwin & Barneby, *Leucaena leucocephala* (Lam.) de Wit e *Inga vera* Willd.

## 2. REFERENCIAL TEÓRICO

### 2.1. Abordagem Histórica

Um dos primeiros pesquisadores a abordar a importância das interações entre as espécies foi Elton em (1927). Em seu trabalho ele definiu o termo *food chains* (cadeias tróficas) como a transferência de energia entre as espécies, e *food cycle* (sinônimo de *food web*) como a combinação de todas as cadeias tróficas em uma comunidade. Elton também buscou entender como a complexidade estava relacionada à estabilidade das populações, defendendo que comunidades mais simples eram também mais susceptíveis a surtos populacionais. MacArthur (1955) propôs através de uma abordagem matemática das interações, que com o aumento da complexidade haveria uma diminuição na possibilidade de ocorrer explosões populacionais, i.e. perturbações dinâmicas, portanto a complexidade seria importante na estabilização das comunidades.

Em 1972, Robert May (1972) foi contra o pressuposto de que as comunidades mais complexas causariam estabilidade nos sistemas. Em seu trabalho ele criou vários cenários (modelos matemáticos) de interações entre as espécies, onde cada cenário seria estático e cada espécie interagiria com as demais de forma e intensidade aleatória. Como resultado observou que o aumento da complexidade (número de espécies, força de interações) causariam efeito contrário nas populações, desestabilizando as comunidades e causando extinções. No entanto, de forma geral, o que observamos na natureza é que as espécies não interagem de forma aleatória, e uma grande crítica ao estudo de May, (1972) foi o fato dele não ter considerado dados realistas em seus modelos matemáticos (HAYDON, 2000). Em contrapartida após seus estudos foi possível observar que as forças de interações e suas intensidades também são relevantes no entendimento do funcionamento das comunidades e ecossistemas.

Desde então, a grande questão não é se a estabilidade aumenta com a complexidade de comunidades aleatórias, mas quais são os padrões estruturais que existem nas comunidades reais que contribuem para que de fato ocorra a estabilidade. Assim muitos trabalhos estão buscando entender padrões e regularidades presentes em redes tróficas empíricas (SUGIHARA; SCHOENLY; TROMBLA, 1989) e componentes capazes de garantir maior estabilidade a comunidades naturais (PIMM, 1984).

Thebault e Loreau (2003) mostraram em seu estudo que o aumento na diversidade de redes multitróficas pode acarretar em diversas respostas, no entanto estas respostas dependem das forças com que as interações ocorrem entre as espécies. Mccann, Hastings e Huxel (1998)

mostraram que forças de interação fracas são super-representadas nas redes tróficas reais, e que elas possuem o potencial de serem estabilizadoras. Em estudos sobre forças de interações é comum utilizar interações direta, ou seja, predador-presa e consumidor-recurso (WOOTTON, 1997). Esse tipo de estudo permite a detecção de efeitos de cascata trófica, que segundo Paine (1980) é definido como os efeitos indiretos que os indivíduos de uma espécie possuem sobre organismos de outra (s) espécie (s) que estão em um nível trófico não adjacente ao seu.

Existem dois tipos de efeito de cascata trófica (ECT), o efeito *bottom-up* (base-topo), em que uma população de indivíduo de um nível trófico inferior controla os níveis tróficos superiores, e *top-down* (topo-base), em que níveis tróficos superiores controlam níveis tróficos inferiores (PACE et al., 1999). Há uma discussão sobre quais forças teriam maior pressão em comunidades de insetos. Alguns estudos indicam uma pressão mais forte das forças *top-down* do que as forças *bottom-up* sobre as populações de herbívoros (BERNAYS; GRAHAM, 1988; HOGG; DAANE, 2015). Outros estudos defendem que ambas as forças *bottom-up* e *top-down* tem efeito na evolução, distribuição e dinâmica populacional de insetos herbívoros (CARPENTER; KITCHELL; HODGSON, 1985; DENNO; LEWIS; GRATTON, 2005; MOONEY; PRATT; SINGER, 2012; PAINE, 1980; PRICE et al., 1980; SINGER; STIREMAN, 2005). Além disso, há também estudos que mostram que interações que ocorrem em um mesmo nível trófico também podem moldar as comunidades de herbívoros (CORNELISSEN et al., 2013; KAPLAN; DENNO, 2007). Desta forma, estudos de redes tróficas são essenciais para detecção de efeitos de cascata tróficas e representam um dos maiores sucessos atualmente (FRETWELL, 1987; POLIS et al., 2000).

## 2.2. O estudo de Redes tróficas

Redes tróficas são sistemas multitróficos, em que recurso, presa e predadores e/ou parasitoides interagem causando mudanças temporais e espaciais sobre sua abundância e riqueza de espécies (MAAR et al., 2002). Os estudos de rede trófica buscam entender como as espécies estão interagindo e como essas interações influenciam na estrutura, estabilidade e funcionamento das comunidades (MOORE; DE RUITER, 1991).

Estudos de redes tróficas devem incluir informações qualitativas e quantitativas sobre as interações entre as espécies em todos os níveis tróficos de uma comunidade (LEWIS et al., 2002). Análises quantitativas são importantes, pois ajudam a distinguir espécies frequentes daquelas que são raras e casuais, o que não é possível se considerarmos apenas os aspectos qualitativos da rede trófica (identidade da espécie e interação resultando em redes binárias).

Nessa perspectiva, interações parasitoides-hospedeiros podem ser um bom sistema de estudo, visto que as interações são estritamente quantitativas (LEWIS et al., 2002; MEMMOTT; GODFRAY, 1994).

Métricas de redes aplicadas a redes tróficas são frequentemente citadas como a variação no número de links tróficos com o número de espécies em uma rede. Conectância é uma dessas análises quantitativas, sendo a proporção de interações realizadas com relação ao total possível, variando de 0 a 1. A conectância mostra o quão interligado estão as espécies, e juntamente com a riqueza fornece uma medida de complexidade da comunidade (PIMM, 1984). Com isso se justifica trabalhos que levem em consideração tanto os aspectos qualitativos como quantitativos de redes tróficas.

### **2.3. Parasitoides e sua importância**

O termo parasitoide foi utilizado pela primeira vez em 1913 (WHEELER, 1914), descrevendo a estratégia em que o parasita se desenvolve internamente ou externamente ao corpo de um único indivíduo hospedeiro, causando sua morte e produzindo um adulto de vida livre que se alimentam de pólen e/ou néctar (QUICKE, 1997). A fêmea do parasitoide pode depositar um ou vários ovos sobre seu hospedeiro que podem ser larvas, ovos, pupas ou imagos de outros insetos. As larvas emergentes dos ovos dos parasitoide se alimentam do tecido do hospedeiro o que no final de seu ciclo biológico causa a morte do mesmo (MARCHIORI et al., 2007). Estima-se que cerca de 10% das espécies de insetos existentes nos ecossistemas terrestres sejam parasitoides (HASSELL, 2000). O parasitismo evoluiu em muitos grupos de insetos como Diptera e Coleoptera, apesar disso a maior parte da diversidade descrita pertence a ordem dos Hymenoptera principalmente parasitoides, que representam cerca de 50% desse número total (QUICKE, 1997; SHARKEY, 2007).

Nos ecossistemas, o parasitismo é responsável por grande parte da regulação dos níveis populacionais de insetos, já que os parasitoides podem parasitar várias espécies de herbívoro e também possuem habilidade de responder rapidamente à densidade dos seus hospedeiros (GAULD; BOLTON, 1988; MAY, 1988). Além disso, parasitoides estão presentes em mais de 50% das cadeias tróficas dos sistemas terrestres, reforçando sua importância na regulação populacional (LA SALLE; GAULD, 1992). Devido a essas e características, eles são amplamente utilizados como agentes de controle biológico, tanto na agricultura como em projetos de conservação no controle de seus hospedeiros, economizando cerca de \$20 milhões



de dólares anuais para indústrias agrícolas nos Estados Unidos (MILLS; WAJNBERG, 2008; PENNACCHIO; STRAND, 2006; PENNISI, 2010).

Os parasitoides podem utilizar diversos grupos taxonômicos de hospedeiros, como aranhas, pulgões, tripes, galhas, e etc. No entanto, os grupos mais comumente utilizados como hospedeiros são: Coleoptera, Diptera, Lepidoptera e Hymenoptera, esses parasitoides que usam herbívoros como hospedeiros, são chamados de parasitoides primários (QUICKE, 1997). Existem ainda parasitoides chamados de secundários ou hiperparasitoides, que são aqueles que utilizam parasitoides primários para completar seu desenvolvimento (SULLIVAN; VÖLKL, 1999). Diferentemente dos níveis tróficos basais (herbívoros, plantas) o nível trófico dos hiperparasitoides não são fixos, visto que existem hiperparasitoides facultativos que podem mudar de parasitoides primários para secundários, sob condições de alta competição (EVELEIGH et al., 2007; MUSTATA, G., MUSTATA, 2009). Outros níveis tróficos de hiperparasitoides foram documentados, incluindo parasitoides terciários e quaternários, mas esses casos são raros (MUSTATA; MUSTATA, 2009).

Uma recente teoria sugere que a habilidade dos predadores de topo (rede trófica) móveis, ou parasitoides, em responder a mudança da densidade de sua presa, tem um papel muito importante na estabilização das redes tróficas. Isto porque, a mobilidade e o comportamento de forrageio tende a escalar com a posição trófica do organismo. Essa teoria prediz que organismos que estão nos níveis tróficos superiores tenham maior resposta a mudança das condições de sua presa no habitat (BECKERMAN; PETCHEY; WARREN, 2006; MCCANN; RASMUSSEN; UMBANHOWAR, 2005). Contudo, apesar da importância dos parasitoides, estudos que os levem em consideração são ainda incipientes, e até onde sabemos poucos estudos buscaram entender e o papel dos parasitoides em múltiplas redes tróficas (MORRIS et al., 2014; TYLIANAKIS; TSCHARNTKE; LEWIS, 2007). Além disso, a estrutura de um sistema (rede trófica) pode ter efeitos importantes sobre a composição e força de interação de outros sistemas (redes tróficas) (INGS et al., 2009; OLESEN et al., 2010).

#### **2.4. Sistemas de estudo família Leguminosae**

A família Fabaceae é a terceira maior família de plantas existentes no mundo em termos de número de espécies, atrás somente de Asteraceae e Orchidaceae (AZANI et al., 2017). Está família possui cerca de 770 gêneros e 19.500 espécies, distribuídas em seis subfamílias: Caesalpinioideae, Cercidoideae, Detarioideae, Dialioideae, Duparquetioideae, and Papilionoideae (Faboideae) (AZANI et al., 2017; LEWIS, 1987; QUEIROZ, 2009). Em nível

de importância econômica, a família Fabaceae está em segundo lugar, atrás somente das plantas da família Poaceae (e.g., arroz, cana de açúcar, milho, aveia, trigo e cevada) (AZANI et al., 2017).

No Brasil, temos cerca de 222 gêneros e 2.822 espécies pertencentes a essa família, o qual compõe a flora dos diversos ecossistemas como o cerrado e a floresta amazônica, e em sua maioria, de forma endêmica (LIMA, 2000; SOUZA; LORENZI, 2005). Essa família é cosmopolita, ocorrendo em todos os habitats como serras montanhosas, florestas tropicais, fragmentos de mata e até mesmo em litorais arenosos. Podem se apresentar como ervas anuais ou perenes, eretas, prostradas, difusas, trepadeiras, lianas, subarbustos, arbustos e árvores de diversos tamanhos (LEWIS, 1987). Os frutos são geralmente do tipo legume, mas podem apresentar de forma diversa como: nucóide, samaróide, legume bacóide, lomento, folículo, sâmara e drupa (BARROSO et al., 1999; DOYLE et al., 2000).

Espécies da família Fabaceae são geralmente descritas como facilitadoras, devido principalmente sua habilidade em fixar nitrogênio no solo, o que pode auxiliar a colonização de outras espécies de planta em determinado habitat (MONTESINOS-NAVARRO et al., 2017). No entanto, algumas espécies de Fabaceae como por exemplo *Leucaena Leucocephala* (Lam.) de Wit, podem se tornar importantes invasoras, trazendo prejuízos econômicos e ecológicos (LOWE, 2007). Entende-se por espécie invasora uma espécie cuja população se expande rapidamente em locais aos quais não ocorre naturalmente, reduzindo a abundância ou deslocando espécies nativas (VITOUSEK, 1986). Essas espécies podem afetar negativamente a estrutura das interações, aumentando a competição por recursos com as espécies nativas (DIDHAM et al., 1998), e desviando inimigos naturais generalistas de herbívoros de espécies nativas para a espécies exóticas, o que pode ter como consequência um aumento da abundância dos herbívoros em espécies nativas (MONTERO-CASTAÑO; VILÀ, 2012).

Estudos anteriores têm demonstrado que plantas da família Fabaceae são interessantes para o estudo de interações, pois, são facilmente coletadas e armazenadas e possuem alta produtividade de sementes e frutos (MORALES-SILVA; MONTEIRO; FARIA, 2018; SILVA; MODESTO ZAMPIERON, 2015; TULLER et al., 2015). Essas características do fruto nesses sistemas, facilitam o estabelecimento de interações hospedeiro-parasitoide. Além disso, outra grande vantagem do uso de plantas dessa família é o fato dos parasitoides permanecerem no fruto durante sua fase larval vivendo dentro das sementes (MORALES-SILVA; MONTEIRO; FARIA, 2018; TULLER et al., 2015). Ademais, outra grande vantagem utilizar esses sistemas

é o fato de que se pode facilmente mensurar os traços das plantas, como seu tamanho e peso, e relacionar com aspectos gerais da rede trófica. Esta informação pode indicar a importância do recurso para o sistema como um todo. Contudo, a maioria dos trabalhos entomológicos não possuem uma abordagem de redes tróficas, e em sua grande maioria desconsideram o terceiro nível trófico (parasitoides), principalmente pelo tamanho dos indivíduos e pela dificuldade em sua identificação (COSTA; DE CAL PAULA, 2012; GARLET et al., 2011; OLIVEIRA; COSTA, 2011). Desta forma, o conhecimento das interações planta-herbívoro-parasioide, apesar de desafiador concretiza-se como um dos parâmetros mais importantes para a conservação de espécies, especialmente diante da problemática perda de diversidade que estamos enfrentando nos últimos anos (LEWINSOHN et al., 2022; SIMMONS et al., 2019)

### **2.5. Uso de DNA *barcoding* auxiliando identificações morfológicas**

A identificação de indivíduos em nível de espécie é essencial para a compreensão da diversidade da vida bem como a delimitação dos locais em que tais espécies ocorrem (DAYRAT, 2005). Tal conhecimento tem sido pré-requisito em estudos científicos, e no estabelecimento de planos de manejo e conservação mais eficientes, auxiliando principalmente no levantamento da biodiversidade local (FRÉZAL; LEBLOIS, 2008).

Com o avanço da tecnologia e do conhecimento científico, surgiram técnicas que permitiram a identificação dos indivíduos, através do seu material genético, auxiliando os estudos morfológicos (HEBERT et al., 2003). Uma dessas técnicas é o DNA *barcoding* em que se utiliza um fragmento de gene mitocondrial do Citocromo C oxidase subunidade I (COI). Esse trecho possui 658 nucleotídeos e foi primeiramente escolhido, pois, está presente em todos os organismos eucariontes, é de fácil manipulação e possui taxas de evolução relativamente rápida (HEBERT et al., 2003). Devido essas características, o uso dessa metodologia permite a diferenciação de indivíduos tanto das mesmas espécies, como de espécies diferentes (HEBERT et al., 2003; HEBERT; RATNASINGHAM; DE WAARD, 2003).

Desta forma, o uso da técnica de identificação molecular por DNA *barcoding* é baseado na ideia da criação de um banco de dados com informações moleculares integrados aos dados taxonômicos e ecológicos de diversas espécies. Esses dados permitem que espécimes não identificadas ou de difícil identificação sejam comparados aos dados existentes, auxiliando assim na atribuição do grupo taxonômico ao qual esses indivíduos pertencem. Esse conhecimento pode auxiliar no descobrimento de espécies crípticas, de novas espécies e resolução de problemas de variação morfológica (HEBERT et al., 2003, 2004). No entanto, o

funcionamento dessa técnica depende da qualidade e quantidade de informações moleculares contidas nos bancos de consulta, o que pode ser um fator limitante. Por isso, para que se tenha confiança nas identidades dos indivíduos, é necessário que os dados moleculares sejam atrelados aos estudos morfológicos e vice-versa (DE CARVALHO et al., 2007).

### **2.5. Objetivos e divisão da tese**

Nesse contexto, para compreendermos melhor as interações entre planta-herbívoros e parasitoides, foram escolhidas três espécies simpátricas de plantas pertencentes a família Fabaceae, duas nativas *Inga vera* e *Senna multijulga* e uma não nativa *Leucaena leucocephala*. Essas espécies estão amplamente distribuídas no campus da Universidade Federal de Lavras, Lavras, Minas Gerais, lugar onde foi realizada essa investigação. Os nossos objetivos foram de forma geral caracterizar as comunidades de herbívoros e parasitoides associadas a esses sistemas, estimando o efeito que cada nível trófico tem sobre o outro, bem como as interações e a relação com tamanho e disponibilidade do recurso (semente e fruto).

No primeiro artigo, tivemos os objetivos de: (1) caracterizar a comunidade de insetos herbívoros e seus parasitoides associados as três plantas mencionadas anteriormente; (2) avaliar os efeitos diretos e indiretos dos traços dos recursos (fruto e sementes) sobre a abundância e riqueza dos insetos herbívoros e parasitoides; (3) estimar o efeito da abundância e riqueza de herbívoros sobre a comunidade de parasitoides e (4) avaliar o grau de especialização dos insetos em seus recursos, utilizando dados morfológicos e moleculares. No segundo artigo, nós caracterizamos as interações da comunidade de herbívoros e parasitoides, bem como os padrões de predação de sementes causadas pelos herbívoros associados à *Leucaena leucocephala*, uma planta considerada invasora em muitos países, inclusive no Brasil. Nesse estudo, nós avaliamos a rede trófica de forma qualitativa (espécies e interação) e quantitativa (abundância, riqueza e métrica da rede trófica), estimando a relação do recurso com os parâmetros quantitativos avaliados e o comportamento de oviposição do principal herbívoro *Acantoscélides macrophthalmus* e por último a relação entre predação de semente e seleção de tamanho do recurso em diferentes grupos taxonômicos.

### **3. CONSIDERAÇÕES GERAIS**

Este trabalho contribuiu para um melhor entendimento da relação tritífica entre o recurso, insetos herbívoros e seus parasitoides. Mostramos um efeito positivo do recurso (semente e fruto) sobre a comunidade de insetos associadas aos mesmos, promovendo um efeito de cascata trófica na abundância e na diversidade de herbívoros e de seus parasitoides. Dessa

forma, sugerimos que futuros trabalhos de redes tritróficas levem em consideração esses atributos como fatores estruturadores das comunidades de insetos, e sugerimos que mais de um traço seja mensurado, já que observamos, que diferentes traços podem ter contribuições diferentes para a comunidade associada. Nós descrevemos 77 espécies de insetos associadas à essas plantas, e mostramos algumas tendências em relação ao grau de especialidade dos herbívoros e parasitoides em seus respectivos hospedeiros. No entanto, para que seja esclarecido a questão da generalidade ou especificidade desses indivíduos e o papel dos parasitoides sobre a estruturação e estabilidade dos sistemas, sugerimos que sejam realizadas coletas com um maior número de espécies de plantas hospedeira com a incorporação dos dados moleculares. Essa informação, facilitaria comparações entre diferentes trabalhos e contribuiria para uma melhor entendimento da distribuição e interação de insetos em diferentes sistemas.

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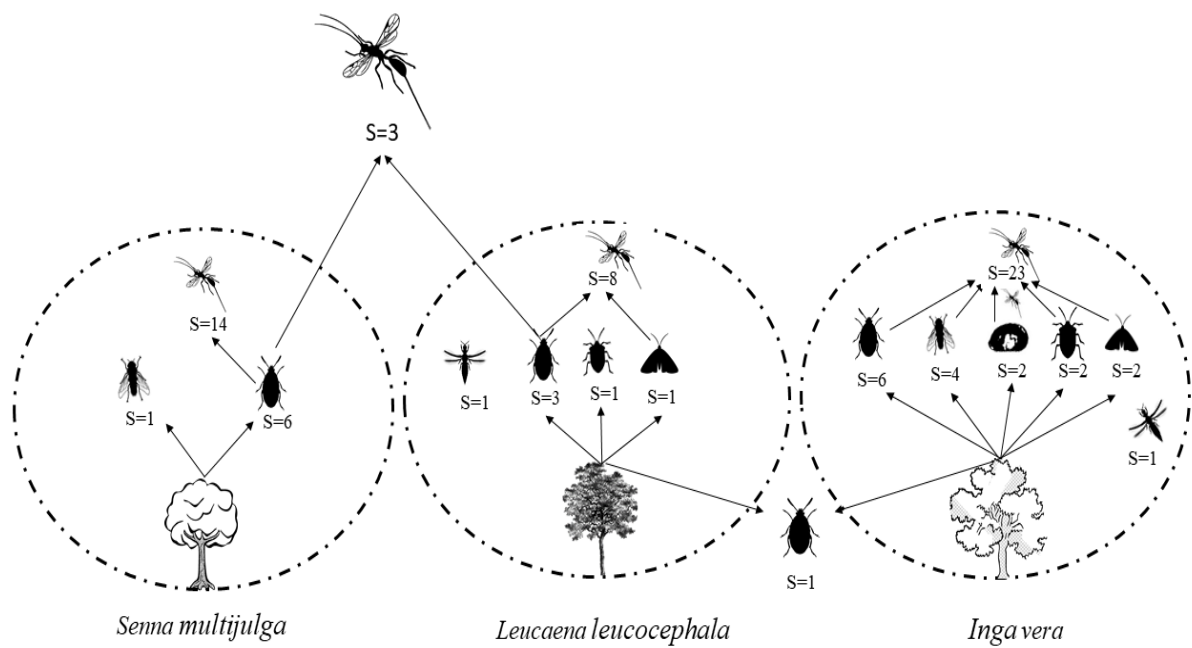
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## **SEGUNDA PARTE-ARTIGOS**

## ARTIGO 1

### The relationship between host plant traits and biodiversity across three sympatric tritrophic systems in a tropical region of Brazil

Normas do periódico Insect diversity and conservation (versão preliminar)



## **The relationship between host plant traits and biodiversity across three sympatric tritrophic systems in a tropical region of Brazil**

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### Abstract

We describe the diverse community of insect herbivores and parasitoids associated with the seeds and fruits of three sympatric Fabaceae species in Lavras, Minas Gerais, Brazil: native *Inga vera* and *Senna multijuga* and invasive the *Leucaena leucocephala*. A total of 5,353 individuals, representing 77 different insect species, were identified via morphology and DNA barcoding. The invasive *L. leucocephala* had the lowest diverse natural-enemy community  $N = 17$  species, while *Inga vera* and *S. multijuga* had  $N=40$  species and  $N= 24$  species in total respectively. Individuals from the insect order Hymenoptera, a group dominated by parasitoid wasps, was the most diverse order recovered from our samples in all three hosts. Additionally, individual herbivore and parasitoid species were more likely to be found on a single host (host plant and herbivore), with only four species found in more than one plant species, including the generalist agricultural pest herbivore *Lasioderma serricorne* found in both native species and three parasitoids connecting native *S. multijuga* and non-native *L. leucocephala* communities, hinting at recent host shifts. Furthermore, different host plant traits had complex effects on the herbivore and parasitoid communities, where total seed number per fruit promoted a cascading effect on the abundance and richness at both higher trophic levels, whereas seed and fruit weight did not. Finally, different taxa have different associations with resources, and the most abundant taxa – Coleoptera, Diptera, galling Hymenoptera, and Lepidoptera promoted a cascading effect on parasitoid abundance and richness. We highlight the importance of including parasitoids in insect community studies because they are highly diverse and provide important ecosystem services. More knowledge of biology, behavior, and parasitoid distributions would be advantageous to conservation and biological control proposals.

**Key words:** Pest reservoir, Conservation biology, DNA barcoding, Tri-trophic interactions.

## Introduction

Insects are responsible for the vast majority of global species richness and ecological processes, such as nutrient cycling, seed dispersal, pollination, and pest control, all of which are critical to the environment and human well-being (Mayhew, 2007; Mora *et al.*, 2011; Jankielsohn, 2018). Despite their importance, insects and arthropods in general are often overlooked in conservation efforts and studies – being omitted as early as in community assessment stages. This is due to the high taxonomic and ecological diversity and the resulting challenge of species identification in highly diverse systems, which makes even a basic understanding of insects distribution and interactions difficult (Haddad, 2009; Wheeler *et al.*, 2012; Eagalle & Smith, 2017).

The majority of global insect biodiversity is made up of complex tri-trophic food webs of parasitoids, their herbivorous insect hosts, and the plants on which the insect hosts feed (Godfray, 1994). In fact, it is estimated that the organisms directly involved in host-parasitoid interactions account for more than half of all known species (Price *et al.*, 1980; Polis & Strong, 1996). The insect order Hymenoptera alone, an order encompassing the bees, wasps, and ants, is arguably the most species-rich animal order on Earth because it includes the highly specialized parasitoid wasps found in almost all terrestrial ecosystems (Forbes *et al.*, 2018). Parasitoids are defined as insect predators that parasitize a single individual of their host species, eventually killing it in the process (Hawkins, 1994). While the biodiversity within these systems can be daunting, the understanding of host-parasitoid dynamics is crucial for a thorough understanding of ecosystem dynamics. Also, importantly, such systems are relevant for biological control, as parasitoid species can be used to combat agricultural pests (Mills & Wajnberg, 2008) and invasive species (Morrison, 2000). Unfortunately, despite their potential relevance, the true diversity of parasitoid species remains unknown (Memmott & Godfray, 1994).

In this aspect, fruit pods of neotropical Fabaceae plants is a good system to study host-parasitoid interactions, this because they shelter a diverse community of insects, primarily Coleoptera (specifically, Bruchines), Lepidoptera, Hymenoptera, and their parasitoids (Tuller *et al.*, 2015; Wood *et al.*, 2017; Morales-Silva *et al.*, 2018, 2019; Lopes, 2020). The resource quality of host plants (e.g., number and size of seeds and fruits) has been identified as a key factor in promoting bottom-up effect in these systems, influencing insect-parasitoid interactions, structure, and community complexity (Bukovinszky *et al.*, 2008a; Morales-Silva

*et al.*, 2018). Furthermore, host plant quality and quantity directly influence not only herbivore performance (e.g., fecundity, body size), and reproduction (Janzen, 1971; Stillwell *et al.*, 2007; Prince *et al.*, 2011; Oliveira *et al.*, 2020a), but also mediate indirect effects in food webs because these herbivores are consumed by others species in complex tri-trophic interactions (Reimchen, 1997; Schmitz, 2008). Thus, tri-trophic systems rooted with host plants in the Fabaceae provide an excellent system for understanding the richness, abundance, distribution, and interaction of herbivores and parasitoids with their resources. Another significant benefit of these systems is that seed consumers and their parasitoids are confined inside of the fruit, making their collection easier and reliable.

Thus, the general aims of this study are to: (1) characterize the community of insect herbivores and their parasitoids associated with three sympatric Fabaceae species: native species *Senna multijulga* and *Inga vera*, and the non-native *Leucaena leucocephala*; (2) evaluate the direct and indirect effects of the resource (i.e., fruits and seeds traits, specifically seed biomass, number of seed, and seed per pod) on the abundance and richness of herbivores and parasitoids; (3) estimate the direct effect of herbivores' abundance and richness on parasitoid communities; and lastly (4) evaluate the specialization of host-parasitoids and host plant-herbivores using both morphological and DNA barcoding data to evaluate connectivity of the food web.

First, we hypothesized that increased resources would have a positive effect on herbivores and parasitoids abundance and richness. Second, we expect different taxa to react differently to the resource availability. This second hypothesis is consistent with competitive coexistence theory, which predicts species' difference in responses to resource variation (Chesson, 2000). Furthermore, ecological specialization is expected since parasitoids and their hosts have an intimate relationship and a strong dependence on one another (Schär & Vorburger, 2013; Clarke *et al.*, 2019). Then, we expect both parasitoids and herbivores to be highly specific to their host with rare species shared among plant species (Memmott & Godfray, 1994; Novotny & Basset, 2005; Novotny *et al.*, 2010).

## **Material and Methods**

### **Study area**

The research was conducted in forest fragments on the campus of the Federal University of Lavras (UFLA) and its surroundings (21° 13' 42.79" S; 44° 59' 11.04" W) in



Lavras, Minas Gerais, Brazil. The predominant climate of the region is highland tropical, characterized by mild summers with precipitation and dry winters. The average annual temperature is 19.4°C and the annual rainfall is 1,529.5 millimeters (Sparovek *et al.*, 2007). We chose the below three sympatric Fabaceae plant species based on their abundance and viability in the study area, as well as for their native or non-native status.

### **Host Plants**

#### ***Leucaena leucocephala***

*Leucaena leucocephala* (Lam.) de Wit (Fabaceae) is a medium-sized, fast-growing tree that can reach up to seven meters in height and belongs to the subfamily Mimosoideae (Lorenzi *et al.*, 2003; Costa & Durigan, 2010). It is a native plant of southern Mexico and northern Central America and has been introduced throughout the tropics. Flowering occurs throughout the year, peaking in between September and November (Lorenzi *et al.*, 2003). The seed pods are thin, flat, with 15–25 seeds, and have a bright brown color when ripe. In our study site, the production of fruit occurs two to four times a year during the dry winter season (June, July and August, personal observation in the study area) (Alcântara & Bufarah, 1988; Raghu *et al.*, 2005). Due to its extremely fast growth, this has been a widely cultivated species for the reforestation of degraded areas, such as pastures and eroded landscapes, and as a protein source for animal feed in agro-silvo-pastoral systems because of its great nutritional value (Nakamanee *et al.*, 2019).

#### ***Senna multijuga***

*Senna multijuga* (Rich.) Irwin & Barneby (Fabaceae) is a tree or shrub that belongs to the subfamily Caesalpinoideae. It may grow as tall as 15 meters. Even though it is native to northern South America and our area of study, it has spread to tropical areas all over the world. Furthermore, due to its lengthy flowering cycle, it is frequently used as an ornamental plant in parks, sidewalks, and in mixed reforestation projects in degraded areas (Lemos-Filho *et al.*, 1997; Lorenzi, 2002). Flowering happens between January and May, while fruits ripen between March and December (Lorenzi, 2002). *Senna multijuga* is considered a pioneer plant that is initially secondary in the succession process or a light-dependent climax species (Carvalho, 2003) with a high dispersion capacity, long-lasting and viable seeds, rapid growth and initial development, and the capacity to germinate in multiple locations (Lorenzi, 2002).

#### ***Inga vera***

*Inga vera* Willd (Fabaceae) is a 5-10 m tall, medium-sized evergreen tree in the subfamily Caesalpinioideae. Its native range is throughout South and Central America, and it has significant economic potential for reforestation (Antunes *et al.*, 2019). The species is primarily found along riversides and its floating fruits are specialized for waterborne dispersion. In northern Brazil and throughout northern South America, the genus *Inga* is highly valued as a food, and some studies have indicated its pharmacological and antioxidant potential (Pompeu *et al.*, 2012). The flowers of *I. vera* bloom from August to September with the fruits maturing in December and January (Lorenzi, 2002).

### **Fruit sampling and resource measurements**

We collected fruits at different times according to sample viability and availability to describe the associated insect species linked with each plant system as well as the relationship between the resource size (fruits and seeds) and the insects (richness and abundance). We collected 450 fruits from each species *L. leucocephala* and *I. vera*, and 150 fruits from *Senna multijuga*. The number of individuals (trees collected within species) and collection data are available in Table SM1 in the Supplementary Material, but ranged from 150-450 individuals per species. We used pruning shears with long stems for the removal of fruits throughout the whole fructification time to make sure all insect community was accessed. Fruits were stored in packages in the field of collection, properly identified by plant and later moved to the laboratory. All fruits were collected while still attached to the mother plant. In the laboratory, we individually stored each fruit in labeled rearing chambers (see below). This allows the development of organisms contained in fruits after their emergence.

After insect emergence (about three months), we assessed both the morphometric measurements (width and length) and biomass of fruits and seeds. The morphometric measurements were taken using a digital caliper. Each fruit had its width measured in three places, with a measure in the region of each end and in the central region of the fruit (Figure SM1). Following, we obtained the average width of each fruit of the three species and multiplied it by its length to obtain the average area. To assess both fruit and seed biomass, we separated them into paper bags and dried them at 40°C for 48 h. Subsequently, fruits and seeds were weighed on the precision analytical balance to obtain their dry biomass. The fruits were individually measured, and to obtain the average seed biomass of each one of these fruits, we weighed all the seeds inside the same fruit together, then divided the value by the total seed number.

### **Rearing insects from host plants**

Rearing chambers, made of PVC tubes covered in veil fabric tissue, were regularly inspected for emerging insect herbivores and parasitoids. During fruit sorting, the adult individuals found outside the seed were classified as “emerged”, whereas those still found inside the seeds after the three months of sorting were removed and classified as “non-emerged”. All insects were stored in microtubes containing 98% ethanol and labeled according to the plant and fruit. We identified Hymenoptera using specific taxonomic keys for the Braconidae family (Wharton *et al.*, 1997; Hanson & Gauld, 2006) and the superfamily Chalcidoidea (Gibson *et al.*, 1997). The seed-consuming Bruchinae species were determined for their occurrence in this host plant, and also by reference to the literature (Johnson, 1990). All other herbivores families were sorted by morphospecies to their respective families (Rafael *et al.*, 2012). The voucher specimens were deposited in the Entomological Collection of the Laboratory of Ecology and Complexity at the Federal University of Lavras, Minas Gerais, Brazil, and in the National Collection of insects in the Instituto de Biología at the Universidad Nacional Autónoma de México, Mexico City, Mexico.

### **Mitochondrial DNA barcoding**

We molecularly characterized 75 specimens belonging to 66 of the 77 collected insect morphospecies with DNA barcoding (Hebert *et al.*, 2003) to complement morphological identification. First, we extracted DNA from the whole specimens using the EZ-10 spin column animal genomic DNA miniprep kit (Bio Basic) following the manufacturer’s protocol with the addition of a pestle crushing step before incubation, using ACL solution (Animal Cell Lysis Solution) and proteinase K. We amplified a length-variable fragment (277-780 bp) of the mitochondrial cytochrome oxidase I (COI) gene was amplified using the Apex Taq RED Master Mix 2X (Apex Bioresearch Products) and the primers reported in the Supplementary material (Table SM2). Reactions consisted of 2:00 at 95° of longer denaturation before cycling started, followed by 35 cycles of 00:45 at 94° C for initial denaturation, 00:15 at the primer’s specific annealing temperature (Table SM2), and 00:30 at 72° C for the extension, followed by a final hold at 4.0°. Amplicons were Sanger sequenced at the University of Arizona Genetics Core. Sequences were manually edited and aligned using the program MEGA 10 software (Kumar *et al.*, 2018), and species identification was carried out by Blasting sequences in NCBI’s GenBank database. We associated our generated sequences with those of described species when they had a genetic distance < 2%.

## Statistical analysis

We performed a rarefaction methodology based on the interpolation/extrapolation method and the Chao1 asymptotic richness species estimator to assess the efficacy of collection sampling and variation in cumulative species richness between plant species (*iNext* package; Hsieh *et al.*, 2016). To gauge the effectiveness of the collection for each of the functional groups, we separated the herbivores and parasitoids. The R package 'vegan' was used to evaluate the insect community composition using non-metric multidimensional scaling (NMDS; Oksanen *et al.*, 2013; R Core Team, 2013). Comparing species composition across plant species was done using the similarity analysis (ANOSIM) in 'vegan' (Oksanen *et al.*, 2022).

To assess the direct and indirect effects of the resource size on the insect community, we constructed two path analysis models. Path analysis consists of multiple regression that calculates causal connections between variables using 'lavaan' package in R (Rosseel, 2012). To answer our questions, we constructed two models: (1) We estimated the direct effects of resource size per fruit (number of seeds in a fruit, seed biomass per fruit, and fruit biomass) on the total abundance and richness of herbivores, as well as the direct effects of these variables on the total abundance and richness of parasitoids per fruit. We also estimated the indirect effect of resource size on the parasitoid abundance and richness. (2) In the second model, we repeated the direct and indirect effects of the first model; however, this time we separated the herbivores into specifically groups according to the insect taxonomic order to which they belong (e.g., Coleoptera, Diptera, Gall Hymenoptera, Parasitoids Hymenoptera, Lepidoptera, Hemiptera and Thysanoptera). However, we excluded Thysanoptera taxa since we only had two individuals collected. Multicollinearity among the explanatory variables were tested using the R package 'corrplot' (Wei & Simko, 2017). Both models presented a non-normal distribution, so we fit them with *bootstrap* bootstrapping method (Kim & Millsap, 2014). All statistical analyses were performed in R environment (Dormann *et al.*, 2009; Hudson *et al.*, 2013).

## Results

### Insect community description and sampling efficiency

A total of 5,353 individual insects, belonging to 77 species, were associated with the three Fabaceae hosts in this study (Figure 1A-H). The relative frequency and distribution of each individual species as well as their DNA sequences can be found in Table SM3 in the Supplementary Material. Overall, the herbivores species were distributed across six orders, 20

families and 29 species (Figure SM2-H). Among herbivores, the most abundant order was Coleoptera with 3,253 individuals followed by Diptera with 329 individuals, Lepidoptera with 225 individuals, gall-forming Hymenoptera with 167 individuals, Hemiptera with 10 individuals, and Thysanoptera with two individuals (Figure 1G). The most diverse taxonomic group were the parasitoids with 11 families, 48 species, and 1,367 individuals, (Figure 1G Figure SM2-H), followed by Coleoptera with 15 species, Diptera with five species, Lepidoptera and Hemiptera with three species, and both galling Hymenoptera and Thysanoptera with one species (Figure 1H).

Our effectiveness in sampling diversity for both *Leucaena leucocephala* and *Senna multijuga* associated herbivore species were well represented, since we observed six and seven species, which matched the number estimated using the Chao1 method (*L. leucocephala*: 6 herbivore species, confidence interval = 6 min 7 max; *S. multijuga*: 7 herbivore species, confidence interval = 7 min-8 max; effectiveness=100% for both host plant species). For *Inga vera*, we observed 17 species, which was 94% of the estimated number (18 herbivore species; confidence interval = 17min -39 max). With regards to the effectiveness of parasitoid sampling, we observed 10 species in the *Leucaena leucocephala* community, which matched the estimated number (confidence interval = 10 min-14 max species). However, the parasitoid species accumulation curves for both *S. multijuga* and *I. vera* showed that we undersampled these communities, documented by a strong tendency towards an increase in the number of parasitoid species as we increased our sampling effort (Figure 2B). We observed 17 and 24 parasitoid species, respectively, which was only 68% of the predicted *Senna multijuga* parasitoid community (25 parasitoids species; confidence interval = 18 min -69 max species) and 79% of the predicted *Inga vera* parasitoids community (29 parasitoids species; confidence interval = 24 min-52 max species; Figure 2B).

Only one species of herbivore, the crop pest and generalist *Lasioderma serricorne* (Fabricius, 1792), was found to feed on multiple species of host plants. Specifically, it was found on both native species (*I. vera* and *S. multijuga*). Three species of parasitoid Hymenoptera were found on more than one host plant (confirmed with DNA- *Eurytoma* sp.1, *Parachas pluteus* and *Stenocorse sudamericanus*). All three spanned the native *Senna multijuga* and the non-native *Leucaena leucocephala* only. No species of herbivore or parasitoid was found across all three host plants.

Accordingly, given the minor overlap in species, the community ordering analysis (NMDS) showed that there were significant differences in the species composition of herbivores and parasitoids across the three plant species (Figure 3). Additionally, plant species had a significant impact on the community organization, according to similarity analysis (ANOSIM) ( $R = 0.99$  (herbivore),  $R=0.91$  (parasitoids),  $p = 0.001$ ).

### **Direct and indirect effects of resource on herbivores and parasitoids.**

Host plant trait resources were the average of seed biomass and number of seeds per pod, and fruit biomass. Significant direct and indirect effects between the host plant resources, herbivores, and parasitoids were shown by the path analysis. We did not find multicollinearity among the explanatory variables, so we keep all of them in both models (Figure SM3). The total number of seeds per fruit significantly increased the abundance of herbivores ( $p<0.001$ ,  $z=6.008$ , Table SM4 in supplementary material) and indirectly increased the richness of parasitoids ( $p<0.001$ ,  $z=4.378$ , Table SM4). Fruit biomass had a direct negative effect on the richness and abundance of herbivores ( $p<0.001$ ,  $z=-7.343$ ;  $p<0.001$ , and  $z=-8.740$ , Table SM4), as well as an indirect negative impact on the parasitoids abundance and richness ( $p=0.039$ ,  $z=-2.062$ ;  $p<0.000$ ,  $z=-5.922$ , Table SM4). The parasitoids abundance and richness were positively correlated with the herbivores abundance and richness ( $p=0.010$ ,  $z=2.584$ ;  $p=0.041$ ,  $z=2.045$ ;  $p=0.011$ ,  $z=2.531$ ;  $p<0.001$ ,  $z=7.028$ , Table SM4). We found no significant direct or indirect effects of seed biomass on parasitoids or herbivores ( $p>0.05$ , Figure 4).

### **Direct and indirect effects of the resource on different insect taxons.**

The second path analysis, which sought to determine how each specific order was influenced by its plant host resources, showed strong direct and indirect relationships among the resources, insect groups, and parasitoids. Specifically, seed biomass had a negative impact on the richness and abundance of the Coleoptera ( $p<0.001$ ,  $z=-4.010$ ;  $p=0.003$ ,  $z=-2.933$ , Table SM5 in supplementary material); and the abundance of the gall-forming Hymenoptera ( $p<0.001$ ,  $z=-3.310$ , Table SM5). The total number of seeds per fruit was strongly correlated with the richness and abundance of Coleoptera ( $p<0.001$ ,  $z=5.561$ ;  $p<0.001$ ,  $z=7.544$ , Table SM5), Lepidoptera abundance ( $p=0.004$ ,  $z=-2.906$ , Table SM5), Hymenoptera gall ( $p<0.001$ ,  $z=-4.569$ , Table SM5), and Diptera richness were negatively influenced by it ( $p=0.003$ ,  $z=-2.999$ , Table SM5). Fruit biomass had a negative direct impact on the richness and abundance of Coleoptera ( $p<0.001$ ,  $z=-5.341$ ,  $p<0.001$ ,  $z=-6.831$ , Table SM5), on the Diptera ( $p=0.002$ ,  $z=-3.175$ , Table SM5), Gall Hymenoptera ( $p<0.001$ ,  $z=-4.003$ , Table SM5), and Lepidoptera

abundance ( $p=0.013$ ,  $z=-2.496$ , Table SM5), as well as indirect negative effects on parasitoid richness and abundance ( $p<0.001$ ,  $z=-6.093$ ;  $p=0.001$ ,  $z=-3.296$ , Figure 5, Table SM5).

## Discussion

Here, we described for the first time the species of herbivores and parasitoids associated with the seeds and fruits of three sympatric Fabaceae trees: the invasive *Leucaena leucocephala*, and natives *Senna multijulga*, and *Inga vera*. Across all communities, parasitoid Hymenoptera were the most species-rich group, whereas the most abundant insect order was Coleoptera. Additionally, we found that insects are highly specialized to their hosts (plant or herbivores) in these three systems, with only one herbivore and three parasitoids (out of 77 total species; 5.2%) found in more than one plant, with none found in all trees. The sampling effort was sufficient to estimate all herbivore species, but rarefaction curves suggested that further sampling is required for a complete assessment of parasitoid species associated with *Senna multijuga* and *Inga vera*. Furthermore, we established the relationship between resources and insects by using path analysis. We discovered that the number of seeds per fruit was more important for both the abundance and richness of herbivores and parasitoids than the seed and fruit weight, which had a negative or non-relationship with the insects, consistent with our first hypothesis. Lastly, we found evidence consistent with our second hypothesis where different taxons responded differently to the resource availability.

### Insect community and sampling effectiveness

It has been long claimed that beetles (Kirby & Spence, 1818) are the most species-rich order on the Earth (Oberprieler *et al.*, 2007; Zhang *et al.*, 2018). This popular claim is corroborated by the fact that the group has the largest number of formally described species (>350,000; Farrell, 1998). However, Forbes *et al.* (2018) recently suggested that Hymenoptera is the most species-rich group instead, estimated to contain 2.5-3.2 more species than Coleoptera. They generated this estimate based on the relationship between insect hosts and parasitoids of well-known systems in North America. Here, we confirm their prediction in a tropical region since the number of parasitoids was 3 times greater than Coleoptera and 1.6 times greater than all herbivore species associated with the three sympatric plant species. The main reasons why Hymenoptera parasitoids have been overlooked in the past are their tiny size, the scarcity of taxonomic resources, and the uncertainty regarding their host ranges, which are often unknown (Gaston, 1993; Shaw & Hochberg, 2001; Forbes *et al.*, 2018).

Furthermore, we found that herbivores and parasitoid species are mostly host-specific in the studied systems. However, there were four biologically interesting exceptions. One species of herbivore, the beetle *Lasioderma serricorne*, was found on both native species analyzed, *I. vera* and *S. multijuga*. Popularly known as cigarette beetle, it is an economically important pest in both agriculture and on stored food products, attacking dozens of species (Hagstrum et al., 2013; Edde, 2019). Our finding suggests that wild Fabaceae plants may act as wild reservoirs for the species. While it is a pest of historically difficult control due to resistance and difficult detectability (e.g. Rajendran & Narasinha, 1994; Edde, 2019), this previously overlooked observation may be a factor in its resilience, and we encourage further studies on this.

Additionally, three parasitoid species were found on both native *S. multijuga* and the invasive *L. leucocephala* (confirmed with DNA barcoding: *Eurytoma* sp.1, *Paracrias pluteus* and *Stenocorse sudamericanus*; Table SM3). Given the more recent introduction of *L. leucocephala*, this overlap hints at either recent host shifts by these parasitoid species, or that they are very broad generalists likely be found in other hosts in the area.

Nonetheless, in general, our findings support a trend of high specialization of herbivores in the tropics (Novotny & Basset, 2005; Novotny *et al.*, 2010) and extend this observation to the parasitoids in this system. Indeed, insects such as the Bruchidae and other seed-eaters, which account for nearly all herbivores in our system, appear to be host-specific rather than generalists (Basset, 1992; Novotny & Basset, 2005; Novotny *et al.*, 2010). Another study that confirmed it evaluated the specialization of insect herbivore networks in five other species of Fabaceae plants in Brazil (Lopes, 2020). They found a highly specialized network between herbivores and their host plants, with only six species shared among plants (Lopes, 2020). In terms of adaptation, it makes sense that some groups of insects are restricted to a few genera or families because of phylogenetically-conserved plant defenses (Basset, 1992; Novotny & Basset, 2005). Nevertheless, we still lack enough evidence to demonstrate that these insects are specialized, as we have not exhaustively sampled all sympatric hosts available. More studies are necessary to solve the specialist versus generalist question, especially over time, space, and host plants of other families and genera. We especially encourage future studies to deposit genetic information on the species in their system when possible, as that greatly facilitates the cross-system comparison of species and identification of possible generalists by DNA sequence similarity.



The NMDS revealed that the community of herbivores and parasitoids differs between plants, which may be related to herbivore host-specificity (Novotny & Basset, 2005) and host constraint to the resource (Janzen, 1969). It is worth mentioning that parasitoid communities obtained from the same plant species in our study differ throughout individual host trees, highlighting the need to collect from multiple sources and places (Reigada *et al.*, 2014). Wood *et al.*, (2017) evaluated the geographic distribution of parasitoids associated with *Acanthoscelides macrophthalmus* feeding on *L. leucocephala* in a large-scale geographical region and discovered more similarities across communities that were geographically close to each other than those that were far apart. We agree with them, but we also noticed that parasitoids play an essential role in biodiversity, even on a smaller local scale. Indeed, parasitoids are underrepresented in insect conservation and are frequently neglected (Shaw & Hochberg, 2001; Ward *et al.*, 2012).

Finally, rarefaction curves revealed that collecting more fruit pods would result in greater parasitoid diversity for both *I. vera* and *S. multijulga*. However, our sampling effort was enough to characterize the herbivore community. Most invertebrate surveys, even those utilizing high sampling efforts over extended periods, fail to achieve an asymptote in species accumulation due to their great diversity and number of rare taxa (Saunders & Ward, 2018). Optimal sampling remains lacking for most groups of terrestrial invertebrates, especially parasitoids (Saunders & Ward, 2018). As a result, we recommend a larger plant pod sample over a long period to access the entire insect community (mainly natural enemies) associated with fruits and seeds.

### **Direct and indirect effects of resource on herbivore and parasitoid community**

The impact of plant traits on the host-parasitoid systems has long been recognized (Ode, 2006; Bukovinszky *et al.*, 2008b). From a food web perspective, plant traits have a direct impact on both insect morphology and size (Silva *et al.*, 2017; Oliveira *et al.*, 2020a, 2020b; Sousa-Lopes *et al.*, 2020), behavior, and life history (Underwood & Rausher, 2000; Ode, 2006). According to Bukovinszky *et al.* (2008b), up to three trophic levels can be affected directly and indirectly by the quantity and quality of the resource.

Here, we found that the second and third trophic levels, represented by herbivores (seed and fruit eaters) and their parasitoids, were influenced by the seed abundance inside the fruit pods. We found that the seed quantity had a direct positive effect on herbivore abundance and an indirect one on parasitoid abundance. Our study corroborates the hypothesis of resource

abundance, which suggests that plants with more resources can support more abundance and diversity of insect herbivore species. As a result, the abundance of a plant part (i.e., seeds) can influence the insect community that relies on it (Janzen, 1969; Hunter *et al.*, 1992). Our research suggests a *bottom-up* effect in these systems across multiple trophic levels, as more seeds support more herbivores, which benefits the community of parasitoids, increasing their abundance and richness.

We did not find any significant relationship between the seed weight and the herbivores and parasitoids abundance and richness. However, previous studies have shown that for some taxa, such as seed beetles (Chrysomelidae: Bruchinae), larger and heavier seeds are related to the number of eggs laid by the female (Lopes, 2020; Pedroso *et al.*, 2021). In this circumstance, the authors argue that larger and heavier seeds are essential for these individuals' offspring success since they are seed concealed, assuming that larger seeds have more nutrients (Modena *et al.*, 2012). Additionally, the evolutionary selection pressure that some Fabaceae plant species have been undergoing in the past century from seed-feeding insect predation, which has caused them to concentrate more energy on producing more seeds instead of on seed weight and defense (Janzen, 1969). Therefore, the lack of the predicted relationship between insect herbivores and seed weight can be a consequence of this evolutionary selection, where the number of seeds seems to be more crucial to those insects than its size.

According to our results, fruit biomass had a negative direct and indirect impact on insect communities. Another study observed that fruit pods with insect gall formers in *Parkinsonia praecox* (Fabaceae) were approximately 3.5 cm shorter than healthy fruits (Cocoletzi *et al.*, 2019). In that study, galling insects alter the natural development of *P. praecox* fruits by changing the production of phytohormones such as Abscisic acid (ABA). Here, we found this pattern for both insect herbivores and parasitoids. A possible explanation could be a change in plant phytohormones or an allocation of energy to healthy fruits. Since we did not measure these aspects here, we suggest more studies to close this gap in our knowledge. However, another plausible explanation is that the predation of fruits by frugivorous insects could give the false impression that smaller fruits are associated with more insects.

Despite what we found, research has revealed a positive correlation between fruit size and insect richness, abundance, and food web complexity (Hunter *et al.*, 1992; Bukovinszky *et al.*, 2008a; Morales-Silva *et al.*, 2018). Additionally, fruit size affects how plants interact with their consumers because it is essential to the plant's dispersal and, consequently, its fitness. It

is because the plant's high nutritional content attracts many herbivorous species (Hunter *et al.*, 1992; Cvrtecka *et al.*, 2016). It is clear that a *bottom-up* effect has been observed here and it is a strong force shaping the insect communities associated with legumes. Further, we recommend using seed numbers instead of seed and fruit weight since insects' consumption may bias the results.

### **Direct and indirect effects of the resource on different insect taxons.**

We found that different insect taxa exhibit distinct relationships with resource size and quantity. For instance, the abundance and diversity of Coleoptera and the abundance of galls were negatively correlated with seed biomass. The number of seeds inside a plant pod was positively associated with Coleoptera abundance and richness and negatively associated with Gall abundance and Lepidoptera and Diptera richness. Fruit biomass had a negative impact on Coleoptera and Diptera abundance and richness, as well as gall-forming Hymenoptera and Lepidoptera abundance.

The negative relationship between seed biomass and gall abundance was most likely due to the biology of the gall former and seed consumption. We found up to seven *Allorhogas* sp. (only gall-forming species) feeding in the same fruit. This species forms isolated expansions, allowing multiple individuals to explore and develop within the same seed (Macêdo & Monteiro, 1989; Tuller *et al.*, 2015). As a result, seeds with higher *Allorhogas* spp. abundance are expected to be smaller, as reported before (Tuller *et al.*, 2015; Oliveira *et al.*, 2020a, 2020b).

Coleoptera was dominated by the Bruchinae subfamily, which had 2,607 individuals, representing 80% of Coleoptera abundance. Bruchidae species have been identified as the most specialized insects for pre-dispersal seed predation (e.g., attacking seeds while still attached to the fruit) (Ramírez & Traveset, 2010). Despite extensive research, evidence regarding the relationship between bruchines and resource size remains mixed. Studies have previously reported positive (larger resource fruit and seed size support more bruchines) (Marcelo & Leonor, 2009; Maia *et al.*, 2017; Morales-Silva *et al.*, 2018), neutral (no relationship), and negative (smaller resource size supports more bruchinae) relationship between bruchinae and resource size (Janzen, 1969; Greig, 1993; Modena *et al.*, 2012; Cvrtecka *et al.*, 2016), suggesting their resource choice strategy might be species or system-specific.

The negative relationship between Coleoptera and seed biomass could be caused due to a plant fitness strategy in which plants expend most of their energy producing more seeds

instead of chemical defenses and seed size. (Janzen, 1969). This strategy allows plants to have more chances to survive and produce more individuals. While smaller seeds may contain fewer nutrients, Bruchinae would have an advantage in consuming them because they would expend less energy overcoming the reduced host seed defenses. We also observed that the seed number inside a pod was more relevant than its biomass for Bruchinae abundance. We recommend that future research measures chemical compounds and their relationships with insect preference and relative seed size to improve our understanding of these results.

We discovered a negative relationship between the number of seeds in a pod and the abundance and richness of Lepidoptera and Diptera. Species that share the same resources (or have similar niches) must develop coexistence strategies to survive. One of these mechanisms is resource partitioning, which can take place in space and time (Albrecht & Gotelli, 2001; Houadria *et al.*, 2015) or in specialization in a range of resources by their quality and quantity (Chesson, 2000; Cuny *et al.*, 2017, 2019). The dominant herbivores in the systems were Coleopterans, and seed biomass was positively correlated with their abundance. The selection of gall-forming Hymenoptera, Lepidoptera, and Diptera fruits with fewer seeds could be driven by resource partitioning and the avoidance of potential competition with coleoptera. Other evidence that suggests it may be happening is that we did not see a Coleopteran sharing fruit with Diptera, and only rarely with a Gall (four fruits). We also observed a few fruits shared between Lepidoptera, Coleoptera, and gall. Our findings suggest that by selecting larger seeds, Coleoptera may have reduced the intensity of interspecies infestation with other taxa that explore the same resource.

Surprisingly, fruit biomass had a negative association with the most abundant taxa, specifically the abundance of Coleoptera, Diptera, Gall, and Lepidoptera, and Coleoptera richness. This could be caused by the higher energy allocation to healthy fruits or the extensive fruit and seed biomass consumption by frugivorous insects. Additionally, Coleoptera abundance and Diptera abundance were the main factors responsible for parasitoid abundance; whereas Coleoptera richness, Diptera, and gall abundance were associated with the parasitoids richness (Hunter *et al.*, 1992; Marques *et al.*, 2000). We expected this could happen because more individuals of herbivores would attract greater natural enemy abundance and diversity. In addition, there are numerous parasitoids associated with these taxa in previous food web studies (Tuller *et al.*, 2015; Morales-Silva *et al.*, 2018, 2019; Lopes, 2020; Oliveira *et al.*, 2020b).

## Conclusion

We conclude that parasitoids (Hymenoptera) are the most abundant taxonomic group in these tritrophic systems associated with the host plant family Fabaceae in our study region and, by far, the most important contributor to insect biodiversity. Because we only found one herbivore and three parasitoid species on more than one plant species, our results suggest that the host plant and host herbivore are highly specialized. Nonetheless, we found some evidence of a host shift from a native host toward the invasive *L. leucocephala*. Furthermore, different resource traits can display different outputs in the abundance and richness of the insects with which they are associated. As a result, we emphasize the importance of measuring multiple resource traits in order to understand the role of resource size in community features. Finally, different taxa have different associations with resources, and the most abundant taxa, Coleoptera, Diptera, Gall, and Lepidoptera, promoted a positive cascading effect on parasitoid abundance and richness. Here, we emphasize the importance of tri-trophic approach in research on insect communities since knowledge of host-parasite associations may help with the employment of parasitoids for biological control and conservation. Further studies should focus on how specific or generalists parasitoids are, and its role in connecting food webs.

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Tables and Figures

Figure 1

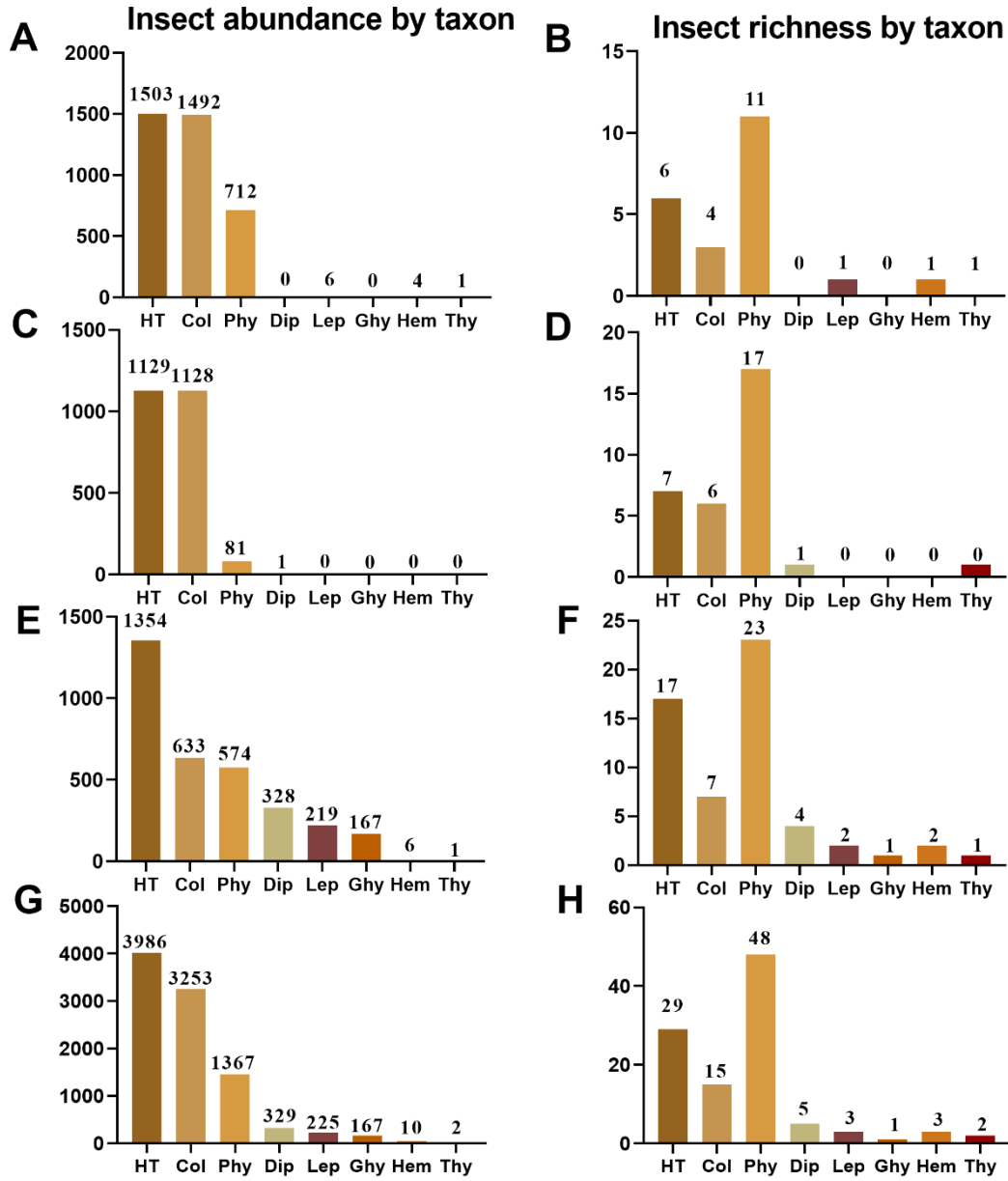


Figure 2

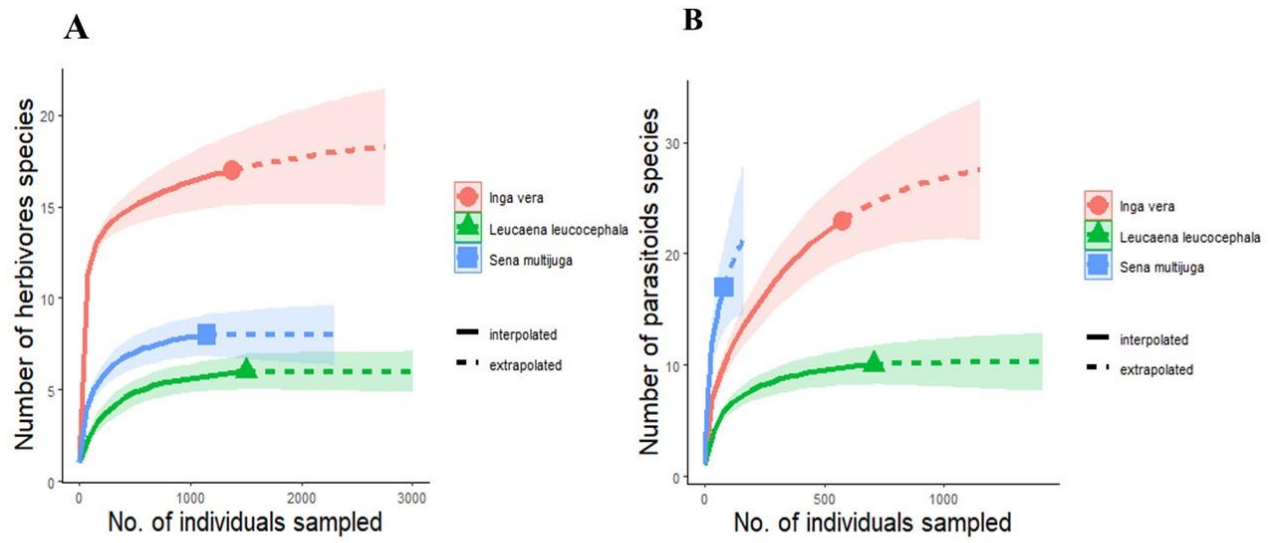


Figure 3

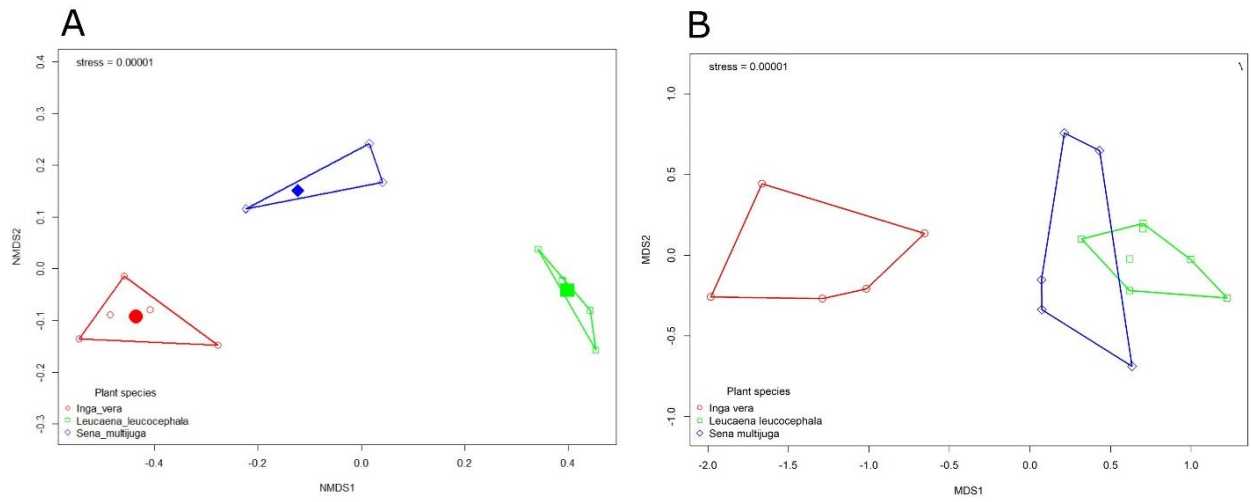


Figure 4

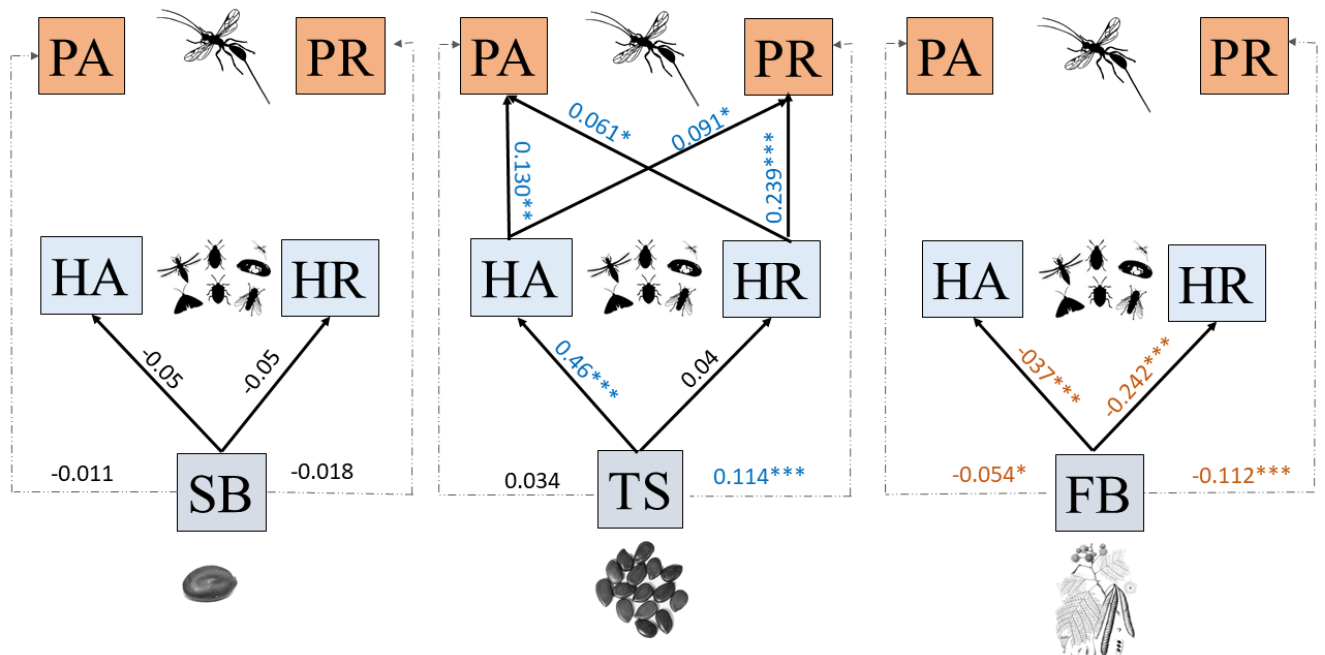
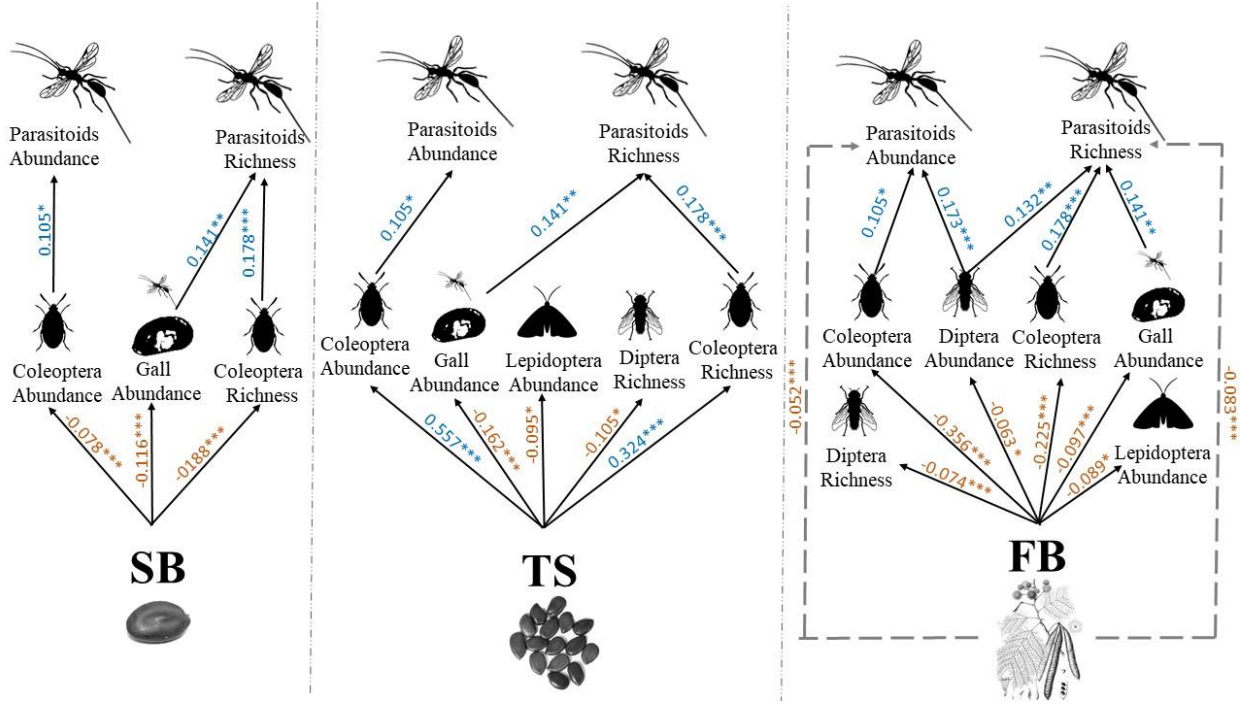


Figure 5



**Figure 1: Distribution of the insect abundance and richness by taxon.** [A-B] *Leucaena leucocephala*; [C-D] *Senna multijuga*; [E-F] *Inga vera* and [G-H] all plants together. [HT: herbivores total, Col: Coleoptera, Phy: Parasitoid hymenoptera, Dip: Diptera, Lep: Lepdoptera, Ghy: Gall hymenoptera, Hem: Himenoptera and Thy: Thysanoptera]

**Figure 2. Species accumulation curves based on specimen sampling for the insect community in plants of the family Fabaceae.** The curves were constructed using the rarefaction method based on interpolation and extrapolation and using the Chao1 species richness estimator and a 95% confidence interval for herbivores [A] and parasitoids [B]. The different colors and symbols represent different plant species. [Pink/circle = *Inga vera*; green/triangle = *Leucaena leucocephala*; blue/square = *Senna multijuga*.]

**Figure 3:** Non-metric multidimensional scaling (NMDS) of the Fabaceae associated insect's communities separated by [A] herbivore and [B] parasitoid communities. The color purple represents insects associated to *Inga vera*; burgundy red represents *Leucaena leucocephala*, and orange *Senna multijuga*.

**Figure 4:** Path analysis with the direct or indirect effect of resources, herbivores, and parasitoids. The relationship between one variable and another is indicated by the black arrows. Significantly negative impact values are shown in orange color, significant positive impact values are shown in blue with an asterisk, and non-significant values by the color black. [ $p < 0.05^*$ ,  $p < 0.01^{**}$ ,  $p < 0.001^{***}$ . SB= Seed biomass, TS= Total number of seed in the pod fruit, FB= Fruit biomass, HA= herbivore abundance, HR herbivore richness, PA= Parasitoid abundance, PR= Parasitoid richness]

**Figure 5:** Path analysis with the direct or indirect effect of resources, herbivores groups, and parasitoids. The relationship between one variable and another is indicated by the black arrows. Significantly positive impact values are shown by the color blue, and significant negative impacts values are shown both by the colour orange and the minus sign. [ $p < 0.05^*$ ,  $p < 0.01^{**}$ ,  $p < 0.001^{***}$ . SB= Seed biomass, TS= Total number of seed in the pod fruit, FB= Fruit biomass]



## Supplementary Material A

**Table SM1:** Distribution and gathering of fruits, and seeds at the Federal University of Lavras in Lavras, Minas Gerais, Brazil, according to the phenology and viability of the fruits of each plant species.

Plant host	Individuals	Sampling dates	Total fruits collected	Total number of seeds	Average seeds per fruit
<i>Leucaena leucocephala</i>	A3, A30,A58, A62	Jun, Jul, Aug 2019	450	5,311	24
<i>Senna multijuga</i>	S1,S2,S3,S4,S 5	Aug 2020	150	3,086	26
<i>Inga vera</i>	I1,I2,I3,I4,I5	Dec 2019, Jan 2020, Dec 2020, Jan 2021	450	1,960	7

**Table SM2:** Primers used to sequence insect's community DNA in this study with the respective annealing temperature.

Primer names	Code	Primer sequences	Annealing temperature	References
<b>Hco 1490</b> <b>Lco 2198</b>	A	5' GGT CAA ATC ATA AAG ATA TTG G 3' 5' TAA ACT TCA GGG TGA AAA AAT CA 3'	52°C	[166]
<b>Lep F</b> <b>Lep R</b>	C	5' ATT CAA CCA ATC ATA AAG ATA TTG G 3' 5' TAA ACT TCT GGA TGT CCA AAA AAT CA 3'	50°C	[167]
<b>Coi pf 2</b> <b>Coi 2437d</b>	D	5' ACC WGT AAT RAT AGG DGG DTT TGG DAA 3' 5' GCT ART CTA AAW AYT TTA ATW CCW G 3'	48°C	[168,169]
<b>Omr 2</b> <b>Coi 2437d</b>	E Coi2435	5' TRG GDG CTC CDG ATA TRG CW 3' 5' GCT ART CTA AAW AYT TTA ATW CCW G 3'	45°C	[169]
<b>TyI460</b> <b>CiN2191</b>	F	5'TAC AAT TTA TCG CCT AAA CTT CAG CC 3' 5' CCC GGT AAA ATT AAA ATA TAA ACT TC 3'	46°C	[168]
<b>Acant F</b> <b>Acant R</b>	H	5'AGA TGT AGA CAC CCG AGC TT 3' 5'ATG ATA GCG AAT ACG GCC CC 3'	50°C	<b>This study</b>

Fonte: Do autor (2022).

**Table SM3:** List of species, guilds, absolute frequency and DNA barcoding code collected at University Federal of Lavras associated with *Leucaena leucocephala*, *Senna multijuga* and *Inga vera*.

Host Plant	Taxonomic affiliation	Guild	Absolute frequency	DNA Barcoding-primer
<i>Leucaena leucocephala</i>	Silvanidae sp.1 (Coleoptera)	Seed consumer	17	-
<i>Leucaena leucocephala</i>	<i>Acanthoscelides macrophthalmus</i> (Coleoptera: Chrysomelidae)	Seed consumer	1471	LL89-H
<i>Leucaena leucocephala</i>	Phalacridae sp. (Coleoptera)	Seed consumer	4	LL59-C
<i>Leucaena leucocephala</i>	Anthocoridae sp. (Hemiptera)	Sucker- predator	4	LL92-E
<i>Leucaena leucocephala</i>	Gelechiidae sp. (Lepidoptera)	Seed consumer	6	LL66-A
<i>Leucaena leucocephala</i>	Phaeothripidae sp. (Thysanoptera)	Fungi consumer	1	-
<i>Leucaena leucocephala</i>	<i>Chelonus</i> sp. (Hymenoptera: Braconidae)	Parasitoid	2	LL142-A
<i>Leucaena leucocephala</i>	<i>Eurytoma</i> sp.1 (Hymenoptera: Eurytomidae)	Parasitoid	4	LL65-D
<i>Senna multijulga</i>			4	SM78-D
<i>Leucaena leucocephala</i>	<i>Heterospilus</i> sp.1 (Hymenoptera: Braconidae)	Parasitoid	8	LL113-D
<i>Leucaena leucocephala</i>	<i>Paracrias pluteus</i> Pikart, 2011 (Hymenoptera: Eulophidae)	Parasitoid	627	LL57-D
<i>Senna multijulga</i>			1	LL108-D
<i>Leucaena leucocephala</i>	<i>Perilampus</i> sp. (Hymenoptera: Perilampidae)	Parasitoid	6	LL58-E

<i>Leucaena leucocephala</i>	<i>Eupelmus (eupelmus) pulchripes</i> sp. (Hymenoptera: Eupelmidae)	Parasitoid	2	-
<i>Leucaena leucocephala</i>	<i>Horismenus abdomicaulis</i> Pikart, Costa & Hansson, 2015 (Hymenoptera: Eulophidae)	Parasitoid	19	-
<i>Leucaena leucocephala</i>	<i>Horismenus</i> sp.1 (Hymenoptera: Eulophidae)	Parasitoid	2	-
<i>Leucaena leucocephala</i>	<i>Urosigalphus</i> sp. (Hymenoptera: Braconidae)	Parasitoid	14	LL177-A
<i>Leucaena leucocephala</i>	<i>Phanerotoma</i> sp. (Hymenoptera: Braconidae)	Parasitoid	1	-
<i>Leucaena leucocephala</i>	<i>Stenocorse sudamericanus</i> González-Joya, Sarmiento & Zaldívar-Riverón, 2019 (Hymenoptera: Braconidae)	Parasitoid	27	LL201-A
<i>Senna multijulga</i>			1	SM109-A
<i>Senna multijulga</i>	<i>Brasema</i> sp. (Hymenoptera: Eupelmidae)	Parasitoid	2	SM100-A
<i>Senna multijulga</i>	<i>Sennius</i> sp.1 (Coleoptera: Chysomelidae)	Seed consumer	1048	SM166-A
<i>Senna multijulga</i>	<i>Sennius</i> sp. 2 (Coleoptera: Chysomelidae)	Seed consumer	3	-
<i>Senna multijulga</i>	<i>Cecidomyiidae</i> sp.1 (Diptera)	Gall former	1	-
<i>Senna multijulga</i>	<i>Eurydinoteloides</i> sp.1 (Hymenoptera : Pteromalidae)	Parasitoid	1	SM98-E
<i>Senna multijulga</i>	<i>Eurydinoteloides</i> sp.2 (Hymenoptera : Pteromalidae)	Parasitoid	1	SM104-E
<i>Senna multijulga</i>	<i>Eurytoma</i> sp. 2	Parasitoid	5	SM77-D

	(Hymenoptera: Eurytomidae)			
<i>Senna multijulga</i>	<i>Eurytoma</i> sp. 3	Parasitoid	5	SM119-A
	(Hymenoptera: Eurytomidae)			
<i>Senna multijulga</i>	<i>Heterospilus</i> sp. 2	Parasitoid	5	SM113-A
	(Hymenoptera: Braconidae)			
<i>Senna multijulga</i>	<i>Heterospilus</i> sp. 3	Parasitoid	6	SM121-B
	(Hymenoptera: Braconidae)			
<i>Senna multijulga</i>	<i>Horismenus</i> sp. 2	Parasitoid	26	SM82-A
	(Hymenoptera: Eulophidae)			
<i>Senna multijulga</i>	<i>Horismenus</i> sp. 3	Parasitoid	9	SM83-A
	(Hymenoptera: Eulophidae)			
<i>Senna multijulga</i>	<i>Lasioderma serricorne</i>	Seed	13	SM84-F
<i>Inga vera</i>	(Fabricius, 1792)	consumer	38	
	(Coleoptera: Ptinidae)			
<i>Senna multijulga</i>	<i>Lyrcus</i> sp.	Parasitoid	1	SM136-E
	(Hymenoptera: Pteromalidae)			
<i>Senna multijulga</i>	<i>Phyloxeroxenus</i> sp.1	Parasitoid	7	SM97-E
	(Hymenoptera: Eurytomidae)			
<i>Senna multijulga</i>	<i>Sennius nappi</i>	Seed	9	Extrain DNA
	Ribeiro-Costa & Reynaud, 1998	consumer		
	(Coleoptera: Chysomelidae)			
<i>Senna multijulga</i>	<i>Sennius puncticollis</i>	Seed	47	SM85-F
	(Fåhraeus, 1839)	consumer		
	(Coleoptera: Chysomelidae)			
<i>Senna multijulga</i>	<i>Sennius lojaensis</i>	Seed	7	-
	(Pic, 1933)	consumer		
	(Coleoptera: Chysomelidae)			
<i>Senna multijulga</i>	<i>Sycophila</i> sp.1	Parasitoid	1	SM118-B
	(Hymenoptera: Eurytomidae)			
<i>Senna multijulga</i>	<i>Aprostocetus (aprostocetus)</i> sp.	Parasitoid	2	-

	(Hymenoptera: Eulophidae)			
<i>Senna multijulga</i>	<i>Eupelmus (Eupelmus) sp.1</i>	Parasitoid	5	-
	(Hymenoptera: Eupelmidae)			
<i>Inga vera</i>	<i>Allorhogas sp.</i>	Seed consumer/gall	167	IV187-A
	(Hymenoptera: Braconidae)			
<i>Inga vera</i>	<i>Anastrepha distincta</i>	Fruit consumer	25	IV19-A
	Greene, 1934 (Diptera: Tephritidae)			
<i>Inga vera</i>	<i>Apanteles sp.</i>	Parasitoid	16	IV2-A
	(Hymenoptera: Braconidae)			
<i>Inga vera</i>	<i>Apion sp.</i>	Seed consumer/ Fruit gall	2	IV39-A
	(Coleoptera: Brentidae)			
<i>Inga vera</i>	<i>Silvanidae sp. 2</i>	Seed consumer	1	-
	(Coleoptera)			
<i>Inga vera</i>	<i>Araecerus fasciculatus</i>	Seed consumer	11	IV12-A
	(De Geer, 1775) (Coleoptera: Anthribidae)			
<i>Inga vera</i>	<i>Hypothenemus seriatus</i>	Seed consumer	11	IV15-C
	Wood & Bright, 1992 (Coleoptera: Curculionidae)			
<i>Inga vera</i>	<i>Brachyplatycerus sp.</i>	Parasitoid	2	IV28-D
	(Hymenoptera: Encyrtidae)			
<i>Inga vera</i>	<i>Cecidomyiidae sp.</i>	Gall fruit former	199	IV52-A
	(Diptera)			
<i>Inga vera</i>	<i>Chelonus (chelonus) sp.</i>	Parasitoid	2	IV189-H
	(Hymenoptera: Braconidae)			
<i>Inga vera</i>	<i>Chelonus (microchelonus) sp.</i>	Parasitoid	1	IV49-C
	(Hymenoptera: Braconidae)			
<i>Inga vera</i>	<i>Eucolinae sp.</i>	Parasitoid	288	IV10-A

	(Hymenoptera: Figitidae)			
<i>Inga vera</i>	<i>Drosophila</i> sp.	Fruit	60	IV17-A
	(Diptera: Drosophilidae)	consumer		
<i>Inga vera</i>	<i>Eupelmus (eupelmus)</i> sp. 2	Parasitoid	40	IV4-A
	(Hymenoptera: Eupelmidae)			
<i>Inga vera</i>	<i>Eupelmus (eupelmus)</i> sp. 3	Parasitoid	112	IV6-A
	(Hymenoptera: Eupelmidae)			
<i>Inga vera</i>	<i>Eupelmus (eupelmus)</i> sp. 4	Parasitoid	1	-
	(Hymenoptera: Eupelmidae)			
<i>Inga vera</i>	<i>Gonatocerus</i> sp.1	Parasitoid	1	IV156-B
	(Hymenoptera: Mymaridae)			
<i>Inga vera</i>	<i>Horismenus</i> sp. 4	Parasitoid	1	IV29-D
	(Hymenoptera: Eulophidae)			
<i>Inga vera</i>	<i>Ichneumonidae</i> sp.1	Parasitoid	1	IV158-B
	(Hymenoptera)			
<i>Inga vera</i>	<i>Ichneumonidae</i> sp. 2	Parasitoid	1	IV31-A
	(Hymenoptera)			
<i>Inga vera</i>	Totricidae sp.	Seed	111	IV22-A
	(Lepidoptera)	consumer		
<i>Inga vera</i>	Cosmopterigidae sp.	Seed	108	IV47-A
	(Lepidoptera)	consumer		
<i>Inga vera</i>	<i>Neosilba</i> sp.	Fruit	44	IV18-A
	(Diptera: Lonchaeidae)	consumer		
<i>Inga vera</i>	Membracidae sp.	Fruit	38	IV20-A
	(Hemiptera)	sucker		
<i>Inga vera</i>	Antocoridae sp.	Sucker-predator	3	IV21-C
	(Hemiptera)			
<i>Inga vera</i>	Nitidulidae sp.	Seed	21	IV43-A
	(Coleoptera)	consumer		

<i>Inga vera</i>	<i>Ooencyrtus</i> sp. Encyrtidae	Parasitoid	1	IV13-A
<i>Inga vera</i>	<i>Phlaeothripidae</i> sp. (Thysanoptera)	Fungi consumer	1	IV23C
<i>Inga vera</i>	<i>Phyloxeroxenus</i> sp. 2 (Hymenoptera: Eurytomidae)	Parasitoid	67	IV35A
<i>Inga vera</i>	<i>Phyloxeroxenus</i> sp. 3 (Hymenoptera: Eurytomidae)	Parasitoid	2	IV53E
<i>Inga vera</i>	<i>Phyloxeroxenus</i> sp. 4 (Hymenoptera: Eurytomidae)	Parasitoid	2	IV50E
<i>Inga vera</i>	<i>Phyloxeroxenus</i> sp. 5 (Hymenoptera: Eurytomidae)	Parasitoid	3	IV152A
<i>Inga vera</i>	<i>Prodecatoma</i> sp. (Hymenoptera: Eurytomidae)	Parasitoid	10	IV7D
<i>Inga vera</i>	<i>Pteromalidae</i> sp. 2 Hymenoptera	Parasitoid	2	IV37A
<i>Inga vera</i>	<i>Sycophila</i> sp. 2 (Hymenoptera: Eurytomidae)	Parasitoid	3	IV159B
<i>Inga vera</i>	<i>Sycophila</i> sp. 3 (Hymenoptera: Eurytomidae)	Parasitoid	15	IV160B
<i>Inga vera</i>	<i>Pteromalidae</i> sp. 1 (Hymenoptera)	Parasitoid	1	-
<i>Inga vera</i>	<i>Tricomidae</i> sp. (Hymenoptera)	Parasitoid	3	-
<i>Inga vera</i>	<i>Conotrachelus</i> sp. (Coleoptera: Curculionidae)	Seed consumer	549	IV193A

Fonte: Do autor (2022)

**Table SM4:** Path analysis demonstrating the association between resource size, insect herbivores, and parasitoids' abundance and richness.

<b>Dependet variable</b>	<b>Independent variable</b>	<b>Estimate</b>	<b>Std. Error</b>	<b>z-value</b>	<b>P(&gt; z )</b>	<b>Std. All</b>
Herbivore abundance	Seed Biomass	-1.951	1.655	-1.178	0.239	-0.052
	Total Seeds per fruits	0.293	0.049	6.008	0.000	0.460
	Biomass fruit	-0.002	0.000	-8.740	0.000	-0.376
Herbivore richness	Seed Biomass	-0.305	0.336	-0.906	0.365	-0.058
	Total Seeds per fruits	0.004	0.925	0.925	0.355	0.042
	Biomass fruit	-0.000	0.000	-7.343	0.000	-0.242
Parasitoid abundance	Herbivore richness	0.175	0.085	2.045	0.041	0.061
	Herbivore abundance	0.053	0.020	2.584	0.010	0.130
	Seed Biomass	-0.357	0.384	-0.930	0.352	-0.011
	Total Seeds per fruits	0.051	0.028	1.845	0.065	0.034
	Biomass fruit	-0.000	0.000	-2.062	0.039	-0.054
Parasitoid richness	Herbivore richness	0.164	0.023	7.028	0.000	0.239
	Herbivore abundance	0.009	0.003	2.531	0.011	0.091
	Seed Biomass	-0.323	0.287	-1.128	0.259	-0.018
	Total Seeds per fruits	0.048	0.011	4.378	0.000	0.114
	Biomass fruit	-0.000	0.000	-5.922	0.000	-0.112

**Fonte: Do autor (2022)**

**Table SM5:** Path analysis demonstrating the association between resource size, insect herbivores separated by taxon, and parasitoids' abundance and richness.

<b>Dependet variable</b>	<b>Independent variable</b>	<b>Estimate</b>	<b>Std. Error</b>	<b>z-value</b>	<b>P(&gt; z )</b>	<b>Std. All</b>
Coleoptera Abundance	Seed Biomass	-2.678	0.913	-2.933	0.003	-0.078
	Total seed per fruit	0.327	0.043	7.544	0.000	0.557
	Fruit Biomass	-0.002	0.000	-6.831	0.000	-0.356
Coleoptera Richness	Seed Biomass	-0.631	0.157	-4.010	0.000	-0.188
	Total seed per fruit	0.019	0.003	5.561	0.000	0.324
	Fruit Biomass	-0.000	0.000	-5.341	-0.000	-0.225
Diptera Abundance	Seed Biomass	1.173	0.689	1.703	0.089	0.108
	Total seed per fruit	-0.007	0.005	-1.493	-0.007	-0.040
	Fruit Biomass	-0.000	0.000	-2.745	0.006	-0.063
Diptera Richness	Seed Biomass	0.188	0.094	2.006	0.045	0.103
	Total seed per fruit	-0.003	0.001	-2.999	0.003	0.105
	Fruit Biomass	-0.000	0.000	-3.175	0.002	-0.074
Lepidoptera Abundance	Seed Biomass	0.089	0.244	0.366	0.715	0.015
	Total seed per fruit	-0.009	0.003	-2.906	0.004	-0.095
	Fruit Biomass	-0.000	0.000	-2.496	0.013	-0.028
Hemiptera Abundance	Seed Biomass	0.105	0.105	1.000	0.317	0.035
	Total seed per fruit	-0.002	0.001	-1.800	0.072	-0.032
	Fruit Biomass	-0.000	0.000	-1.119	0.263	-0.028
Gall Hymenoptera Abundance	Seed Biomass	-0.640	0.193	-3.310	0.001	-0.116
	Total seed per fruit	-0.015	0.003	-4.569	0.000	-0.162



	Fruit Biomass	-0.000	0.000	-4.003	0.000	-0.097
Parasitoid Abundance	Coleoptera Abundance	0.046	0.022	2.072	0.038	0.105
	Coleoptera Richness	0.116	0.136	0.847	0.397	0.026
	Diptera Abundance	0.192	0.037	5.204	0.000	0.137
	Diptera Richness	-0.021	0.209	-0.102	0.919	-0.003
	Lepidoptera Abundance	-0.027	0.055	-0.493	0.622	-0.010
	Hemiptera Abundance	-0.093	0.105	-0.885	0.376	-0.018
	Gall Hymenoptera Abundance	0.179	0.132	1.356	0.175	0.065
Parasitoid Richness	Coleoptera Abundance	0.004	0.003	1.289	0.197	0.040
	Coleoptera Richness	0.192	0.035	5.479	0.000	0.0178
	Diptera Abundance	0.044	0.017	2.553	0.011	0.132
	Diptera Richness	0.080	0.059	1.358	0.175	0.040
	Lepidoptera Abundance	0.026	0.028	0.935	0.350	0.042
	Hemiptera Abundance	-0.016	0.027	-0.605	0.545	-0.013
	Gall Hymenoptera Abundance	0.093	0.029	3.168	0.002	0.141
Parasitoids Abundance	Seed Biomass	-0.102	0.233	-0.439	0.661	-0.007
	Total seed per fruit	0.014	0.007	1.879	0.060	0.052
	Fruit Biomass	-0.000	0.000	-3.296	0.001	-0.056
Parasitoid Richness	Seed Biomass	-0.124	0.080	-1.561	0.118	-0.034
	Total seed per fruit	0.003	0.001	1.999	0.046	0.044
	Fruit Biomass	-0.000	0.000	-6.093	0.000	-0.083

Fonte: Do autor (2022)

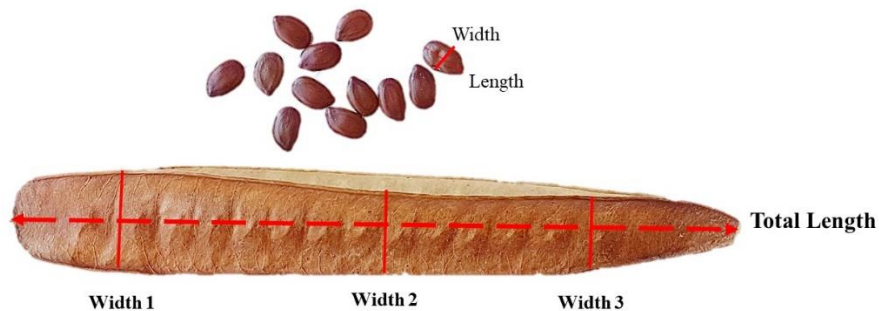


Figure SM1: Measurements made to determine the pods' fruit and seed areas.

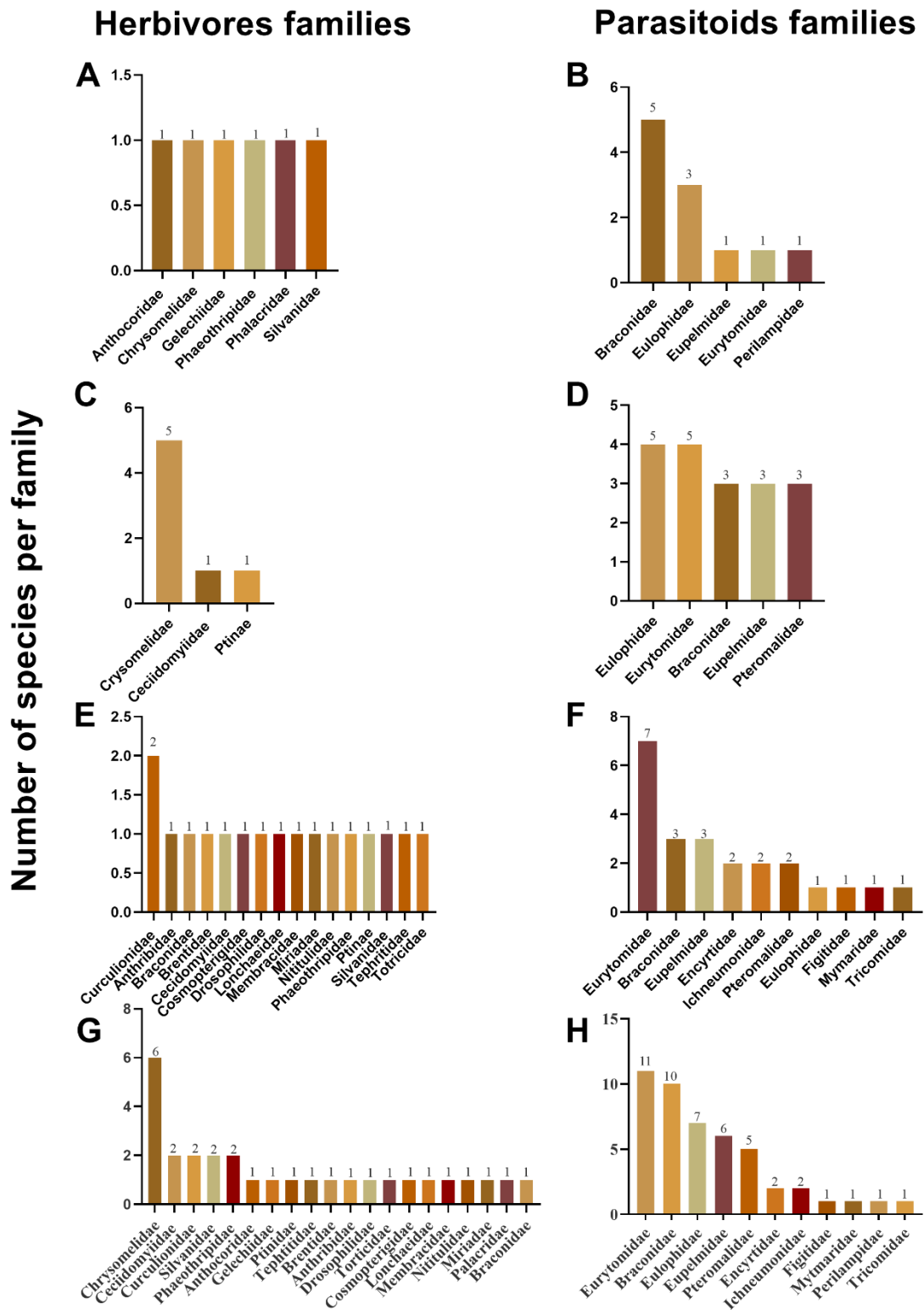


Figure SM2: Insects families and number of species collected at University Federal of Lavras associated with [A-B] *Leucaena leucocephala*, [C-D] *Senna multijuga* and [E-F] *Inga vera*, and [G-H] all together. On the left side are the herbivores families and on the right parasitoids.

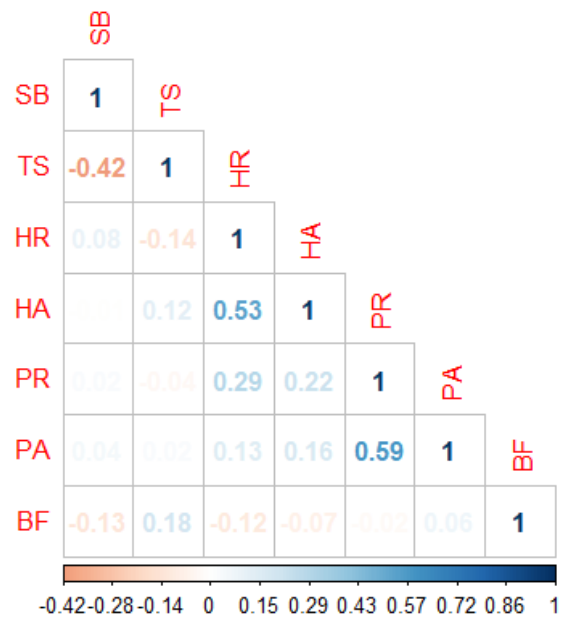
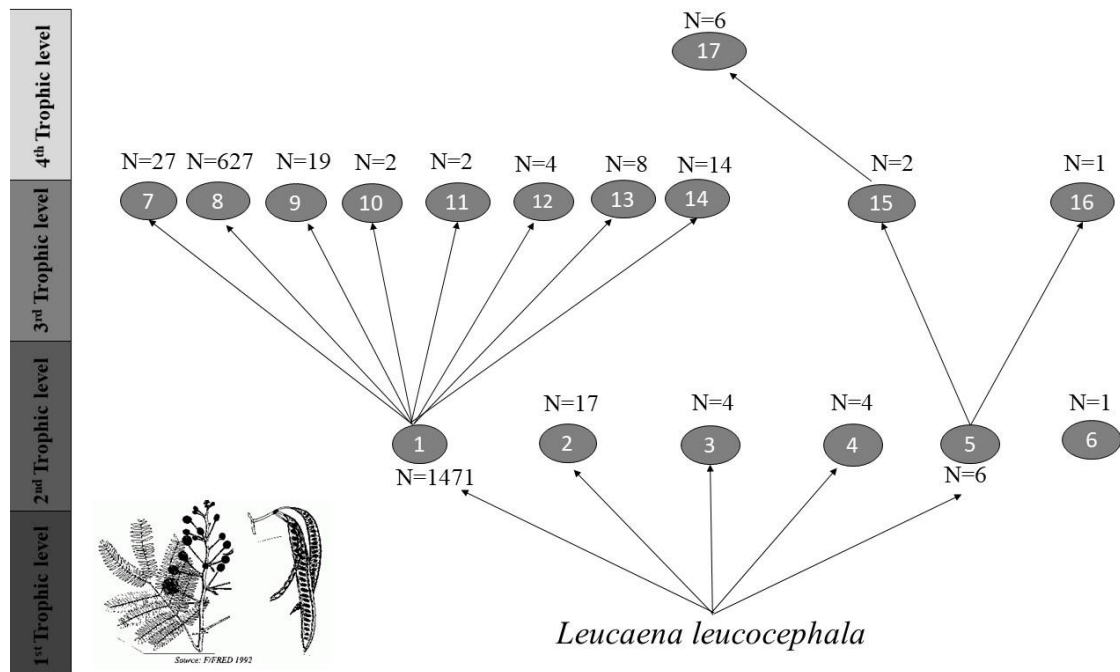


Figure SM3: Matrix with the results of a multicollinearity test using the corrplot R package. Values less than 0.85 indicate low multicollinearity among the explanatory variables. SB: seed biomass, TS: total seed per fruit, HR: herbivore richness, HA: herbivore abundance, PR: parasitoids richness, PA: parasitoid abundance, BF: fruit biomass.

## ARTIGO 2

### Description of the herbivore and natural enemy community associated with the seeds of the invasive *Leucaena leucocephala* (Lam.) de Wit in Lavras, Minas Gerais, Brazil.

Normas do periódico Ecological Entomology (versão preliminar)



**Description of the herbivore and natural enemy community associated with the seeds of the invasive *Leucaena leucocephala* (Lam.) de Wit in Lavras, Minas Gerais, Brazil.**

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**Abstract**

1. We describe the food web of insects associated with fruits and seeds of *Leucaena leucocephala* (Fabaceae) and their relationship with seed biomass. A total of 450 fruits and 7114 seeds of *L. leucocephala* were sampled across seven trees in Lavras, Minas Gerais, Brazil.

2. We found seventeen species of insects distributed across three trophic levels. The main herbivore species sampled was *Acanthoscelides macrophthalmus* (Coleoptera, Chrysomelidae, Bruchinae), which was responsible for most of the seed predation (89.78% of predated seeds) and associated with the highest parasitoid biodiversity (N= 10). Other herbivorous species (N=4) were lower in abundance. This includes a previously unreported Lepidopteran species, responsible for 9.32 % of the seed predation, with two parasitoids and one hyperparasitoid associated with it. Overall, we observed a parasitism percentage of 40.62%, including 41.28% for *A. macrophthalmus*, and 33% for the Lepidopteran species.

3. Fruits containing heavier seeds promoted an increase in insect abundance, insect richness, and the number of links and connectivity. We observed two native parasitoids species, *Paracrias pluteus*, and *Stenocorse suldamericanos*, shifting from herbivores on native plants to the invasive *L. leucocephala*, consistent with a host shift. However, more investigation is required to ascertain the effects of recent shifts of native insect communities (on native plants) to non-native ones and their consequences for plant fitness (e.g., seed germination).

**Key words:** Seed predation, Alien species, Connectivity, Network, Tritrophic interactions.

## Introduction

Human activity in natural habitats has had a significant impact on the dynamics and structure of communities (Foley *et al.*, 2005). In recent years, one of the primary contributors to biodiversity loss and ecosystem degradation has been the introduction of new species (McNeely *et al.*, 2001). Exotic species harm native species mainly by increasing competition for resources (e.g., soil, nutrients, and space) (Gioria & Osborne, 2014) and/or facilitating the invasion of other plants (Keiichiro & Shuichi, 2004). The success and establishment of exotic plants in a new environment depends on the quality and quantity of interactions in which those species can develop with the native community (e.g., with herbivores, competitors, and parasitoids) (Sanabria-Silva & Amarillo-Suárez, 2017) and the disturbance regime of an area (e.g. housing development, agriculture, and proximity to roads) (Xiao *et al.*, 2016; Ariori *et al.*, 2017; Nievas *et al.*, 2019). Therefore, the establishment of a species in a new habitat can alter the functioning of ecological processes, such as community composition (e.g., changing richness and abundance of native species), species interactions (Aragón *et al.*, 2014), and host-parasitoid dynamics (Roland & Taylor, 1997).

Food web studies, which primarily focus on trophic interactions, are suitable for describing community structure, understanding the role of interactions, and determining the impact of these interactions on natural communities (Paine, 1992). An efficient community evaluation should include qualitative (e.g., species identification and interaction) and quantitative (e.g., abundance, richness and food webs metrics) attributes of the community at all trophic levels (Lewis *et al.*, 2002). However, exploring the entire food web is difficult, especially in tropical ecosystems, so an alternative strategy is to concentrate on community subgroups such as plant-herbivore-parasitoids interactions (Lewis *et al.*, 2002; Huxham *et al.*, 2017).

In this aspect, the Fabaceae family has shown that fruits and seeds are interesting systems to study the relationship between herbivores and parasitoids and their interaction with their associated host plants (Tuller *et al.*, 2015; Wood *et al.*, 2017; Morales-Silva *et al.*, 2018, 2019b; Lopes, 2020; Camacho-Eraza *et al.*, 2021). These systems have the advantage of being very rich in species and relatively easy to sample (Tuller *et al.*, 2015). In addition to describing biodiversity, certain plant characteristics or traits can influence species interactions across trophic levels in a food web (Bukovinszky *et al.*, 2008). Indeed, in these systems, interactions can be regulated by resource traits, in which larger fruits and seeds may offer more resources

and support a larger number of species and interactions, highlighting the importance of adding resource traits in interaction studies (Marcelo & Leonor, 2009; Tuller *et al.*, 2015; Morales-Silva *et al.*, 2018).

We chose *Leucaena leucocephala* (Lam.) de Wit (Fabaceae, Mimosoideae) to describe interactions among resources and its associated insects due to its wide distribution as well as its economic importance and biological invasiveness (Sharma *et al.*, 2022). *Leucaena leucocephala* is a plant that is referred to as a "controversial tree" because it provides several services, including food for livestock, firewood, and coal and cellulose production (Lorenzi *et al.*, 2003; Sharma *et al.*, 2022). However, the species has established itself as an invasive in several places across the globe, harming the local communities (Keiichiro & Shuichi, 2004; Olckers, 2011; Machado *et al.*, 2020; Sharma *et al.*, 2022).

Given this problem, an attempt to control *L. leucocephala* spread was made with the introduction of the biological control agent in Africa and Australia, *Acantoscelides macrophthalmus* (Schaeffer) (Chrysomelidae, Bruchinae), the primary consumer of *L. leucocephala* seeds (Olckers, 2004; Raghu *et al.*, 2005; Tuda *et al.*, 2009). The hatching larvae burrow into a seed, and they are unable to move between seeds during their immature stages. Therefore, depending on the seed size, they can support the development of one or two individuals (English & Olckers, 2014). Indeed, resource quality (size of seeds and pods) is known to influence egg-laying in seed beetles. In particular, larger seeds and seed pods provide more resources and thus are expected to be more infested by their eggs (Mitchell, 1975; Cope & Fox, 2003a; English & Olckers, 2014). However, this oviposition behaviour can be variable depending on the insect population and the local habitat (English & Olckers, 2014; Camacho-Erazo *et al.*, 2021).

Thus, the objective of this work was to describe qualitatively and quantitatively multitrophic interactions and predation characteristics of seed-feeding insects associated to the plant *L. leucocephala* in a location where it is invasive. Additionally, we sought to understand the links between resource traits, the direct and indirect interactions of herbivores and parasitoid insects accumulated on this non-native species, and food web functioning (connectance, interaction richness, and density of links). Specifically, we tested whether: (1) heavier seeds increase the abundance and richness of herbivores and their parasitoids, increasing the food web's complexity (number of links and species); (2) seeds consumed by herbivores and with



parasitoids are heavier than other categories; and (3) *A. macrophthalmus* females will choose larger resources to lay their eggs.

## **Material and Methods**

### **Study area**

The study was carried out in forest fragments on the Federal University of Lavras (UFLA) in Lavras, Minas Gerais state, Brazil (21°13'42.79"S, 44°59'11.04"W). The highland tropical climate that predominates in the area is marked by two defined stations: hot summers with precipitation and dry winters. The average annual temperature is 19.4 °C, and rainfall average of 1,529mm yearly (Sparovek *et al.*, 2007).

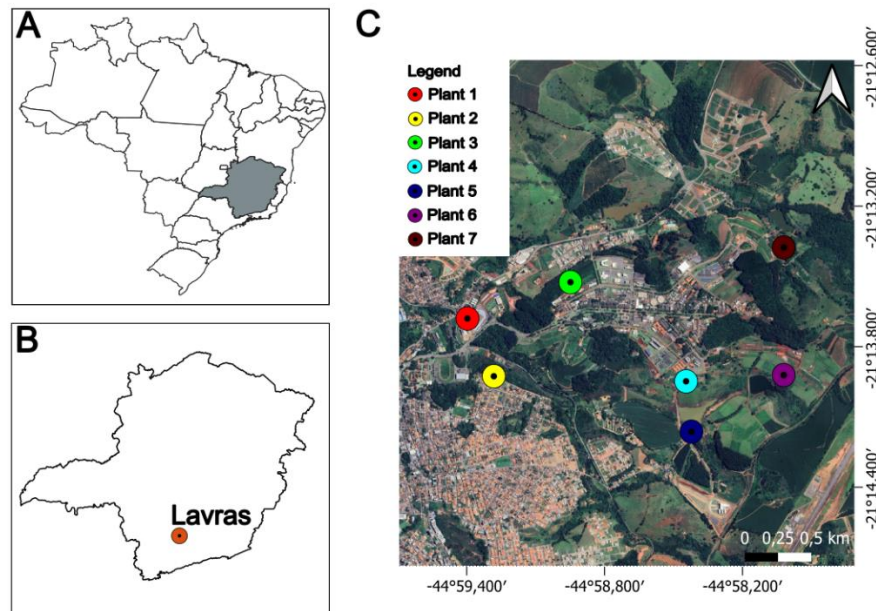
### **Host Plant**

*Leucaena leucocephala* is a leguminous species of the Fabaceae family, a medium-sized fast-growing tree (Fabaceae) (sub-family: Mimosoideae) that can reach up to seven meters (Lorenzi *et al.*, 2003; Costa & Durigan, 2010). It is originally native to Southern Mexico and Northern Central America and has now naturalized throughout the tropics (Batisteli *et al.*, 2020). Flowering occurs throughout the year, primarily between September and November (Lorenzi *et al.*, 2003). The pods ripen 10–15 weeks after seeding and produce around 15–25 seeds. They are flat, and when fully mature they are bright brown (Alcântara & Bufarah, 1988; Lorenzi *et al.*, 2003). Fruit production occurs typically two to four times a year during the dry winter season, but can be variable according to the region environmental conditions (Alcântara & Bufarah, 1988; Raghu *et al.*, 2005).

*Leucaena leucocephala* is a widespread species in the tropics and it is considered an invasive species in many regions across at least 77 countries and five continents (Sharma *et al.*, 2022). The widespread use of this species, particularly in agroforestry, livestock (as a source of animal protein), and restoration of damaged landscapes and pastures, has led to its dissemination throughout the world (Lorenzi *et al.*, 2003; Costa & Durigan, 2010). Indeed, this plant is one of the top five species of invasive terrestrial plants and is currently ranked among the 100 worst invasive species in the world (Lowe, 2007; Turbelin *et al.*, 2017). In Brazil, the first official report of *L. leucocephala* was made for the São Paulo state in November 1940 (Vilela and Pedreira 1976). However, new evidence suggests that it was introduced in Brazil in 1831 or earlier, and it is now found throughout Brazil (Machado *et al.*, 2020).

### **Fruit sampling, seed categories and resource measurements**

From June to August 2019, a total of 360 mature fruits were collected from four individuals of *L. leucocephala* to establish the relationship between the resource size of fruits and seed and the insect species richness and abundance. Additionally, to describe the insect species associated with this system, we collected 90 extras fruits from the other three individuals of *L. leucocephala*, totaling 450 fruits from seven individual trees (Figure 1). We used pruning shears with long stems for the removal of fruits. Fruits were stored in packages in the field and properly identified by plant and later moved to the Laboratory of Ecology and Complexity of UFLA. All fruits were collected while still attached to the mother plant and closed. In the laboratory, we individually stored each fruit in labeled PVC tubes covered with *voile* fabric tissue to enable air circulation. This allows the development of organisms contained in fruits after their collection.



**Figure 1:** A map with the location where plants and fruits of *Leucaena leucocephala* were collected at the campus of the Federal University of Lavras, Minas Gerais State, Brazil. [A] Minas Gerais state (gray) location within Brazil, [B] Lavras municipality location, and [C] *L. leucocephala* tree individual locations where pods were collected.

After insect emergence (about three months), we assessed both the fruit and seed morphometric measurements (width and length) using a digital caliper and its dry biomass. Each fruit had its width measured in three places, with a measure in the region of each end and in the central region of the fruit (Figure SM1). We examined seeds in a stereo microscope, and we separated them into the general categories as follows: non-predated, predated, predated with

parasitoids, and with parasitoids, and into the specific categories: predated by Coleoptera (pco), predated by Lepidoptera (ple), predated by Coleoptera and Lepidoptera (pco/ple), seed with Coleoptera parasitoid (parco), seed with Lepidoptera parasitoid (parle), seed with both Coleoptera and Coleoptera parasitoid (pco/parco), seed with both Lepidoptera and Lepdoptera parasitoid (ple/parle) and non-predated (np). We found a single seed with multiple predations (pco/parco/ple), but due to its rarity (1 sample), we opted to exclude it from the analysis. For these categories of classification, we observed insect feeding patterns and examined the cephalic capsules of herbivore larval instars left inside the respective fruit.

After that, we obtained the average width of each fruit of *L. leucocephala* and multiplied it by its length to obtain the average area. To access both fruit and seed biomass, we separated them into paper bags and dried them at 40°C for 48 h. Subsequently, they were weighed on the precision analytical balance in order to obtain their dry biomass. The fruits were individually measured, and to obtain the average biomass of each seed category in each fruit, we grouped the seeds of the same category and fruit and measured them together, then divided by the total number of seeds per fruit.

### **Obtaining and handling of insects**

PVC tubes were regularly inspected for the handling and preserving of emerging insect herbivores and parasitoids. During fruit sorting, the adult individuals found outside the seed were classified as “emerged” and those still found inside the seeds were removed and classified as “non-emerged”. All insects were stored in microtubes containing ethanol (98%) and labeled according to the plant and fruit collected.

To know which insect the emerging parasitoids develop within, we examined all the seeds from which parasitoids had emerged, inspecting the cephalic capsule, oral apparatus, and remnants of the host to determine the interaction. To complement these links, we also based interactions on previous literature descriptions (Morales-Silva *et al.*, 2019b). Lastly, we counted the eggs of the Bruchinae species *Acanthoscelides macrophthalmus* (Schaeffer) located under the outer surface of the fruit using a stereo microscope. For the identification of Hymenoptera, we used specific references: for the Braconidae family - Wharton *et al.*, (1997); Hanson & Gauld,( 2006), for Chalcidoidea (Gibson *et al.*, 1997). The seed-consuming Bruchinae species were determined for their occurrence in this host plant and also by reference to the literature (Johnson, 1990). All other herbivore families were sorted by morphospecies (Rafael *et al.*, 2012). The voucher specimens were deposited in the Entomological Collection of the

Laboratory of Ecology and Complexity at the Federal University of Lavras, Minas Gerais, Brazil.

### Statistical analysis

We constructed a species accumulation curve with the rarefaction method and first-order Jackknife estimator based on 1,000 randomizations to evaluate the sampling efficiency of the fruit pod in the accumulated species richness using the R package ‘*vegan*’ (Oksanen *et al.*, 2022; Team, 2022).

To correlate food web characteristics (response variables: number of links, abundance of insects, and number of insect species per fruits) with resource traits (average seed biomass per fruit, fruit biomass, and total number of seeds per pod), we first chose the best structure for random factors using the ‘*bbmle*’ package and second-order Akaike information criteria (Zuur *et al.*, 2009; Oliveira *et al.*, 2020; Ben Bolker and R Development Core Team, 2021). To do so, we used the R package ‘*gamlss*’ to compare four models with the same response and explanatory variables we wanted to test with generalized additive models, changing only the random factors (Rigby & Stasinopoulos, 2005). The GAMLSS framework (Generalized additive models for location, scale, and shape) has the benefit of being able to fit a wide range of distributions to the response to the characteristics of the food web (number of links, abundance of insects, and number of insect species) with resource traits (seed and fruit biomass and total number of seeds per pod), we first selected the best structure for random factors using the package ‘*bbmle*’ and Akaike information criteria (Zuur *et al.*, 2009; Oliveira *et al.*, 2020; Ben Bolker and R Development Core Team, 2021).

The first (I) model assumed a linear relationship between the explanatory variable and each response variable (without a random factor). The remaining three models assumed mixed effects with various random variables: (II) the sampling date, (III) the plant (individuals), and (IV) both the sampling date (intercept) and the plant (individuals)(slope). The restricted maximum likelihood method was used to estimate these models (Zuur *et al.*, 2009). The best model was chosen using the Akaike information criterion corrected for small samples (AICc), where the lowest value depicts the best model, and Akaike weight ( $w$ ), which estimates the likelihood of each model being chosen as the best model if the process is repeated several times (Burnham *et al.*, 2011). The *fitDist* function was used to find the best model distribution based on the response variable. As a result, we employed the ‘*count*’ function to investigate the

relationship between food web parameters and plant resources (Rigby & Stasinopoulos, 2005). We used the worm plots proposed by Buuren & Fredriks (2001) for model diagnostics.

To estimate the oviposition behavior of *Acanthoscelides macrophthalmus* on the *L. leucocephala* fruit, we considered the number of eggs as a response variable and the seed biomass as an explanatory variable using the negative binomial error distribution and the R package ‘*glmmTMB*’ (Brooks, Mollie *et al.*, 2017). We only considered fruits with eggs in this analysis. Similar to the previous analysis, we first selected the best structure of random factors using the package ‘*bbmle*’ comparing the same four models of random structures as before (I, II, III, IV) (Zuur *et al.*, 2009). This time we used the ‘*lme4*’ package and constructed GLM (general linear model) and GLMM (generalized linear mixed model) models (Bates *et al.*, 2008). The best model was selected through the second-order Akaike information criterion corrected for small samples (AICc) (Burnham *et al.*, 2011).

We created two distinct models to compare seed biomass amongst seed categories for predation and parasitism. The first one was with general seed categories (seeds predated, non-predated, seeds with parasitoids, and seeds predated and with parasitoids), and the second one was with the specific parasitism and predation categories. First, we selected the best model structure to evaluate which random effect was the best fit for each model structure (I, II, III, IV) (Zuur *et al.*, 2009). We used the *gamma* error distribution for both approaches. We found differences among predation categories, so we performed a *post-hoc* Turkey test and used the R package ‘*emmeans*’ to check those differences (Lenth, 2022). Food web metrics were calculated using ‘*cheddar*’ and ‘*igraph*’ packages per fruit and for the *L. leucocephala* system as a whole (with all fruits and insets) (Csardi & Nepusz, 2006; Hudson *et al.*, 2013).

Following that, we use path analysis models with the R package ‘*lavaan*’ to assess the direct and indirect effects of resource size on the abundance and richness of herbivores and parasitoids (Rosseel, 2012). Multicollinearity among the explanatory variables were tested using the R package ‘*corrplot*’ (Wei & Simko, 2017) and the model presented a non-normal distribution, so we fit them with bollen-stine bootstrapping method (Kim & Millsap, 2014). First, we calculated the direct influence of seed and fruit biomass and the number of seeds per fruit on herbivore abundance and richness, as well as the direct effect of herbivores on parasitoids abundance and richness. Then we calculated the indirect influence of resource size on parasitoid abundance and richness. The parasitism percentage was determined using the formula below (Equation 1).

$$PR = \frac{PT}{PT + AH} \times 100$$

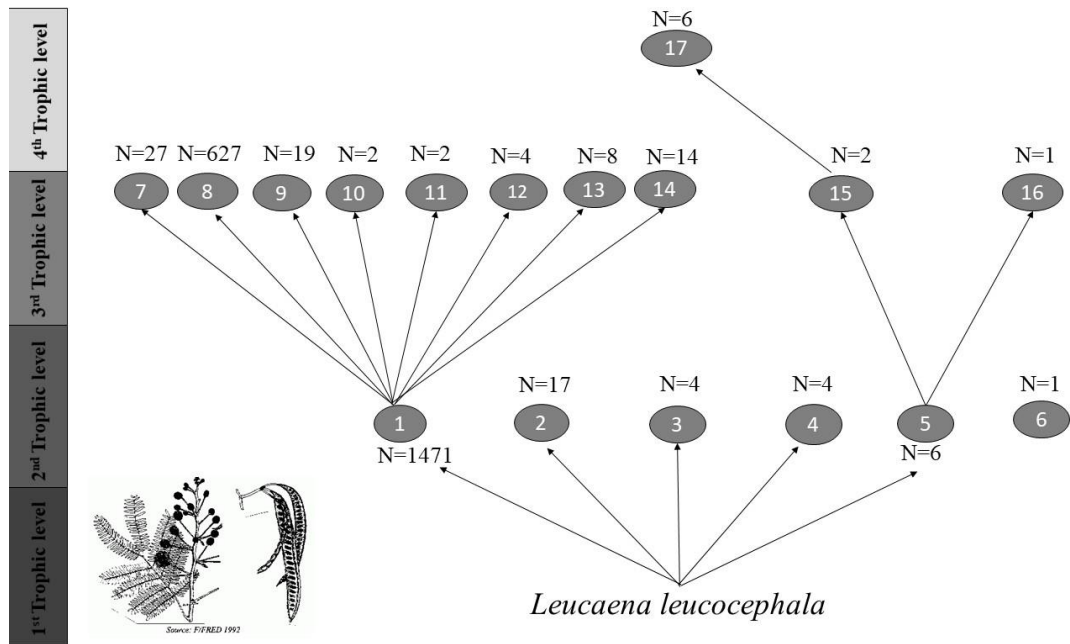
**Equation 1:** PR is the parasitism percentage total, PT is the abundance total of parasitoids in the fruit, and AH is the abundance total of herbivorous in the fruit.

Lastly, we estimate the relationship of food web structure (link density that is the average number of links per species in each fruit, interaction richness which is number total of interactions per fruit, and connectance that represents the proportion of realized interactions from the pool of all possible interactions between the species of a network per fruit) with seed biomass (Sb), fruit biomass (Fb), total seed per fruit (Ts) Parasitoid abundance (Pa), Parasitoid richness (Pr), Herbivore abundance (Ha), and Herbivore richness (Hr) as explanatory and fixed factors. Multicollinearity was tested for these variables by a variance inflation factor (VIF) in the R '*corrplot*' package (Fox & Weisberg, 2019), VIF values below five suggest a lack of collinearity among them (Akinwande *et al.*, 2015). We first selected the best structure of the models (GLM versus GLMM, I, II, II, IV structure). Then, we used the function '*dredge*' to run an automated model (Ben Bolker and R Development Core Team, 2021). We considered that models with  $\Delta AICc < 2$  were statistically equivalent. If more than one model was selected, we used the '*MuMin*' package to perform multimodel inferences, calculating the mean effect of the variables in the best models, weighted by the value of  $w$  in each model (Burnham *et al.*, 2011). We evaluated the importance of each variable by counting the number of times each variable occurred in the comparison of the best model, weighted by the value of  $w$  in the models in which they occurred (Burnham *et al.*, 2011). All statistical analyses were performed in the R environment (Dormann *et al.*, 2009; Hudson *et al.*, 2013; Team, 2022).

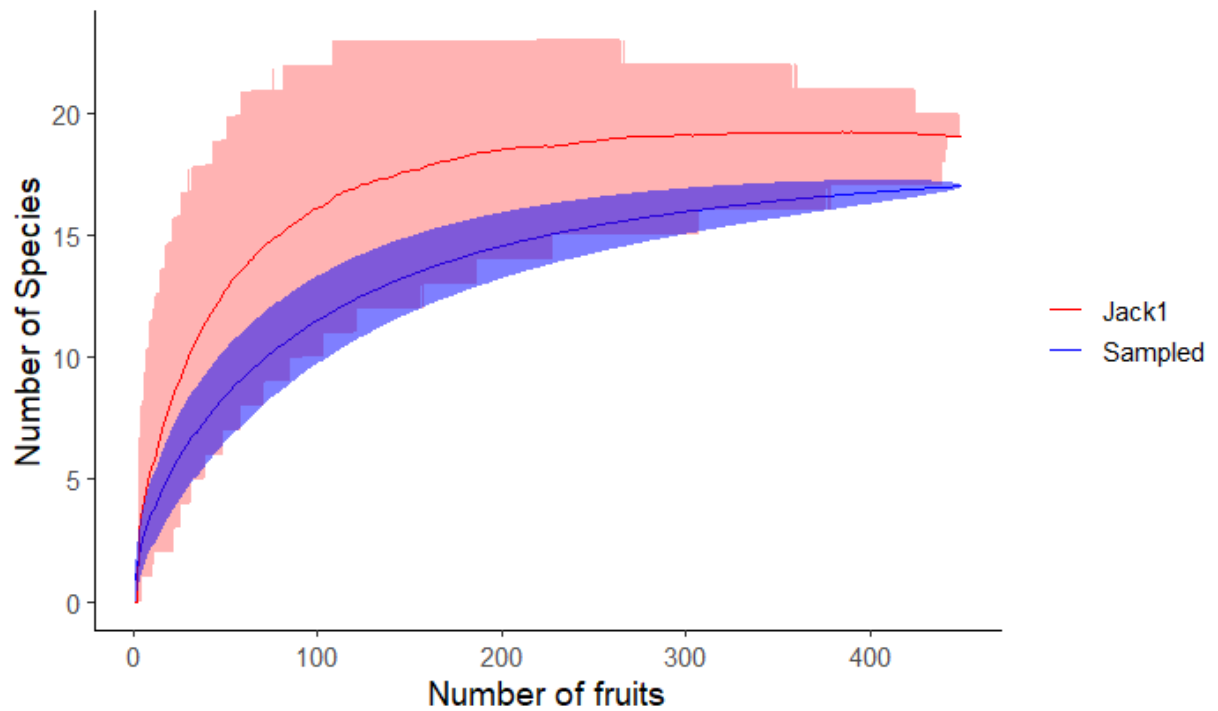
## Results

### Food web characterization

We observed a total of 17 insect species of insects associated with the fruits of *L. leucocephala* (Figure 2, Table 1, Table SM2). These spawned three trophic levels, including five herbivores, 10 parasitoids, one hyperparasitoid, and one unknown species of Thysanopter (Phalaeothripidae sp.) that we could not determine the relationship to the resource. The species accumulation curve showed the sampling effort was sufficient to access 89.5% of the insect richness associated with the *L. leucocephala* seeds, since the first-order Jackknife estimated 19 species (confidence interval: 17-20 species) (Figure 3).



**Figure 2:** *Leucaena leucocephala* food web. The first level is represented by the plant, the second one by the herbivorous species, the third one by the parasitoids, and the fourth trophic level by the hyperparasitoid species. 1- *Acanthoscelides macrophthalmus*; 2-Silvanidae sp.; 3-Phalacridae sp.; 4-Anthocoridae sp.; 5-Lepidoptera sp.; 6- Phalaeothripidae sp. ;7- *Stenocorse sudamericanus*; 8-*Paracrias pluteus*; 9-*Horismenus abnomicaulis*; 10-*Horismenus* sp.; 11-*Eupelmus (eupelmus) pulchriceps*; 12-*Eurytoma* sp.; 13- *Heterospilus* sp.; 14- *Urosigalphus* sp.; 15-*Chelonus* sp.; 16-*Phanerotoma* sp.; 17-*Perilampus* sp.

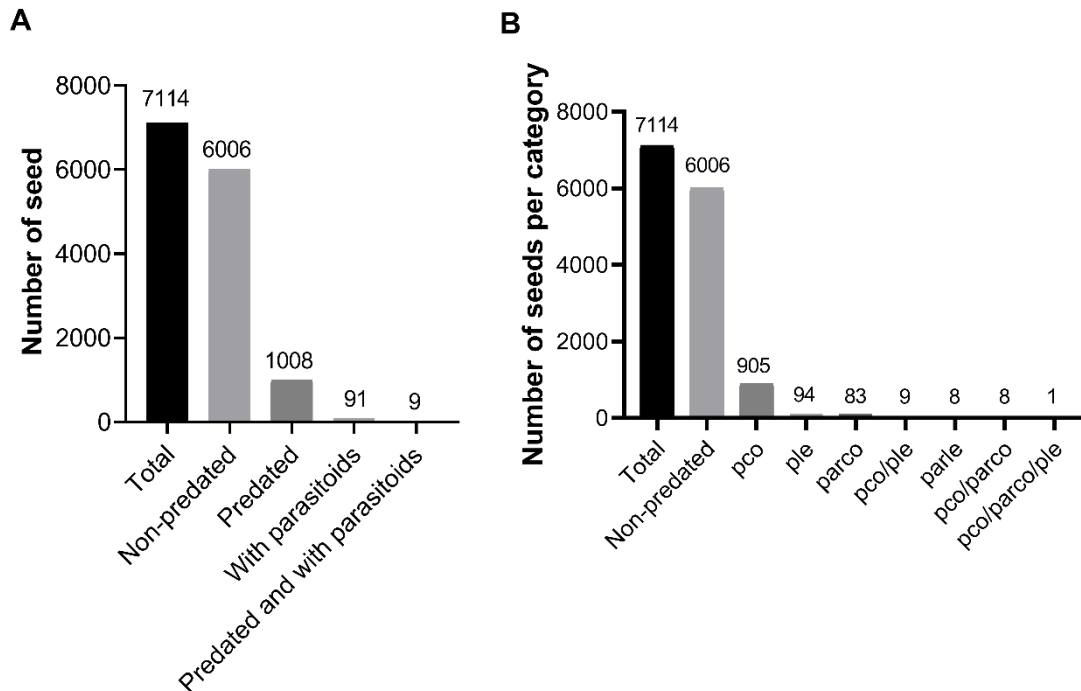


**Figure 3:** Species accumulation curves for insects based on fruit samples from *L. leucocephala*. The curve was created using the rarefaction method, the Jackknife first-order estimator, on 1,000 randomizations, and a confidence interval of 95%. The blue color is the number of species recovered in this study and pink color is the number of species estimated by the Jackknife estimator.

The first trophic level, represented by the plant species *L. leucocephala*, was composed of 7,599 seeds of the 360 fruits (21.18 seeds/fruit on average) with a maximum of 50 seeds per fruit across the seven individual trees. From this total, 474 seeds (6.2%) were aborted and excluded from the analysis. Among the remaining 7,114 viable seeds, 6,006 were non-predated (84.42%), 1,017 seeds were predated (14.29%), and 100 seeds had parasitoids (1.40%) (Figure 4 A). The seeds predated by Coleoptera (pco) was the category with the most seed predation with 905 seeds (89.78% of the predated seeds), followed by seeds predated by Lepidoptera (ple)



(9.32% of the predated seeds) (Figure 4B).



**Figure 4:** Categories of *Leucaena leucocephala* seed predation and its distribution. On the left are the general predation categories [A], and on the right are the specific predation categories [B]. Seeds were separated into: predated by Coleopterans (pco), by Lepidoptera (ple), with coleopteran parasitoids (parco) and with Lepidoptera parasitoids (parle) [B].

We found 2,215 insects associated with the 360 fruit pods of *L. leucocephala*, with 1,503 individuals (67.45%) belonging to the second trophic level, composed of six species distributed in six families and four orders. The main herbivore species in this trophic level is *Acanthoscelides macrophthalmus* (Schaeffer) (Coleoptera, Chrysomelidae, Bruchinae) with 1,471 individuals (66.41%), followed by Silvanidae sp. (Coleoptera) with 17 individuals (0.76%), Lepidoptera sp. with six (0.27%), Palacridae sp. (Coleoptera) and Anthocoridae sp. (Hemiptera) with four individuals each (0.18%), and Phalaeothripidae sp. (Thysanoptera) with one (0.04%). For the third trophic level, we found 716 parasitoids individuals (Hymenoptera) (32.31%), belonging to ten species distributed in nine genera and four families (Braconidae, Eurytomidae, Eulophidae, and Eupelmidae). From these, 703 (31.73% of total insects) are associated with *A. macrophthalmus* and three individuals of two species are parasitoids of Lepidoptera sp. (0.13%). The fourth and last trophic level was composed of six individuals of *Perilampus* sp. (Hymenoptera, Perilampidae) (Table 1), which were found to parasitize the

parasitoid *Chelonus* sp. New interactions were observed in this study, which are described in **Table 2**.

**Table 1:** Insect species are classified into four trophic levels, with their abundance and frequency associated with the seeds of *Leucaena leucocephala*. The fruit pods were collected at the Federal University of Lavras, Minas Gerais.

<b>Trophic position/species</b>	<b>Plenty</b>	<b>RF(%)</b>	<b>DNA cod.</b>
<b>PRIMARY CONSUMERS</b>	<b>1,503</b>	<b>67.85</b>	
<i>Acanthoscelides macrophthalmus</i>	1,471	66.41	LL89-H
Silvanidae sp.	17	0.76	-
Lepidoptera sp.	6	0.27	LL66-A
Palacridae sp.	4	0.18	LL59-C
Anthocoridae sp.	4	0.18	LL92-E
Phalaeothripidae sp.	1	0.04	LL59-C
<b>PARASITOID</b>	<b>706</b>	<b>31.87</b>	
<b>Associated to <i>A. macrophthalmus</i></b>	703	31.73	
<i>Paracrias pluteus</i>	627	28.30	LL57-D
<i>Stenocorse sudamericanus</i>			LL201-A
González-Joya, Sarmiento & Zaldívar-Riverón, 2019	27	1.21	
<i>Horismenus abnormicaulis</i> Pikart 2011	19	0.85	-
<i>Urosigalphus</i> sp. Ashmead	14	0.63	LL177-A
<i>Heterospilus</i> sp. Halliday	8	0.36	LL113-D
<i>Eurytoma</i> sp. Lliger	4	0.18	LL65-D
<i>Horismenus</i> sp. Walker	2	0.09	-
<i>Eupelmus (eupelmus) pulchriceps</i> sp. (Cameron, 1904)	2	0.09	-
<b>Associated to Lepidoptera sp. 1</b>	<b>3</b>	<b>0.13</b>	
<i>Chelonus</i> sp. Panzer	2	0.09	LL142-A
<i>Phanerotoma</i> sp. Wesmael	1	0.04	-
<b>HYPERPARASITOID</b>			
<b>Associated to <i>Chelonus</i> sp. 1</b>	<b>6</b>	<b>0.27</b>	
<i>Perilampus</i> sp. Latreille	6	0.27	LL58-E
<b>Total</b>	<b>2,215</b>	<b>100</b>	

RF relative frequency; - Fail DNA sample

**Table 2:** Interactions observed in laboratory conditions of the *Leucaena leucocephala* food web and records based on the literature.

Resource	Consumer	Interaction evidence
<i>L. Leucocephala</i>	<i>Acanthoscelides macrophthalmus</i> (Coleoptera, Chrysomelidae, Bruchinae)	The species was observed consuming seeds of this plant in Brazil by Wood et al., 2017.
<i>L. Leucocephala</i>	<i>Silvanidae</i> sp. (Coleoptera)	This order was observed in seed pods of this plant in Hawaii-USA (Follett <i>et al.</i> , 2016).
<i>L. Leucocephala</i>	Lepidoptera sp.	We did not find interaction evidence of Lepidoptera sp. consuming seeds of this plant. However, this order was observed infesting foliage of this plant species in Florida USA (Lee & Hayden, 2019), and plant inflorescences in Peru (Alonso-Amaro <i>et al.</i> , 2020). *
<i>L. Leucocephala</i>	Phalacridae sp. (Coleoptera)	Individuals of this family are associated with pollen of seed plats as adults, while their larvae can feed on plant fluids (Gimmel, 2013).*
<i>L. Leucocephala</i>	Anthocoridae sp. (Hemiptera)	This family was recorded as predator in plants of <i>L. leucocephala</i> in Mexico (Ahmed <i>et al.</i> , 2016)
<i>L. Leucocephala</i>	Phlaeothripidae sp.	This family was recorded as a predator in seeds of <i>L. leucocephala</i> in Colombia (Sanabria-Silva & Amarillo-Suárez, 2017)

<i>Acanthoscelides macrophthalmus</i>	<i>Paracrias pluteus</i> Pikart, 2011 (Hymenoptera, Eulophidae)	Parasitism record of the species in this plant (Wood <i>et al.</i> , 2017), and known to parasitize bruchinae species (Pikart <i>et al.</i> , 2011).
<i>Acanthoscelides macrophthalmus</i>	<i>Stenocorse sudamericanus</i> (Hymenoptera, Braconidae)	Parasitism record of this species associated with <i>A. macrophthalmus</i> in Brazil ( <i>L. leucocephala</i> ) and in Colombia ( <i>L. leucocephala</i> ) (Zaldívar-Riverón <i>et al.</i> , 2019; Camacho-Eraza <i>et al.</i> , 2021)
<i>Acanthoscelides macrophthalmus</i>	<i>Horismenus abdomicaulis</i> Pikart, Costa & Hansson, 2015 (Hymenoptera, Eulophidae)	Parasitism record of the species interaction in this plant (Oliveira <i>et al.</i> , 2022).
<i>Acanthoscelides macrophthalmus</i>	<i>Urosigalphus</i> sp. (Hymenoptera, Braconidae)	Parasitism record of the genus in this plant (Wood <i>et al.</i> , 2017).
<i>Acanthoscelides macrophthalmus</i>	<i>Heterospilus</i> sp. (Hymenoptera, Braconidae)	Record of <i>Heterospilus</i> sp. associated with <i>A. macrophthalmus</i> in seed pods of <i>L. leucocephala</i> in Tolima, Colombia (Camacho-Eraza <i>et al.</i> , 2021).
<i>Acanthoscelides macrophthalmus</i>	<i>Eurytoma</i> sp. (Hymenoptera, Eurytoma)	Parasitism record for <i>Acanthoscelides clitellarius</i> in <i>Pepitadenia gonoacantha</i> (Morales-Silva <i>et al.</i> , 2018). *
<i>Acanthoscelides macrophthalmus</i>	<i>Horismenus</i> sp. (Hymenoptera, Eulophidae)	Parasitism record of the genus associated with <i>A. macrophthalmus</i> in this plant (Wood <i>et al.</i> , 2017; Pérez-Benavides <i>et al.</i> , 2019).

<i>Acanthoscelides macrophthalmus</i>	<i>Eupelmus (eupelmus) pulchripes</i> sp.	Parasitism record of the species in this plant associated with <i>A. macrophthalmus</i> (Wood <i>et al.</i> , 2017).
Lepidoptera sp. 1	<i>Chelonus</i> sp. (Hymenoptera, Braconidae)	Generally, found as a lepidopteran parasite (Wharton <i>et al.</i> , 1997).*
Lepidoptera sp. 1	<i>Phanerotoma</i> sp. (Hymenoptera, Braconidae)	They are endoparasitoids of eggs and larval forms of Lepidoptera in general (Shaw, 1997). *
<i>Chelonus</i> sp.1	<i>Perilampus</i> sp.	This genus is a hyperparasitoid of Lepidoptera via Braconidae (Darling, 2006).

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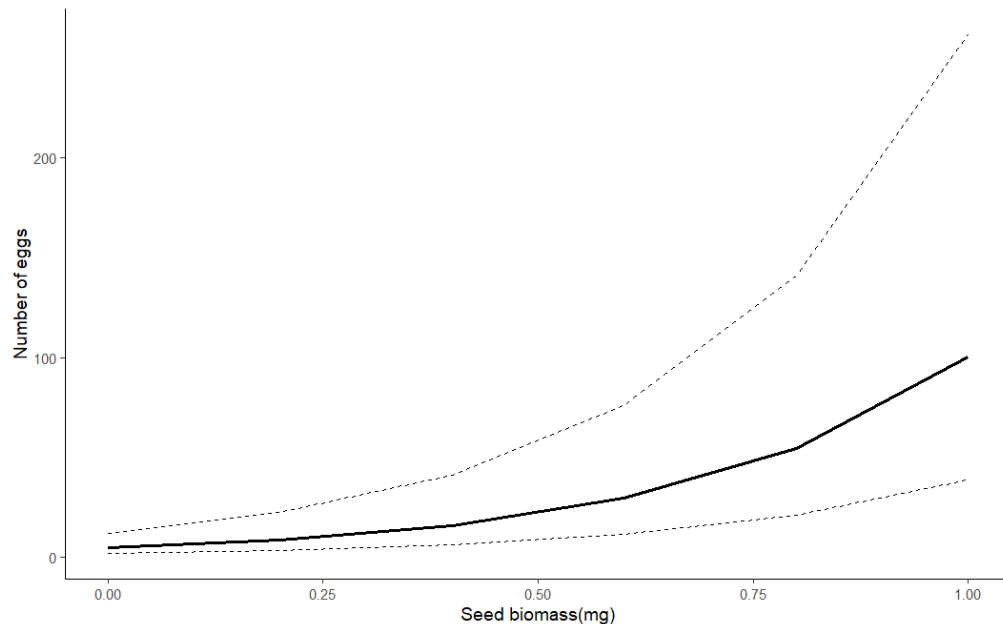
\*New interaction record in this plant/host

Regarding the estimated metrics of the food web, we found 18 species (S) with a linkage density of 0.94 (LS), a connectance of 0.05 (C), proportion of basal, intermediate and superior species of 0.05, 0.16, and 0.77, respectively. There was a parasitoid–host ratio of 4.25, trophic generality of 1.00, trophic vulnerability of 4.25, and an average chain length of 2.61. In terms of parasitism percentages, we observed 33% for Lepidoptera parasitism, 41.28% for *A. macrophthalmus* parasitism the only herbivores parasitized in this food web, and 40.62% for the overall food web.

### **Relationship between seed biomass and *Acanthoscelides macrophthalmus* oviposition**

We observed that females of *A. macrophthalmus* laid their eggs irregularly on the outer surface of fruits. The hatching larvae enter the fruit and burrow into a single seed, where they remain confined due to their inability to move between seeds, consuming the seed content before pupating and emerging as adults (English & Olckers, 2014). The total number of eggs laid on the fruit surface was 2,603 eggs, with a minimum of zero and a maximum of 177 eggs (an average of 7.26 eggs per fruit). Therefore, *A. macrophthalmus* adult survivorship was

56.51% of total initial eggs. We found a positive relationship between the number of eggs laid by *A. macrophthalmus* and *L. leucocephala* seed biomass ( $p < 0.001$ , Figure 5).



**Figure 5:** Generalized linear mixed models (GLMM) of the number of *A. macrophthalmus* eggs as function of the seed biomass of *L. leucocephala* ( $p < 0.001$ ).

### Categories of predation and parasitism and seed biomass relationship

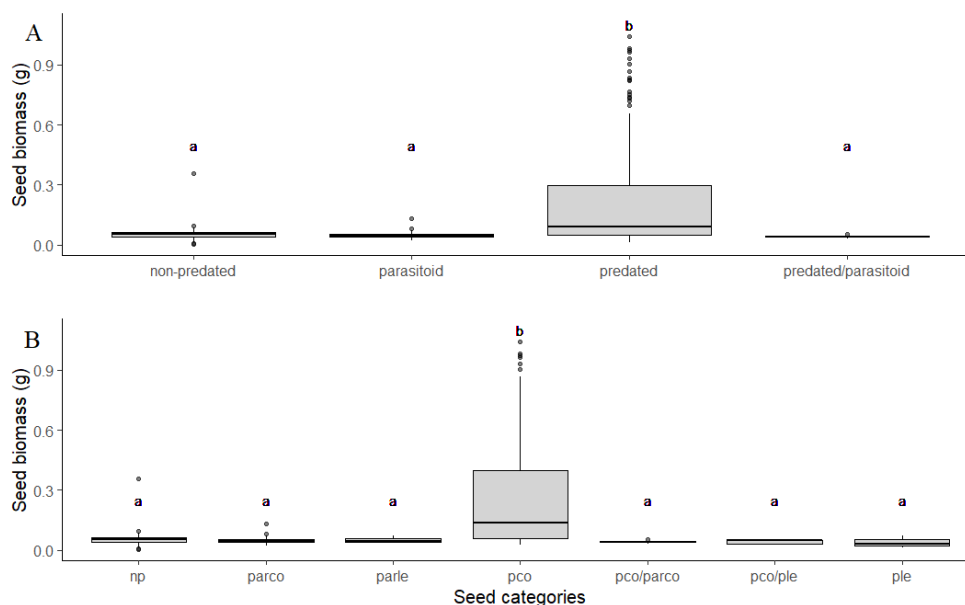
We observed one to two individuals of *A. macrophthalmus* consuming the same seed. These insects exit holes are distinctive, with a discrete round shape. A total of 884 seeds were consumed by them (12.42% of the total seeds and 87.69% of the predated seeds). In comparison, Silvanidae sp. caused more damage to the seeds than *A. macrophthalmus*, eating the seeds in a disorderly way. We found up to six Silvanidae sp. individuals in the same fruit. However, they were found in low numbers, with only eight seeds predated by this insect (0.11% of the total seeds and 0.79% of the predated seeds). The Lepidoptera sp. immature individuals consumed a greater number of seeds of *L. leucocephala*, since we observed that a single larva consumed multiple seeds inside the same fruit. Often, when we examined seeds predated by these individuals, we found silk threads and feces produced by the immatures. In total, 94 seeds were predated by Lepidoptera sp. (1.32% of the total seeds and 9.32% of the predated seeds).

We found nine seeds being predated by both *A. macrophthalmus* and Lepidoptera sp. (0.12% of the total seeds and 0.14% of the predated seeds). Eight seeds with both *A. macrophthalmus* and its parasitoid *Paracrias pluteus* (0.11% of the total seeds and 0.13% of

the predated seeds), and only one seed with *A. macrophthalmus*, *Paracrias pluteus*, and *Lepidoptera* sp. 1 (0.01% of the total seeds and 0.01% of the predated seeds). *Phalacridae* sp. (Coleoptera), *Phalaeothripidae* sp. (Thysanoptera) and *Anthocoridae* sp. (Hemiptera) were found and low abundance inside of fruits, but were not associated with seed predation. We found eight seeds with *Lepidoptera* parasitoids (0.11% of the total seeds and 0.79% of seeds with parasitoids) and 83 seeds with *A. macrophthalmus* parasitoids (1.16% of the total seeds and 8.23% of seeds with parasitoids). The parasitoids cause a circular opening when they emerge from the fruits, being around half of the size of *A. macrophthalmus* exit hole.

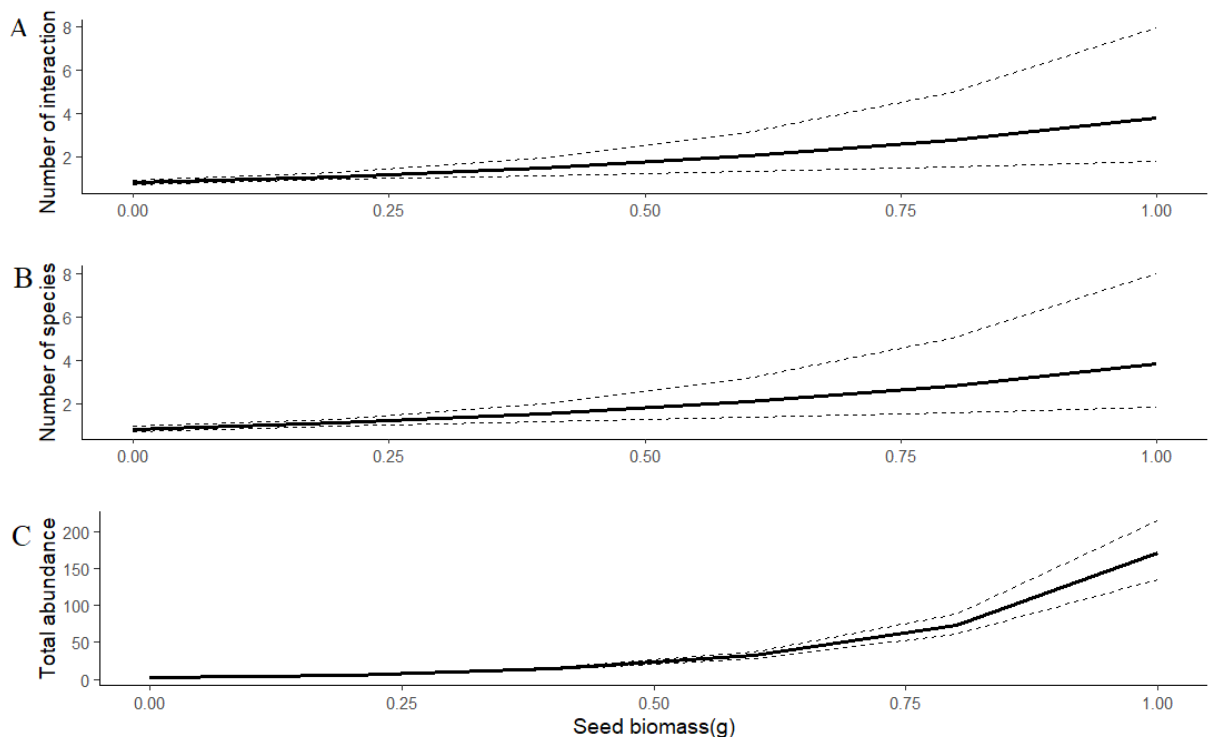
The seed biomass analysis across the four categories (non-predated, with parasitoids, predated, and both predated and with parasitoids) showed herbivore preference for heavier seeds, with predated seed biomass on average four times heavier than the other categories (Figure 6A,  $p < 0.001$ , Table SM3). We did not find any significant biomass difference among the other three categories (Figure 6A,  $p > 0.05$ ).

The specific seed categories showed that seeds predated by Coleoptera (pco) were the ones with heavier seeds and were significantly different from the other categories (Figure 6B,  $p < 0.001$ , Table SM4). Among the other categories, seeds predated by *Lepidoptera* (ple) showed the lowest biomass with an average of 0.03 g (Table SM4), but no significant difference with seeds non-predated (np), with Coleoptera parasitoid (parco), *Lepidoptera* parasitoid (parle) the combined categories pco/parco, pco/ple and predated by *Lepidoptera* (ple) ( $p > 0.005$ , Table SM4).



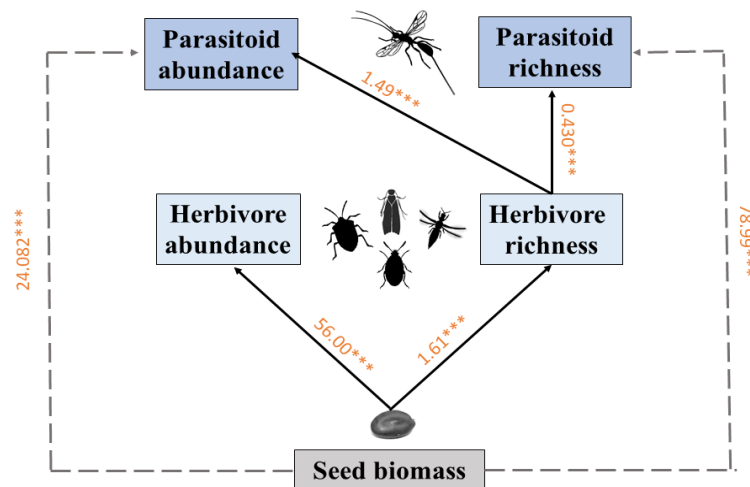
**Figure 6:** The average biomass per fruit according to general seed categories [A] and specific seed categories [B]. The GLMM model was significantly different from the null model for both [A] and [B] categories ( $p < 0.001$ ). Different letters indicate category differences by the Tukey test. Specific categories: non-predated (np), with Coleoptera parasitoid (parco), with Lepidoptera parasitoid (parle), the combined categories with coleopteran and its parasitoid (pco/parco), predated by coleopteran and Lepidoptera (pco/ple), and predated by Lepidoptera (ple).

We found that average seed biomass per fruit has a positive effect on the number of interactions, the number of species, and the abundance of insects, in which higher seed biomass shows a greater number of insect species, abundance, and interactions ( $p < 0.001$ , Figure 7). Additionally, path analysis showed that seed biomass has a strong direct and indirect effect on herbivore and parasitoid abundance and richness (Figure 8, Table SM5). Specifically, we found that seed biomass has a positive effect on both herbivore and parasitoid abundance and richness (Figure 8, Table SM5,  $p < 0.001$ ). Parasitoid abundance and richness were positively correlated with herbivore richness but not with herbivores abundance (Figure 8).



**Figure 7:** Generalized linear mixed models of the seed biomass effect of *L. leucocephala* on the number of interactions per fruit [A] ( $p < 0.001$ ), number of insect's species per fruit [B] ( $p < 0.001$ ), and on the abundance of insects per fruit [C] ( $p < 0.001$ ).





**Figure 8:** Path analysis with the significant direct and indirect effects of seed biomass, herbivores, and parasitoids' abundance and richness. The direct relationship between one variable and another is indicated by solid black arrows, while the indirect one is indicated by dotted grey arrows. Significant values are indicated by the color orange, (\*\*\*) =  $p < 0.001$ .

Lastly, we estimated structural food web metrics. We observed an average of interactions of 0.91 (mean number of interactions), linkage density of 0.94, and connectance of 0.05. To connectivity, individual multicollinearity was not detected among all variables according to VIF (values  $< 5$ ), e.g., seed biomass = 3.04; fruit biomass = 1.50; total number of seeds per fruit = 1.46; parasitoid abundance = 1.35; parasitoid richness = 1.35; herbivore abundance = 3.10; herbivore richness = 1.15. The model selection for interaction richness provided only one model with  $\Delta AICc < 2$ . Herbivores and parasitoids richness positively influenced connectivity, while seed biomass, parasitoid and herbivore abundance were less important and did not show significance (Table 3). In the Supplementary Material, we presented the analysis of linkage density and connectance (Tables SM6 and SM7,  $p > 0.05$ ).

**Table 3:** Model average and importance for models evaluating the effect of the seed biomass (SB), Fruit biomass (FB), Total Seed (ST) parasitoid abundance (PA), parasitoid richness (PR) Herbivore abundance (HA) and herbivore Richness (HR) on interaction richness.

	Estimate	Std. Error	Adjusted SE	z value	Importance	Pr(> z )
(Intercept)	0.492	0.161	0.162	2.403		<0.016
SB	0.14	0.417	0.420	0.335	0.17	0.737
PA	0.011	0.014	0.014	0.803	0.22	0.422
PR	0.492	0.074	0.074	6.615	1	<0.001

HA	0.002	0.006	0.006	0.366	0.17	0.714
HR	0.492	0.074	0.074	6.615	1	< <b>0.001</b>

## Discussion

We described the quantitative and qualitative food web of insect herbivores, parasitoids, and hyperparasitoids associated with the fruits and seed pods of the non-native and invasive *L. leucocephala*. We discovered 17 species of insects across three trophic levels associated to *L. leucocephala*. Furthermore, a strong positive correlation between resource size (average seed biomass per fruit), the number of interactions (links), and the richness and abundance of insects (herbivores and parasitoids) was found. These results suggest a cascading effect from the lowest trophic level (seed biomass) to higher trophic levels, wherein heavier seeds supported a greater abundance and richness of insect herbivores and, as a result, a greater abundance and richness of their parasitoids. It increased the complexity of the food web, in support of our first hypothesis which states that heavier seeds increase the abundance and richness of herbivores and their parasitoids, increasing the food web's complexity. In terms of seed predation, our results suggest the preference of herbivores for larger seeds since, on average, the seed biomass in the predated seed category was four times bigger than the other ones. The dominant herbivore, *A. macrophthalmus*, also supported this hypothesis that herbivores prefer bigger resources since they laid more eggs on fruits that contained heavier seeds, supporting our second and third hypothesis, which states that seeds consumed by herbivores are heavier than other categories, and *A. macrophthalmus* would chose larger resources to lay their eggs. Lastly, the food web structure was determined and compared to characteristics of the insect community associated with *L. leucocephala* seeds. Connectivity was explained by the herbivore and parasitoid richness, but the density of links and connectance was not related to any variable tested.

## Food web characterization

The most abundant herbivore species in the food web was *A. macrophthalmus* (N= 1,471). This species has been released as a biological control in many countries to contain the *L. leucocephala* spread (Olckers, 2004, 2011). Nonetheless, its biological control capacity has been arguable since seed damage by this taxon barely exceeds 50%, while at least 80% of seed damage (predation) is required for effective control of the host plant (Raghu *et al.*, 2005; Rodrigues *et al.*, 2012; Sharratt & Olckers, 2012). We observed a seed predation rate of 12.42%, which was similar to other studies and considered insufficient to control the spread of the invasive plant (Raghu *et al.*, 2005; Sharratt & Olckers, 2019). Furthermore, a significant

number of parasitoid species (N = 8) were associated with *A. macrophthalmus*, which reduced their abundance and released the seeds from predation, making even more seeds available consistent with a *top-down* effect (Vidal & Murphy, 2018).

The second most abundant species of granivore was Silvanidae sp. (N = 17). Their ecology is poorly understood, but they are known to feed on small mites and fungi or on stored grain as pests (Majka, 2008). We observed Silvanidae sp. consuming seeds of *L. leucocephala* and, usually, causing more damage to the seeds than *A. macrophthalmus*, but in low abundance. Regarding Lepidoptera sp. (N = 6), they were responsible for damaging 9.32% of the seeds in the predated category. To our best knowledge, the biology and ecology of Lepidoptera consuming seeds of *L. leucocephala* are not well explored, and most of the information on Lepidoptera predation on this plant is regarding its leaves or inflorescences (Lee & Hayden, 2019; Alonso-Amaro *et al.*, 2020). Thus, our study adds an important new connection between Lepidopteran herbivores and the *L. leucocephala* host plant that requires further study.

The parasitoid families Eulophidae and Braconidae were found to be the most abundant taxa in this and also in a previously study that looked at the geographic distribution of *A. macrophthalmus* parasitoids in four Brazilian states: Minas Gerais, São Paulo, Paraná, and Santa Catarina. Aside from the species described by Wood *et al.* (2017), we recorded four more species in the state of Minas Gerais associated with *A. macrophthalmus*: *Heterospilus* sp., *Eurytoma* sp., *Stenocorse sudamericanus* (Zaldívar-Riverón *et al.*, 2019), and *Horismenus abdomicaulis* (Oliveira *et al.*, 2022 in revision). Moreover, we described the parasitoids associated with the Lepidoptera sp.: *Chelonus* sp., *Phanerotoma* sp., and *Perilampus* sp. Given that Lepidoptera have not been previously described to consume *L. leucocephala*, these parasitoid associations may also be new.

The dominant parasitoid, *Paracrias pluteus* (N = 627), was first reported in Brazil as a parasitoid of *Sennius spodiogaste* and *Sennius cupreatus* (Chysomelidae, Bruchinae) on seeds of *Melanoxilin brauna* (Fabaceae) (Pikart *et al.*, 2011), and later on as a parasitoid of *A. macrophthalmus* on seeds of *L. leucocephala* (Wood *et al.*, 2017). Although this interaction has been previously reported, little is known about the biology and ecology of this species. Here, we add that this species' parasitism in *L. leucocephala* is polyembryonic, with the development of multiple identical embryos from a single egg through clonal division, and gregarious, which indicates that more than one offspring can complete development on or in a host (Godfray, 1994; Segoli *et al.*, 2010). We observed up to four parasitoids emerging from the same host individual, which explains its high abundance in *L. leucocephala*. The second most abundant

species, *Stenocorse sudamericanus* (N = 27), was previously reared with some Bruchinae species, including *A. macrophthalmus* in *Pseudosamanea gruchapele* (Kunth) and *L. leucocephala* (Pérez-Benavides *et al.*, 2019; Camacho-Erazo *et al.*, 2021). This species was recorded as a parasitoid of seed beetles feeding on seeds of native legumes in Colombia, such as *Senegalia riparia* (Kunth), *Chloroleucon bogotense* Britton & Killip, *P. gruchapele* and *Acacia farnesiana* (Amarillo-Suárez, 2010; Sanabria-Silva & Amarillo-Suárez, 2017) and was recently reported to colonize the exotic species *L. leucocephala* there (Camacho-Erazo *et al.*, 2021). We also observed the shift of parasitoids from native to invasive plant species, since *Stenocorse sudamericanus* and *Paracrias pluteus* (we were unable to determine the species level for other parasitoids) are found primarily as natural enemies of seed-feeding insects in native species (e.g., *P. guachapele* and *M. brauna*).

Regarding the estimated food web metrics, our results are similar to the one presented by Morales *et al.* (2019), which evaluated the food web based on insects (herbivores and parasitoids) associated with the seed of *Piptadenia gonoacantha* (Mart.) Macbr. (Fabaceae) in the same area as this study was conducted. The main differences are that they found more species (N = 21 spp.), higher parasitoids-host ratio (19), vulnerability (5.25), and lower chain length (1.83). These differences are mainly because their system had more parasitoid species (N = 14) and less abundance of herbivores (N=629) and parasitoids (N = 146), which can be explained due the fact *P. gonoacantha* is native in Brazil, while the *L. leucocephala* is not.

#### **Relationship between seed biomass and *Acantoscelides macrophthalmus* oviposition**

The choice of the oviposition site is a crucial factor for female offspring success, especially for those insects that are constrained in their resources throughout their life, such as bruchinae seed-feeding (Mitchell, 1975). We discovered a positive relationship between the number of eggs laid by *A. macrophthalmus* on fruits with heavier seeds. Many studies have previously reported bruchinae preferences for larger seeds and fruits (Messina & Renwick, 1985; Campbell, 2002; Cope & Fox, 2003b; English & Olckers, 2014; Morales-Silva *et al.*, 2018; Pedroso *et al.*, 2021). Indeed, larger seeds are thought to provide more resources for larvae development, improving their chances of survival and producing larger adults (Mitchell, 1975; Cipollini & Stiles, 1991; Oliveira *et al.*, 2020).

Researchers investigated whether resource size affects *A. macrophthalmus* oviposition behavior on *L. leucocephala* fruits in the field and in the lab. In two of three site surveys, they discovered that *A. macrophthalmus* has a strong oviposition preference for larger seeds and fruit pods (English & Olckers, 2014). We found no correlation between oviposition and fruit size or

the total number of seeds within a fruit, but we did find an association with fruits containing seeds heavier. This finding demonstrates that resource traits, selected by this species, can vary across different populations and emphasizes the significance of measuring more than one plant trait in studies that seek to establish a connection between oviposition and resource size. Given that seed size in the Fabaceae family is typically connected with germination and, hence, plant success, our findings suggest a favorable consequence for *L. leucocephala* biological control in scenarios where dehisced seeds are utilized.

### **Categories of predation and parasitism and seed biomass relationship**

We discovered a clear preference of herbivores for larger seeds among the seed predation categories, as the predated seed categories had four times the average biomass of the others (including the non-predated category). The 'pco' category (average of seeds predated by Coleoptera per fruit), primarily composed of *A. macrophthalmus*, was discovered to be mainly responsible for this result. This result was expected because similar system studies have revealed bruchinae species' preference for larger seeds (English & Olckers, 2014; Maia *et al.*, 2017; Morales-Silva *et al.*, 2019a; Parra-Gil *et al.*, 2020). Furthermore, while we found no statistical differences between the specific categories, we did observe a lower biomass for the 'Ple' category (Lepidoptera), which was also expected given that seeds predated by them are normally entirely consumed (Oliveira, T.C.T personal observation).

The 'Parco' category (seeds with parasitoids of Coleoptera), on the other hand, produced an unexpected result. We expected this category to have seeds that were heavier or as heavy as the Pco category because the majority of the Coleopteran parasitoids in this study are idiobiont parasitoids. Idiobiont parasitoids prevent host development after parasitism by injecting paralyzing venom into adult females and typically involve an immobile host life stage (e.g., egg or pupa) (La Salle & Gauld, 1992). Thus, seed with parasitoids would not be efficiently consumed by the herbivore. However, there is a well-known relationship between parasitism percentages and selection for larger gall sizes (Weis *et al.*, 1985), suggesting plant tissues act as barriers for parasitoid oviposition into herbivores. Therefore, we hypothesize that the higher parasitism percentage of smaller seeds observed here is because the thicker seed capsules of the larger seeds protect the herbivores inside from oviposition by parasitoids. This dynamic generates a second selective pressure beyond resource quality for herbivores to choose larger seeds over smaller ones (Dias *et al.*, 2010).

A positive influence of seed biomass on the complexity of food web was found. Fruits with larger seeds (seed biomass) shows a greater increase in both insect abundance, insect

richness and number of interaction. Our findings support the hypothesis of resource abundance (or parts of the plant: fruits and seeds), which states that plants with more resources can support more species and have higher abundances of insect herbivores (Teragushi *et al.*, 1981; Hunter & Willmer, 1989; Hunter, 1992; Ohgushi, 1992). In fact, it has been suggested that the abundance of resource is an important factor structuring insect herbivores communities, but only few studies tested it in community level (Teragushi *et al.*, 1981; Hunter, 1987; Hunter & Willmer, 1989; Marques *et al.*, 2000) Thus, host plant with higher biomass can attract more insect species (Teragushi *et al.*, 1981), such as herbivores and consequently their parasitoids. We did not found the same pattern for fruit biomass and number of seeds in the fruit, although it has been previously verify in other systems (Ctvrtecka *et al.*, 2016; Morales-Silva *et al.*, 2018; Cocolletzi *et al.*, 2019). Hence, we emphasize the importance of measure more than one plant trait since it can be more or less important for some taxa of insects.

Lastly, we measured which parameters of the community (abundance and richness of herbivores and parasitoids, and seed biomass) would affect interaction richness (total number of interactions), linkage density (average number of links by species), and connectance (proportion of realized interactions among all possible ones), which are three of other uncountable attributes that can be associated with ecosystem function measures (Thompson *et al.*, 2012). In general, our results suggested that interaction richness was influenced by the communities' attributes, while connectance and density of links were not. Interaction richness significantly increased when the richness of herbivores and parasitoids increased, and although seed biomass and insect abundance were selected through the model selection method, we did not observe a significant relationship between the interaction richness measure and the variables. This result was expected, particularly in terms of insect richness, because our food web is simple, with only one interaction per species (herbivore-host and parasitoid-herbivore host). This means that the more species there are in the system, the more links there are and, as a result, the greater resilience in the system. The same result was found in a food web based on *Senegalia tenuifolia* seeds, which is also a simple system with one interaction per species (Pedroso *et al.*, 2021). Regarding connectance and link density, no variable tested affected these measures. Connectance is a measure of stability in ecosystems and is less sensitive to food web structure changes (Martinez, 1991; Dunne *et al.*, 2002), and link density in our case, with only one interaction per species, did not vary among fruits.

## Conclusion

In conclusion, we described the community of herbivores and their associated parasitoids and hyperparasitoids and then applied a food web approach to understand the relationship between resource size and the qualitative and quantitative characteristics of the community of insects associated with the seeds of the invasive plant *Leucaena leucocephala*. We showed that resource size is an important attribute to the food web complexity, in which seed biomass promoted a cascading effect on both insect abundance and richness and functional measures (number of links and n). This highlights the importance of including resource traits in studies of species interactions. Moreover, we observed the preference of herbivores, especially the *Acantoscelides macrophthalmus*, for heavier seeds, since they laid more eggs in fruits with heavier seeds. In terms of biology, our study has provided a complete analysis of the community of insects associated with the seeds and fruits of the invasive plant *L. leucocephala*, documenting an initial shift of natural enemies from native plants to this non-native species and highlighting the potential for local adaptation (Carroll *et al.*, 1998) and speciation associated with a host shift (Forbes *et al.*, 2017).

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## Contribution of authors

L.D.B.F., T.C.T.O. designed the manuscript. T.C.T.O., G.M.O, V.H.D.S. collected data. T.C.T.O. and L.D.B.F performed the analyses. T.C.T.O and P.F.P.B.D performed the molecular

analysis, T.C.T.O. wrote the first draft, and all authors contribute substantially to the final version.

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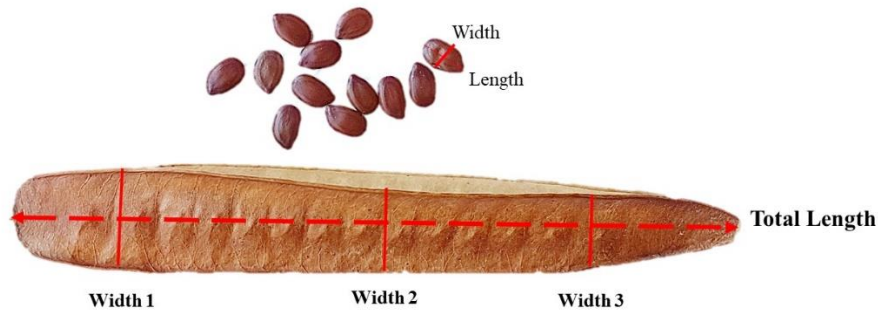
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## Supplementary Material B



**Figure SM1:** Measurements made to determine the pods' fruit and seed areas.

**Table SM1:** Primers used to sequence insect's community DNA in this study with the respective annealing temperature.

Primers	Code	Sequencing	Annealing	References
<b>Hco 1490</b> <b>Lco 2198</b>	A	5' GGT CAA ATC ATA AAG ATA TTG G 3' 5' TAA ACT TCA GGG TGA AAA AAT CA 3'	52°C	(Folmer <i>et al.</i> , 1996)
<b>Lep F</b> <b>Lep R</b>	C	5' ATT CAA CCA ATC ATA AAG ATA TTG G 3' 5' TAA ACT TCT GGA TGT CCA AAA AAT CA 3'	50°C	(Hebert & Gregory, 2005)
<b>Omr 2</b> <b>Coi 2437d</b>	E	5' TRG GDG CTC CDG ATA TRG CW 3' 5' GCT ART CTA AAW AYT TTA ATW CCW G 3'	45°C	(Kaartinen <i>et al.</i> , 2010)
<b>TyI460</b> <b>CiN2191</b>	F	5'TAC AAT TTA TCG CCT AAA CTT CAG CC 3' 5' CCC GGT AAA ATT AAA ATA TAA ACT TC 3'	46°C	(Simon <i>et al.</i> , 1994)
<b>Acant F</b> <b>Acant R</b>	H	5'AGA TGT AGA CAC CCG AGC TT 3' 5'ATG ATA GCG AAT ACG GCC CC 3'	50°C	<b>This study</b>

**Table SM2:** List of species, guilds, absolute frequency and DNA barcoding code collected at University Federal of Lavras associated with *Leucaena leucocephala*.

Host Plant	Taxonomic affiliation	Guild	Absolute frequency	DNA Barcoding-primer
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<i>Leucaena leucocephala</i>	Silvanidae sp.1 (Coleoptera)	Seed consumer	17	-
<i>Leucaena leucocephala</i>	<i>Acanthoscelides macrophthalmus</i> (Coleoptera: Chrysomelidae)	Seed consumer	1471	LL89-H
<i>Leucaena leucocephala</i>	Phalacridae sp. (Coleoptera)	Seed consumer	4	LL59-C
<i>Leucaena leucocephala</i>	Anthocoridae sp. (Hemiptera)	Sucker- predator	4	LL92-E
<i>Leucaena leucocephala</i>	Gelechiidae sp. (Lepidoptera)	Seed consumer	6	LL66-A
<i>Leucaena leucocephala</i>	Phaeothripidae sp. (Thysanoptera)	Fungi consumer	1	-
<i>Leucaena leucocephala</i>	<i>Chelonus</i> sp. (Hymenoptera: Braconidae)	Parasitoid	2	LL142-A
<i>Leucaena leucocephala</i>	<i>Eurytoma</i> sp.1 (Hymenoptera: Eurytomidae)	Parasitoid	4	LL65-D
<i>Leucaena leucocephala</i>	<i>Heterospilus</i> sp.1 (Hymenoptera: Braconidae)	Parasitoid	8	LL113-D
<i>Leucaena leucocephala</i>	<i>Paracrias pluteus</i> Pikart, 2011 (Hymenoptera: Eulophidae)	Parasitoid	627	LL57-D
<i>Leucaena leucocephala</i>	<i>Perilampus</i> sp. (Hymenoptera: Perilampidae)	Parasitoid	6	LL58-E
<i>Leucaena leucocephala</i>	<i>Eupelmus (eupelmus)</i> <i>pulchripes</i> sp. (Hymenoptera: Eupelmidae)	Parasitoid	2	-
<i>Leucaena leucocephala</i>	<i>Horismenus abdomicaulis</i> Pikart, Costa & Hansson, 2015	Parasitoid	19	-



	(Hymenoptera: Eulophidae)			
<i>Leucaena leucocephala</i>	<i>Horismenus</i> sp.1 (Hymenoptera: Eulophidae)	Parasitoid	2	-
<i>Leucaena leucocephala</i>	<i>Urosigalphus</i> sp. (Hymenoptera: Braconidae)	Parasitoid	14	LL177-A
<i>Leucaena leucocephala</i>	<i>Phanerotoma</i> sp. (Hymenoptera: Braconidae)	Parasitoid	1	-
<i>Leucaena leucocephala</i>	<i>Stenocorse sudamericanus</i> González-Joya, Sarmiento & Zaldívar-Riverón, 2019 (Hymenoptera: Braconidae)	Parasitoid	27	LL201-A

**Table SM3:** GLMM results to general categories of predation. Different letters indicate categories differences by Turkey test.

General categories	Emmean*	SE	Average seed biomass (g)	Group
Predated	6.1	1.15	0.20	a
Non-predated	21.21	1.31	0.05	b
Parasitoids	22.7	2.12	0.04	b
Predated/ parasitoid	26.1	6.7	0.04	b

\* Results are given on the inverse (not the response) scale.

**Table SM4:** GLMM results to specific categories of predation. Different letters indicate categories differences by Turkey test. Specific categories: non- predated (np), with coleoptera parasitoid (parco), with lepidoptera parasitoid (parle) the combined categories with coleopteran and its parasitoid (pco/parco), predated by coleopteran and lepidoptera (pco/ple) and predated by lepidoptera (ple).

General categories	Emmean*	SE	Average seed biomass (g)	Group
pco	5.73	1.22	0.24	a
parle	20.39	4.71	0.04	b
np	21.30	1.34	0.05	b
parco	23.21	2.04	0.04	b
pco/parco	26.10	5.83	0.04	b
pco/ple	26.81	6.2	0.04	b
ple	29.38	3.09	0.03	b

\*Results are given on the inverse (not the response) scale.

**Table SM5:** Path analysis demonstrating the association between resource size, insect herbivores, and parasitoids' abundance and richness of insects associated with *Leucaena leucocephala* in Lavras-Minas Gerais, Brazil.

Dependent variable	Independent variable	Estimate	Std. Error	z-value	P(> z )	Std. All
<b>Herbivore abundance</b>	Seed Biomass	56.009	5.239	10.691	0.000	0.810
	Total Seeds per fruits	0.035	0.053	0.665	0.506	0.024
	Biomass fruit	-0.0442	1.088	-0.407	0.684	-0.012
<b>Herbivore richness</b>	Seed Biomass	1.616	0.337	4.796	0.000	0.293
	Total Seeds per fruits	0.003	0.008	0.433	0.665	0.029
	Biomass fruit	-0.257	0.167	-1.539	0.124	-0.085
<b>Parasitoid abundance</b>	Herbivore richness	1.409	0.318	4.433	0.000	0.249
	Herbivore abundance	0.045	0.046	0.991	0.322	0.100
	Seed Biomass	78.995	17.922	4.408	0.000	0.231
	Total Seeds per fruits	0.050	0.078	0.639	0.523	0.009
	Biomass fruit	-0.635	1.581	-0.402	-0.635	-0.011
<b>Parasitoid richness</b>	Herbivore richness	0.430	0.069	6.257	0.000	0.448
	Herbivore abundance	-0.000	0.004	-0.075	0.322	0.100
	Seed Biomass	24.082	4.3520	5.533	0.000	0.362
	Total Seeds per fruits	0.015	0.023	0.659	0.510	0.010
	Biomass fruit	-0.190	0.460	-0.413	0.679	-0.005

#### *Linkage density*

We evaluated the relationship among linkage density and the effect of the seed biomass (SB), Fruit biomass (FB), Total Seed (ST) parasitoid abundance (PA), parasitoid richness (PR) Herbivore abundance (HA) and herbivore Richness (HR). *Dredge* function selected eleven models. However, no significant effect was found by the variables tested.

**Table SM6:** Model average and importance for models evaluating the effect of the seed biomass (SB), Fruit biomass (FB), Total Seed (ST) parasitoid abundance (PA), parasitoid richness (PR) Herbivore abundance (HA) and herbivore Richness (HR) on linkage density.

Variables	Estimate	Std. Error	Adjusted SE	z value	Importance	Pr(> z )
(Intercept)	-5.972	0.117	0.118	5.054	0.18	0.0001
SB	3.549	0.053	0.053	0.006	0.18	0.9472
FB	-7.194	0.052	0.052	0.13	0.18	0.8917
ST	5,68E+03	0.002	0.002	0.0019	0.18	0.9984
PA	3.424	0.01	0.01	0.3207	0.18	0.6733
PR	7.072	0.1110	0.111	0.631	0.36	0.527
HA	9.527	0.003	0.003	0.025	0.18	0.979
HR	2.550	0.060	0.060	0.421	0.16	0.673

### Connectance

We evaluated the relationship among connectance and the effect of the seed biomass (SB), Fruit biomass (FB), Total Seed (ST) parasitoid abundance (PA), parasitoid richness (PR) Herbivore abundance (HA) and herbivore Richness (HR). *Dredge* function selected five models. However, no significant effect was found by the variables tested.

**Table SM7:** Model average and importance for models evaluating the effect of the seed biomass (SB), Fruit biomass (FB), Total Seed (ST) parasitoid abundance (PA), parasitoid richness (PR) Herbivore abundance (HA) and herbivore Richness (HR) on connectance.

Variables	Estimate	Std. Error	Adjusted SE	z value	Importance	Pr (> z )
(Intercept)	-5.972	0.117	0.118	5.054	0.18	<0.001
SB	3.549	0.053	0.053	0.006	0.18	0.9472
FB	-7.194	0.052	0.052	0.13	0.18	0.8917
ST	5,68E+03	0.002	0.002	0.0019	0.18	0.9984
PA	3.424	0.01	0.01	0.3207	0.18	0.6733
PR	7.072	0.1110	0.111	0.631	0.36	0.527
HA	9.527	0.003	0.003	0.025	0.18	0.979
HR	2.550	0.060	0.060	0.421	0.16	0.673

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