Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Free-feeding organisms and galling insects (Hymenoptera) interactions on *Caryocar brasiliense* (Malpighiales: Caryocaraceae) trees, a savanna plant from Brazil

Interações entre organismos de alimentação livre e insetos galhadores (Hymenoptera) em árvores de *Caryocar brasiliense* (Malpighiales: Caryocaraceae), uma planta da savana do Brasil

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Abstract

Galling insects are abundant in nature, found in many ecosystems globally, with species attacking plants of economic importance. We studied the effects of free-feeding organisms on the abundance of galling insects on *Caryocar brasiliense* (Caryocaraceae) trees in the Brazilian Cerrado (Savanna). Percentage of defoliation and the number of phytophagous mites or number of phytophagous Hemiptera correlated negatively with percentage of galled leaves and the parasitoid *Eurytoma* sp. (Hymenoptera: Eurytomidae) adults, respectively. Percentage of galled leaves and the numbers of *Eurytoma* sp. adults and phytophagous mites correlated positively with spiders. Numbers of mites and Hemiptera phytophagous correlated positively with those of lady beetles and *Sycophila* sp. (Hymenoptera: Eurytomidae), respectively. The number of *Ablerus magistretti* Blanchard (Hymenoptera: Aphelinidae) adults correlated negatively with *Sycophila* sp. The number of *Agistemus* sp. (Acari: Stigmaeidae) correlated, negatively and positively, with those of lady beetles and phytophagous mites, respectively. Free-feeding herbivores affected the presence of galling insects (Hymenoptera) on *C. brasiliense* trees, competing for food and space. The same was observed between two parasitoids of *Eurytoma* sp. galling insect, which can reduce the natural biological control of this pest.

Keywords: competition hypothesis, parasitoids, pequi, predators, gall niche overlap.

Resumo

Insetos galhadores são abundantes na natureza, encontrados em muitos ecossistemas globalmente, com espécies atacando plantas de importância econômica. Os efeitos de herbívoros de alimentação livre na abundância de insetos galhadores em árvores de *Caryocar brasiliense* (Caryocaraceae) no Cerrado brasileiro (Savana) foram estudados. A porcentagem de desfolha e o número de ácaros fitófagos ou de Hemiptera fitófagos correlacionaram-se, negativamente, com a percentagem de folhas galhadas e de adultos do parasitoide *Eurytoma* sp. (Hymenoptera: Eurytomidae), respectivamente. Porcentagem de folhas galhadas e o número de *Eurytoma* sp. adultos e ácaros fitófagos correlacionaram-se, positivamente, com o de aranhas. Os números de ácaros e fitófagos Hemiptera correlacionaram-se, positivamente, com os de joaninhas e *Sycophila* sp. (Hymenoptera: Eurytomidae), respectivamente. O número de adultos de *Ablerus magistretti* Blanchard (Hymenoptera: Aphelinidae) correlacionou-se, negativamente, com *Sycophila* sp. O número de *Agistemus* sp. (Acari: Stigmaeidae) correlacionou-se, negativa e positivamente, com os de joaninhas e ácaros fitófagos, respectivamente. Herbívoros de alimentação livre afetaram a presença de insetos galhadores (Hymenoptera) em árvores de *C. brasiliense*, competindo por alimento e espaço. O mesmo foi observado entre dois parasitoides de *Eurytoma* sp., o que pode reduzir o controle biológico natural dessa praga.

Palavras-chave: hipótese de competição, parasitoides, pequi, predadores, sobreposição de nicho de galhas.

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Received: November 4, 2021 – Accepted: April 7, 2022

1. Introduction

The *Caryocar brasiliense* Camb. (Caryocaraceae) trees are widely distributed in the Brazilian Cerrado (Savanna) where this plant can reach over 10 m high and 6 m of canopy width (Leite et al., 2006). Fruits of *C. brasiliense* are used by humans as food, being the primary income source of many communities. Federal laws in Brazil protect this tree, and, therefore, they are left in deforested areas of the Cerrado (Leite et al., 2006). Isolated *C. brasiliense* individuals in the agro-landscape are subjected to higher leaf, flower, and fruit damage by herbivorous insects (e.g., Hemiptera and Coleoptera), including galling insects and mites (Leite et al., 2012, 2016, 2017, 2020a, b, 2021, 2022; Demolin-Leite, 2024).

Galling insects are among the most sophisticated herbivores because they modify the tissues of their host plants to produce highly specialized structures, named galls, where their larvae develop protected from the hostile environment while feeding on a rich food source (Price et al., 1987; Shorthouse et al., 2005). Galling insects are highly abundant in nature and found globally in many ecosystems, besides damaging plants of economic importance (Araújo et al., 2013). The system with a common and economically important Cerrado tree species, *C. brasiliense*, and its four galling herbivores, and several free-feeding organisms in central Brazil is appropriate to evaluate the hypotheses of competition for free space and food between galling and free-feeding species. Competition between predatory mites, tending ants, and galling insects, among others, has been reported and related to the dispute over food, protection and space (van der Hammen et al., 2012; Macke et al., 2012; Leite et al., 2017; Liu et al., 2020; Supriya et al., 2020).

Competing organisms use various ways to avoid each other. Indirect competition, via chemical defenses or nutritional quality of their shared host plants, is found between phytophagous insects from different feeding guilds (Cunan et al., 2015). The content of amino acid is five-fold lower in ungalled leaves than on galled ones and, consequently, can affect attractiveness to free-feeding insects (Koyama et al., 2004). Highest leaf damage by *Trirhabda* sp. (Coleoptera: Chrysomelidae) on *Solidago altissima* L. (Asteraceae) plants reduced the gall numbers by *Eurosta solidaginis* (Fitch) (Diptera: Tephritidae) (Cunan et al., 2015). Stemboring insects (an unknown lepidopteran) reduced *Asphondylia borrichiae* Rossi and Strong (Diptera: Cecidomyiidae) gall size and increases midge crowding within galls, which likely reduced midge size and fecundity in the apical meristem of *Iva frutescens* L. (Asteraceae) (Stokes and Stiling, 2015). The number of the sap-sucking *Liothrips setinodis* (Reuter) (Thysanoptera: Phlaeothripidae) feeding on *Fagus sylvatica* L. (Fagaceae) leaves with *Hartigiola annulipes* (Hartig) (Diptera: Cecidomyiidae) galls was higher than in ungalled leaves, probably, due to the change of chemical content of the leaves with young developing galls, attracting this thrips (Pilichowski et al., 2019). The thrips might trigger defensive mechanisms of the host plant resulting in the galling insect larvae mortality. Competition between free-feeding organisms and galling increases the importance of the latter to host plants in the tropics. The study of food webs is complex due to interactions among host plants and phytophagous organisms, and few studies have examined food webs in complex ecosystems such as the Cerrado (Morris et al., 2004).

We tested the competition hypothesis between free-feeding organisms (Coleoptera and Lepidoptera), sucking insects (Hemiptera), and phytophagous mites (Acari) with galling insects (Hymenoptera) at a population level.

2. Materials and Methods

This study was performed in the municipality of Montes Claros, Minas Gerais state, Brazil, for three consecutive years (Jun 2008 through Jun 2011). The region has dry winters and rainy summers with climate Aw: tropical savanna according to Köppen (Alvares et al., 2013). The study was performed in three areas: *strict sensu* Cerrado, pasture formerly with Cerrado vegetation and an urban area in the *Campus* of the "Instituto de Ciências Agrárias da Universidade Federal de Minas Gerais (ICA/UFMG)". Details of these areas as latitude, longitude, altitude, soil classifications, physical and chemical characteristics, floristic and arthropod diversities, and *C. brasiliense* canopy height and width have been described (Leite et al., 2006, 2012, 2016, 2020a, b).

The study design was completely randomized with 12 replications (12 tree individuals) growing in these three areas. At each site, data were collected on *C. brasiliense* adult trees (producing fruits) at every 50 m along a 600 m transect. For the 12 replications, we collected data for three consecutive years to capture more arthropod species (including rare species) in a given year or area with no application of fertilizers or pesticides. Permission to collect in these locations/activities was granted by the landowner (UFMG). The collected arthropods are not endangered and do not represent protected species.

The distribution of galling insects and their galls, predators, parasitoids, percentages of leaves infested with galls (three leaflets/leaf) and defoliation by insects, and phytophagous Hemiptera was recorded by examining 12 fully expanded leaves of the 36 *C. brasiliense* trees (one leaf in each vertical and horizontal stratifications of the canopy) (Leite et al., 2020a). Sampling was performed in the morning (7:00-11:00 AM) by direct visual observation every month (Silva et al., 2020). Insects were collected with tweezers, brushes, or aspirators and preserved in vials with 70% alcohol for identification by taxonomists. Insect defoliation was evaluated visually by the leaf area losses on a 0–100% scale with 5% increments for removed leaf area (Silva et al., 2020) for the 36 trees, per evaluation. These leaves were collected and transported to the laboratory, where the numbers of the nymphs and adults (sum) of mites (phytophagous and predators) were counted. The counting started within 2 h after material collection and was performed by examining the leaves under a binocular microscope with 12.5× magnification. The mites were counted in three fields located in the central area (equidistant between the principal vein and the margin) of each leaf (abaxial and adaxial surface) (Leite et al.,

2020b). The mites on *C. brasiliense* leaves were collected with a brush and preserved in vials with 70% alcohol for identification by Dr. A. L. Matioli (several families) and Dr. E. A. Ueckermann (*Agistemus*). Subsequently, leaves were placed inside a white plastic pot (temperature 25ºC). We evaluated the emergence of galling insects, parasitoids, and hyperparasitoids for each collected sample at every alternate day during the 30 days. The emerged insects were collected and preserved as described for the identification by taxonomists. The voucher number for spiders is IBSP 36921-36924 (Instituto Butantan, São Paulo state, Brazil) and for insects are 1595/02 and 1597/02 (CDZOO, Universidade Federal do Paraná, Paraná state, Brazil).

Averages of the abundance of arthropods and percentages of leaves infested with galls and defoliation were realized by reducing the data per leaf/tree in each area. Correlations of the arthropods data were subjected to principal component regressions (PCR) ($P< 0.05$) using the statistical program System for Analysis Statistics and Genetics (SAEG), version 9.1 (SAEG, 2007) (Supplier: "Universidade Federal de Viçosa"). The regression model known as PCR, or regression on principal components, uses principal component analysis to perform regression based on the covariance matrix (Bair et al., 2006). Thus, it can reduce the regression dimension by excluding the dimensions that contribute to causing multicollinearity problems, that is, linear relationships between the independent variables. The parameters used in these regressions were those significant ($P < 0.05$) to select the variables for the method "Stepwise".

3. Results

Percentage of defoliation and number of phytophagous mites or phytophagous Hemiptera correlated negatively with the percentage of leaf galled and the number of *Eurytoma* sp. (Hymenoptera: Eurytomidae) adults, respectively (Figure 1A, B). Percentage of leaf galled and the number of *Eurytoma* sp. adults correlated negatively with those of mites and Hemiptera phytophagous, respectively (Figure 1C, D).

Percentage of leaf galled and numbers of *Eurytoma* sp. adults and phytophagous mites correlated positively with spiders (Figure 1A-C). Numbers of mites and Hemiptera phytophagous correlated positively with those of lady beetles *Neocalvia fulgurata* (Mulsant) (Coleoptera: Coccinellidae) and *Sycophila* sp. (Hymenoptera: Eurytomidae), respectively (Figure 1C, D). The number of *Ablerus magistretti* Blanchard (Hymenoptera: Aphelinidae) adults correlated negatively with that of *Sycophila* sp. and vice-versa, and both positively, with that of *Eurytoma* sp. adults (Figure 1E, F). The number of *Agistemus* sp. (Acari: Stigmaeidae) correlated, negatively and positively, with those of lady beetles and phytophagous mites, respectively (Figure 1G).

4. Discussion

The competitions between defoliators, Hemiptera, and mites phytophagous with hymenopteran galling insects observed on *C. brasiliense* trees confirm our hypothesis: free-feeding herbivores can affect galling insects (Hymenoptera) presence. The competition between free-feeding and galling insects (phytophagous Hemiptera x galling insects) and galling insect's parasitoids (*A. magistretti* x *Sycophila* sp.) on *C. brasiliense* trees are probably related to the dispute over food and space. Competitions between insects were recorded for sap-sucking galls, Cecidomyiidae, and two caterpillar parasitoids (De Moraes et al., 1999; Pilichowski et al., 2019). The competition between species can be a key factor in the dynamics of the plant community, herbivores, and parasitoids. Another interesting competition mechanism reported on leaves of *Lycium barbarum* L. (Solanaceae), after the infestation of *Aceria pallida* Keifer (Eriophyoidea) (phoretic gall mite) galls, did not favor the development of *Bactericera gobica* (Loginova) (Hemiptera: Psylloidea) nymphs (prolonged in 5.6%) and increased its mortality (from 19.0 to 36.3%), due to, probably, this mite, besides to be a galler, it is also phoretic in this sucking insect (Li et al., 2018). This association between this gall mite and *B. gobica* is for transportation to its hibernation sites for survival and is seasonably taken back to the *L. barbarum* leaf (Li et al., 2018). This fact can explain the negative effect of infestation by these psyllids in the number and size of mite galls on *L. barbarum* leaves. Both arthropods live and feed on the same leaf and compete for plant resources during the growing season, with almost no direct contact between them. However, how the *A. pallida* gall mite, possibly a superior competitor, interacts with *B. gobica* (its vector) during the growing season to achieve the phoretic association is not clear (Li et al., 2018).

Numbers of *Eurytoma* sp. adults and phytophagous mites increased with that of spiders, probably, due to these organisms being important predators of Coleoptera defoliators (< defoliation) (Venturino et al., 2008; Leite et al., 2012; 2016) and, consequently, more free space and food for galling insects and mites, increasing their numbers on *C. brasiliense* trees. The numbers of mites and Hemiptera phytophagous increased with those of lady beetles *N. fulgurata* and *Sycophila* sp., respectively, on *C. brasiliense* trees. Lady beetles feed on sternorrhynchan hemipteran (e.g., aphids) and phytophagous mites, when these predators are very small (non-case of *N. fulgurata*) (Wäckers et al., 2017; Kaneko, 2018), and, consequently, favoring the phytophagous mite population, maybe, due to less competition with sucking insects. Another possibility is this lady beetle feeding also on *Agistemus* sp., bigger than species of the Tetranychidae genus, increasing phytophagous mites and reducing this important mite predator (Leite et al., 2020b). *Sycophila* sp. is a major *Eurytoma* sp. parasitoid and its high population can reduce the numbers of these galling insects (Leite et al., 2013) and, maybe, leaving more space for the sucking insects. The negatively correlation between the number of *A. magistretti* adults with *Sycophila* sp. and vice-versa (both are *Eurytoma* sp. parasitoids), suggests competition between them. Negative interactions such as intra- or interspecific competition occur when several parasitoid females simultaneously search for the same host (Barakat et al., 2020). This competition can occur among adult parasitoids (extrinsic competition) or between larvae developing (intrinsic competition) of: i) same species (superparasitism) or ii) different species (multiparasitism)

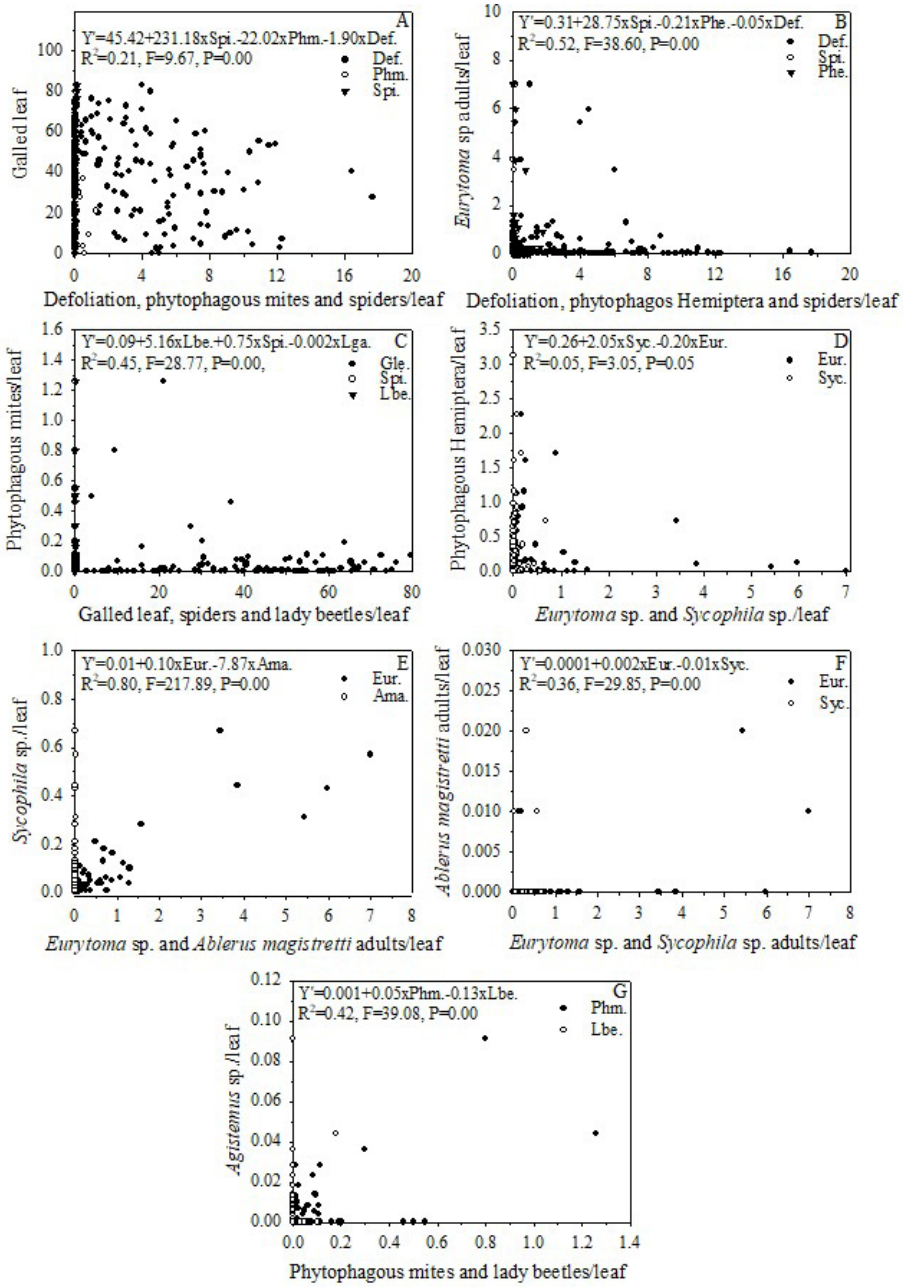

Figure 1. Principal components regressions among: (A) galled leaf (%) (Gle.) with spiders (Spi.), phytophagous mites (Phm.), and defoliation (%) (Def.); (B) *Eurytoma* sp. adults (Eur.) with Spi., phytophagous Hemiptera (Phe.), and Def.; (C) Phm. with lady beetles (Lbe.), Spi, and Gle.; (D) Phe. with *Sycophila* sp. adults (Syc.) and Eur.; (E) Syc. with Eur. and *Ablerus magistretti* adults (Ama.); (F) Ama. with Eur. and Syc.; and (G) *Agistemus* sp. with Phm. and Lbe. on 36 *Caryocar brasiliense* trees during three years. The symbols represent averages per leaf/tree. n=111.

(Barakat et al., 2020). This competition was reported for *Cardiochiles nigriceps* Viereck and *Microplitis croceipes* (Cresson) (Hymenoptera: Brachonidae) in *Heliothis virescens* F. (Lepidoptera: Noctuidae) caterpillars and *Trissolcus basalis* Woll. and *Ooencyrtus telenomicida* (Vassiliev) (Hymenoptera: Encyrtidae) in *Nezara viridula* L. (Hemiptera: Pentatomidae) eggs (De Moraes et al., 1999; Cusumano et al., 2012). Competitive interactions between parasitoids are relevant in the biological control since these interactions affect the mortality of the targeted hosts and more species that are introduced; the greater the effect of reducing pest density, but multiple species can also disrupt biological control (Cusumano et al., 2012; Barakat et al., 2020).

Free-feeding herbivores reduced the numbers of galling insects (Hymenoptera) on *C. brasiliense* trees, competing

for food and space. The same was observed between two parasitoids of *Eurytoma* sp. galling insect, which can reduce the natural biological control of this pest.

Acknowledgements

To the Brazilian institutions "Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq)", "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-Finance Code 001)", "Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG)" and "Programa Cooperativo sobre Proteção Florestal (PROTEF) do Instituto de Pesquisas e Estudos Florestais (IPEF)" for financial support.

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