

## SELECTION OF LEAVES OF *PALICOUREA PADIFOLIA*, *PLATANUS MEXICANA* AND *ERIOBOTRYA JAPONICA* BY *ATTA MEXICANA* (HYMENOPTERA: FORMICIDAE) AND ITS INFLUENCE ON THE GROWTH OF THE MUTUALISTIC FUNGUS *LEUCOAGARICUS GONGYLOPHORUS*

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### Abstract

**Background:** The foraging preference of *Atta mexicana* for leaves of three plant species was analyzed under laboratory conditions. Physico-chemical characterizations, bioassays with leaf material and crude extracts, and tests on the effect on the mutualistic fungus *Leucoagaricus gongylophorus* were conducted.

**Question:** Does the interaction between *A. mexicana* and its mutualistic fungus influence the selection of *Palicourea padifolia* leaves over *Platanus mexicana* and *Eriobotrya japonica*?

**Species study:** *Atta mexicana*, *Leucoagaricus gongylophorus*, *Palicourea padifolia*, *Platanus mexicana*, *Eriobotrya japonica*.

**Study site and date:** Xalapa, Veracruz, Mexico, 2023.

**Methods:** Foraging preferences were evaluated under laboratory conditions. Leaves were physically and chemically characterized. Insecticidal and antifungal tests with methanolic extracts were performed, and specialized metabolites were detected through qualitative assays.

**Results:** *A. mexicana* preferred leaves of *P. padifolia*. Less selected species exhibited trichomes, higher toughness, and elevated levels of phenols, flavonoids, and tannins. In contrast, *P. padifolia* had glabrous leaves, high moisture, and low metabolite content, favoring its acceptance. Methanolic extracts showed no insecticidal activity. The extract of *P. padifolia* promoted the growth of *L. gongylophorus*, while those of *P. mexicana* and *E. japonica* moderately inhibited it.

**Conclusions:** *Atta mexicana* preferred *P. padifolia* leaves due to their characteristics, and the extract promoted mutualistic fungus growth. Leaf toughness and trichomes were barriers to foraging. Trichome-removing behavior of *A. mexicana* is reported for the first time.

**Keywords:** antifungal, herbivory, mutualism, specialized metabolites, trichomes.

### Resumen

**Antecedentes:** Se analizó la preferencia de forrajeo de *Atta mexicana* por hojas de tres especies vegetales en condiciones de laboratorio. Se realizaron caracterizaciones fisicoquímicas, bioensayos con material foliar y extractos crudos, así como pruebas sobre el efecto en el hongo mutualista *Leucoagaricus gongylophorus*.

**Pregunta:** ¿La interacción entre *A. mexicana* y su hongo mutualista influye en la selección de hojas de *Palicourea padifolia* frente a *Platanus mexicana* y *Eriobotrya japonica*?

**Especies estudiadas:** *Atta mexicana*, *Leucoagaricus gongylophorus*, *Palicourea padifolia*, *Platanus mexicana*, *Eriobotrya japonica*.

**Sitio de estudio y fecha:** Xalapa, Veracruz, México, 2023.

**Métodos:** Se evaluaron las preferencias de forrajeo bajo condiciones de laboratorio. Las hojas se caracterizaron física y químicamente. Se realizaron pruebas insecticidas y antifúngicas con extractos metanólicos, y los metabolitos especializados se detectaron mediante ensayos cualitativos.

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**Resultados:** *A. mexicana* prefirió hojas de *P. padifolia*. Las especies menos seleccionadas presentaron tricomas, mayor dureza y niveles elevados de fenoles, flavonoides y taninos. En contraste, *P. padifolia* tuvo hojas glabras, con alta humedad y bajo contenido de metabolitos, lo que favoreció su aceptación. Los extractos metanólicos no mostraron actividad insecticida. El extracto de *P. padifolia* promovió el crecimiento de *L. gongylophorus*, mientras que los de *P. mexicana* y *E. japonica* lo inhibieron moderadamente.

**Conclusiones:** *Atta mexicana* prefirió hojas de *P. padifolia* por sus características, y el extracto promovió el crecimiento del hongo mutualista. La dureza foliar y los tricomas actuaron como barreras al forrajeo. Se reporta por primera vez el comportamiento de remoción de tricomas en *A. mexicana*.

**Palabras clave:** antifúngico, herbivoría, metabolitos especializados, mutualismo, tricomas.

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Leaf-cutting ants belong to the genera *Acromyrmex* (Mayr 1865) and *Atta* (Fabricius 1805) and are distributed in the neotropics of the Americas (Barrera *et al.* 2022). Leaf-cutting ants are known to search for natural plant materials and determine if they are suitable for the colony. This acceptance is influenced by various characteristics of plants, as well as the communication of information about food among workers from the colony (Travaglini *et al.* 2015).

Leaf-cutting ants can cause significant damage to crops of agricultural or commercial interest, and therefore these insects are considered a pest (Infante-Rodríguez *et al.* 2020). Leaf-cutting ants account for about 25 % of all herbivory in Neotropical forest ecosystems, moving 10-15 % of leaves in their foraging range to their nests (Swanson *et al.* 2019). However, they also play important ecological roles as large defoliators and participate in ecological and biogeochemical processes, such as modifying plant communities around nests, influencing carbon cycling and nutrient fluxes (Haines 1978). For example, leaf-cutter ants disturb the soil structure during nest excavation changing soil aeration and temperature. They mix relatively nutrient-poor soil from deeper layers with the upper organic-rich layers increasing the heterogeneity of carbon and nutrients within nest soils or creating canopy openings that facilitate the recruitment of new plant species, changing the light, wind and temperature regimes, which affects ecosystem processes (Swanson *et al.* 2019). In addition, they act as soil modifiers by altering the soil horizon through removal and by concentrating on nutrients released by the waste material dumped outside of mushroom gardens (Abril & Bucher 2004).

Mutualism between ants and fungi involves ant species belonging to the subfamilies Dolichoderinae, Formicinae, Myrmicinae, and Pseudomyrmecinae. Among them, the myrmicinae species of the subtribe Attini, which primarily cultivate Basidiomycota fungi of the Agaricaceae family for food (Dejean *et al.* 2023a). The leaf-cutting and fungus-growing abilities are not limited to the higher Attini subtribe, which is restricted to the Americas. These abilities are also found in a *Crematogaster* species (*C. clariventris*) from Africa. In this case, fungal mycelia are used to reinforce the carton of the nests by forming a hard-composite material that resists heavy equatorial rains, rather than being cultivated for food (Dejean *et al.* 2023b).

Leaf-cutting ants depend on fungus cultivation as their primary food source (Mehdiabadi & Schultz 2010). These ants cultivate the fungus with such efficiency that they can maintain it free of microbial pathogens (Currie 2001). For example, they defend their cultivated fungus gardens from parasitic fungi by combining specialized anti-parasite behaviors with their mutualistic bacterial symbionts (actinomycetous bacteria), which produce antibiotics to control fungal pathogens. This symbiotic relationship extends beyond the ants and their fungus gardens to encompass this protective bacterial community, demonstrating a complex defense system involving multiple species (Little *et al.* 2006).

The cultivated fungus species is *Leucoagaricus gongylophorus* (Möller) Singer, which belongs to the Agaricaceae family (Basidiomycota: Agaricales). This mutualistic symbiotic interaction is obligatory (Silva *et al.* 2006). The ants' capacity to cultivate fungi for consumption has been traced back approximately 15 million years (Schultz & Brady 2008, Branstetter *et al.* 2017), coinciding with a transition from a low-specialized open cultivation system to a highly specialized domesticated fungal cultivar (Mueller & Rabeling 2008).

The cultivation of *L. gongylophorus* by ants involves the collection of substrates, such as leaves, flowers, fruits, or seeds, from a variety of plant sources (Infante-Rodríguez *et al.* 2020). *Leucoagaricus gongylophorus* provides the enzymes necessary to degrade plant polymers, releasing sugars for their energy needs. Recent research suggests that autophagy regulates gongyliid morphogenesis in *L. gongylophorus*, constituting the mechanism by which exclusive

nutritional rewards are generated for leaf-cutting ants, reflecting a unique fungal domestication process within this symbiosis (Leal-Dutra *et al.* 2023).

Ants benefit from this interaction when they consume the modified structures of the hyphae, called gongylidia, which provide them with glucose, proteins, and hydrolytic enzymes (Rønhede *et al.* 2004). However, this fungus can degrade complex polymers such as cellulose or lignocellulose (Abril & Bucher 2004, Viguera *et al.* 2017) using lignocellulolytic enzymes such as endoglucanase, xylanase, or laccases (Maya-Yescas *et al.* 2021).

Plants have developed diverse strategies for protection against herbivores and microorganisms, including chemical protection by specialized metabolites (Fürstenberg-Hägg *et al.* 2013). In this sense, there has been an increase in the study of toxic plants with insecticidal or fungicidal properties as an agro-ecological alternative for the control of leaf-cutting ants and their symbiotic fungus (Bueno *et al.* 2005, Boulogne *et al.* 2012).

Extracts from 11 plant species have been reported as antifungal against *L. gongylophorus*, where intrinsic factors of the plant material, as well as the method and type of solvent used to obtain the extracts, may enhance their antifungal activity (Araújo *et al.* 2022). Most active compounds reported against the members of this symbiosis are alkaloids, terpenes, and phenols (Infante-Rodríguez *et al.* 2020). Leaf extracts of *Capsicum baccatum* (L.) and *Capsicum frutescens* (L.), which are rich in alkaloids, terpenes, and phenols, have been reported as alternatives for controlling *Atta cephalotes* and its symbiont fungus (Lobo Echeverry *et al.* 2016). Additionally, the presence of lignans and flavonoids in the leaves of *Virola sebifera* (Aubl.) show insecticidal effect on *Atta sexdens* and a fungicidal effect on its symbiont fungus (Bicalho *et al.* 2012). Tannins cause death in herbivorous insects by affecting their digestive processes and growth (Perkovich & Ward 2020). Some tannins may also harm leaf-cutting ants and their mutualistic fungus (Littleldyke & Cherrett 1976).

The most complete phylogeny of the genus *Atta* was reconstructed using ultra-conserved elements (UCEs), confirming its monophyly and the validity of its four main clades. The results revealed that the extant species of *Atta* arose through a recent and rapid radiation from the Miocene to the early Pleistocene. This radiation originated in North, Central, and northwestern of South America was followed by successive dispersals into distinct South American biomes (Barrera *et al.* 2022). *Atta mexicana* (F. Smith 1858) is distributed from the United States of America to Colombia (Mintzer 1979, AntWeb 2020). This species is a pest of agricultural interest in Mexico (Serratos-Tejeda *et al.* 2017), and it is responsible for the defoliation of numerous crops and ornamentals in tropical regions (Rivera *et al.* 2016). Mintzer (1995) estimated that an active colony collects approximately 43,000 to 252,000 leaf fragments per day, which is equivalent to 230 kilograms per year based on dry weight. Valdés-Rodríguez *et al.* (2015) documented moringa tree defoliation in individuals less than 1.5 meters tall. *Atta mexicana* is a foraging pest of sugarcane, particularly during the dry season (Murguía-González *et al.* 2018). Also, it is able to defoliate maize and bean plants during the early growth stages and recent reports show that it damages crops of commercial opuntia species in Mexico (Zárate-Castrejón *et al.* 2020). However, there is limited information on its behavior, foraging preferences, diet, and control methods (Infante-Rodríguez *et al.* 2020). The plant material selection criteria by leaf-cutting ants include characteristics such as moisture (Cherrett 1972, Bowers & Porter 1981) and nutrients (Mundim *et al.* 2009). Factors involved in plant rejection include hardness, thickness (Rockwood 1976), leaf structural defenses such as waxes (Hubbell *et al.* 1984) and trichomes (Kitayama *et al.* 2010), presence of endophytic fungi (Rocha *et al.* 2014), and concentration of certain specialized metabolites (Vasconcelos & Cherrett 1995, Van Bael *et al.* 2011, Infante-Rodríguez *et al.* 2020).

Preliminary observations made in 2018 by our group while maintaining laboratory colonies using plant material obtained from mountain cloud forest fragments in central Veracruz, Mexico, revealed that *Palicourea padifolia* (Humb. & Bonpl. ex Roem. & Schult.) C.M. Taylor & Lorence (Rubiaceae), *Platanus mexicana* Moric. (Platanaceae), and *Eriobotrya japonica* (Thunb.) Lindl. (Rosaceae) are foraged with different intensities by *A. mexicana*. The leaves of these species exhibit a range of varied characteristics. A notable difference is that *P. padifolia* lacks trichomes, while *E. japonica* and *P. mexicana* have them.

Based on these observations, this study tested the hypothesis that less-foraged species have structural defenses or contain phytochemicals detrimental to the ant-fungus symbiosis. To evaluate this hypothesis, the objectives of the

present study were: I) to evaluate the foraging preferences of *A. mexicana* on leaf material of *P. padifolia*, *P. mexicana*, and *E. japonica* in laboratory-maintained colonies; II) to determine if physical characteristics and moisture content of the leaves from this species affect their selection; III) to perform a qualitative phytochemical profile of the foliar methanolic extracts; and IV) to determine the effect of the foliar methanolic extracts on the survival of the ants and the growth of the symbiont fungus.

## Materials and methods

**Plant material.** To determine the foraging preferences of *A. mexicana* on selected species during spring 2019, young leaves of each species were collected from three different individuals at the Botanical Garden of the Institute of Ecology, Xalapa, Veracruz, Mexico (19° 30' 51.5" N, 96° 56' 32.31" W). As a proxy, young leaves can be differentiated from older ones based on their morphological characteristics. They are generally smaller because they are still in the process of expanding. They are also lighter green or reddish in color. Young leaves are tender, thin, and flexible, while older leaves become rigid or leathery. Their position on the plant also helps distinguish them. Young leaves are mainly located at the tips of stems and branches, while older leaves are mainly located toward the base of shoots or older branches. The curator of the herbarium XAL confirmed the identification of these species, and voucher specimens were deposited as: *Palicourea padifolia* (voucher XAL0106254); *Eriobotrya japonica* (voucher XAL59120) and *Platanus mexicana* (voucher XAL59120).

**Foraging preference assays.** Leaf foraging assays.- Six sub-colonies were created from six maternal nests established in the laboratory to evaluate foraging preferences. A 250 mL plastic bottle containing 10 g of mutualistic fungal mycelium (containing ant pupae, larvae, and workers) was used to establish each sub-colony. The 250 mL plastic bottle was connected to a 1.5 L rectangular plastic container that served as a foraging area. Leaves were obtained from three individuals of each plant species (two leaves per individual). Those leaves were selected with similar size within each species. There was no observable damage from herbivory or necrosis, and the leaves were offered randomly to the six experimental sub-colonies.

One leaf of each plant species was placed in each foraging arena. Before exposure to ants, the surface area of each leaf was measured in square centimeters using millimetric paper. The leaves were exposed to the ants for 24 h, and then the plant material was removed. The consumed leaf area was measured in square centimeters using millimetric paper.

**Leaf-disc foraging assays.**- The leaf disc foraging experiment was performed using the protocol described by Infante-Rodríguez *et al.* (2020). Six new sub-colonies were established similarly to the leaf consumption tests, but in this experiment, we offered 30 leaf discs of 0.5 cm in diameter for each plant species; these discs were obtained from leaf material from three different individuals (10 discs per plant) for each species and were offered randomly to each ant sub-colony for 24 h. The number of discs brought into the sub-colonies was counted and the remaining discs were removed from the food area. Then, each sub-colony was left without food for two hours. After that, a new set of 30 discs of each species was offered and the process repeated until all sub-colonies were exposed to the three test plant species.

**Characterization of leaf material.** To determine whether mechanical or phytochemical characteristics could be involved in the acceptance or rejection of plant species, leaf attributes such as hardness, thickness, presence or absence of trichomes, moisture content, and qualitative tests for specialized metabolites were evaluated using the same plant individuals tested in the foraging assays. Trichomes are reported as present or absent on the leaves. To determine leaf thickness, 10 leaves were randomly collected from the three individuals of each species. The leaf thickness (mm) was measured in the apical, central, and basal part of each leaf (avoiding the leaf veins) using a digital micrometer (Mitutoyo IP65). The average of the three measurements was used as the leaf thickness value per leaf. From 10 new leaves collected as it is described above, the hardness was estimated using a handmade leaf penetrometer (Dirzo 1987). The average of the three measurements was used as the leaf hardness value (kg/cm<sup>2</sup>).

Leaf moisture.- For each species, the moisture content (%) was estimated from 10 leaves randomly selected from three individuals for each species. For each leaf, the fresh and dry weights were obtained using an analytical balance (OHAUS explorer ex224). The leaves were placed in a drying oven at 45 °C for 72 h to get the dry weight. The moisture content (MCL) was estimated as a percentage using the equation:

$$\text{MCL} = \left[ \frac{\text{LFW}-\text{DW}}{\text{LFW}} \right] \times 100$$

where MCL = the moisture content (%), LFW = is the leaf fresh weight, DW = is the leaf dry weight (Jin *et al.* 2017).

Freeze-dried leaf material.- Leaves (250 g) from each individual per species were in a freeze dryer (LABCONCO FreeZone with an 86 L/min vacuum pump) at -56 °C for seven days. Then, the freeze-dried leaves were pulverized using a mortar and pestle and stored in plastic bags at 4 °C.

*Methanolic leaf extract preparation.* Using the extraction protocol reported by Infante-Rodríguez *et al.* (2020), 3 g of lyophilized leaves were used to obtain methanolic extracts using an accelerated solvent extraction system (ASE 350, Dionex-Thermo Scientific, USA). Then, a rotary evaporator (RII, Büchi, Flawil, Switzerland) was used at 40 °C and a vacuum of 337 mbar until the leaf extracts were completely dried and solvent eliminated.

Qualitative phytochemical profile.- Phytochemical analyses were performed on 1 mg of the methanolic extract of each species to determine the presence of alkaloids, terpenoids, steroids, coumarins, phenolics, flavonoids, and tannins according to the methodology described by Domínguez (1973) and Infante-Rodríguez *et al.* (2022). All qualitative assays were performed in triplicate. The results were classified according to the formation of precipitates for alkaloids, UV fluorescence for coumarins, and color intensity for the rest of the metabolites. The presence of each group of metabolites in the samples was categorized as absent, low, medium, or high.

Effect of methanolic extracts on ant survival.- Ants were divided into treatment groups of 20 individuals from the same worker caste and deprived of food and water for 24 h. The bioassay was performed with one of the following treatments: 1 mL of purified water (negative control), 1 mL of spinetoram (Palgus TM, Dow Agrosciences, Mexico) at 0.43 mg/mL (positive control) The methanolic extracts of *P. padifolia*, *P. mexicana* and *E. japonica* were evaluated at a concentration of 10 mg/mL. For each replicate, a microcentrifuge tube sealed with cotton wool containing 1 mL of each treatment was used. Ants were placed in 100 mL plastic jars with lids. Mortality rates were recorded after 48 hours. Major workers were selected from the laboratory mother nests and collected from the jars containing the foraging material supplied to each colony. This allowed to ensure that each experimental replicate had only foraging workers.

These experiments were performed with 4 replicates and the results are reported as a percentage of mortality corrected using the Abbott formula:

$$\text{Mortality (\%)} = \left[ \frac{\text{mt\%} - \text{mtc\%}}{100 - \text{mtc\%}} \right] \times 100$$

Where mt is the mortality in the treatment and mtc is the mortality in the control (Abbott 1925).

*In vitro isolation of L. gongylophorus.* A strain of the mutualistic fungus of *A. mexicana* was isolated from a nest maintained under the laboratory conditions described by Infante-Rodríguez *et al.* (2024a). Samples of 1 mm<sup>3</sup> of mycelium were taken from the fungal garden (n = 50) of the ant nest. These mycelium fragments were placed in Petri dishes (60 × 15 mm) containing 12 mL of potato dextrose agar (PDA) medium and incubated at 25 °C. The strain was maintained by serial cultures using 1 mg samples of mycelium, which were reseeded into Petri dishes containing 20 mL of PDA medium and incubated at 25 °C. The mutualistic fungal strain was previously genetically identified as *Leucoagaricus gongylophorus* (Infante-Rodríguez *et al.* 2024a).

*Mycelium growth in PDA medium plus leaf extracts.* To evaluate the effect of leaf extracts on *L. gongylophorus*, we performed mycelial growth tests on a PDA medium supplemented with 2 mg/mL of leaf methanolic extracts of the evaluated species. PDA medium supplemented with thiabendazole (T5535-50G Sigma-Aldrich) and PDA supplemented with methylthiophanate (Axione® M 70 PH) at concentrations of 2 mg/mL were used as positive controls. PDA medium was used as a negative control. Mycelial growth (mm) was measured weekly for 35 days. Mycelial growth inhibition (%) was calculated using the equation

$$GI = \left[ \frac{DC - DT}{DC} \right] \times 100$$

where GI represents the percentage of growth inhibition (%), DC is the average diameter of the mycelium of the positive control treatment (mm), and DT measures the growth of the mycelium diameter (mm) (Badawy & Abdelgaleil 2014).

*Statistical analyses.* The values obtained for leaf area consumed, hardness and thickness, and the percentage of mortality in the extracts were analyzed using the Kruskal-Wallis nonparametric test and Dunn's *post hoc* test. Leaf disc selection data, moisture content values, and mycelial growth of *L. gongylophorus* were analyzed by one-way analysis of variance followed by Tukey's *post hoc* test. Statistical analyses were performed using R software version 4.1.2 (R Core Team 2020) and the Agricolae library (De Mendiburu 2010). To group the most significant variables and to identify the main characteristics observed among the tested plants, PCA analyses and biplot were made using the factoextra library (Kassambara & Mundt 2020) in R software version 4.1.2.

## Results

*Foliar consumption.* *Atta mexicana* foragers showed a higher preference for *P. padifolia* leaves and a lower preference for *P. mexicana* and *E. japonica* leaves ( $H_{(2)} = 12.016$ ,  $P < 0.01$ ). Leaf consumption (mean  $\pm$  SE) was  $53.75 \pm 0.95$  cm<sup>2</sup> for *P. padifolia*,  $14.81 \pm 5.6$  cm<sup>2</sup> for *E. japonica*, and  $9.91 \pm 6.4$  cm<sup>2</sup> for *P. mexicana* (Figure 1).

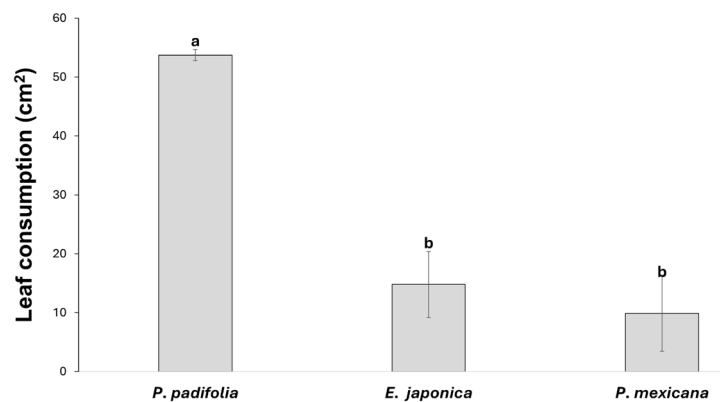


Figure 1. Leaf consumption in 24 h (cm<sup>2</sup>) (mean  $\pm$  SE) by experimental subcolonies of *A. mexicana*.

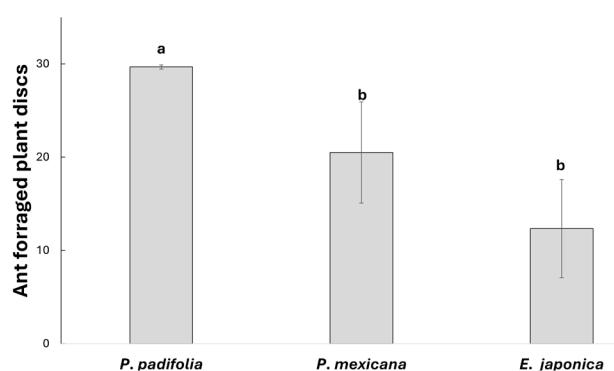
*Foraging on leaf discs.* Leaf discs were more attractive to ants than whole leaves. The ants foraged a significantly higher number of *P. padifolia* discs, which was  $29.66 \pm 0.51$  discs (mean  $\pm$  SE), compared to *P. mexicana* with  $20.5 \pm 5.42$  foraged discs, and *E. japonica* with  $12.33 \pm 5.27$  foraged discs ( $F = 3.99$ ,  $df = 2$ ,  $df_{\text{error}} = 15$ ,  $P < 0.05$ ) (Figure 2).

*Characterization of leaf material.* *Palicourea padifolia* was characterized by having glabrous leaves with low hardness ( $H_{(2)} = 37.91$ ,  $P < 0.001$ ) but thick ( $H_{(2)} = 30.646$ ,  $P < 0.001$ ) with high moisture content ( $F_{2,117} = 28.144$ ,  $P < 0.001$ ) compared to *E. japonica* and *P. mexicana*. The leaves of *E. japonica* showed the highest hardness value with  $18.4 \pm 8.57$  Kg/cm<sup>2</sup> (mean  $\pm$  SD), followed by *P. mexicana* with  $10.9 \pm 3.94$  Kg/cm<sup>2</sup> and *P. padifolia*

with  $8.7 \pm 2.44$  Kg/cm<sup>2</sup> (Table 1). In the experimental subcolonies, *A. mexicana* workers were observed to clean the trichomes of *E. japonica* to collect leaf fragments; this cleaning behavior is described for the first time in *Atta mexicana* (Figure 3).

**Qualitative phytochemical analysis.** The presence of terpenes, phenols, flavonoids, tannins, and coumarins was detected, along with an intermediate presence of alkaloids in the leaf extracts from *P. padifolia*. In *P. mexicana*, a high presence of flavonoids, a moderate presence of phenols and tannins, and a low presence of alkaloids, terpenes, and coumarins were detected. In *E. japonica*, a high presence of phenols and flavonoids, a moderate presence of tannins, and a low presence of alkaloids, terpenes, and coumarins were detected (Table 2).

**Correlation of leaf traits of studied plant species.** To reduce the complexity of our data, identify grouping patterns, and determine which variables explain the most variability in the dataset, we performed a principal component analy-



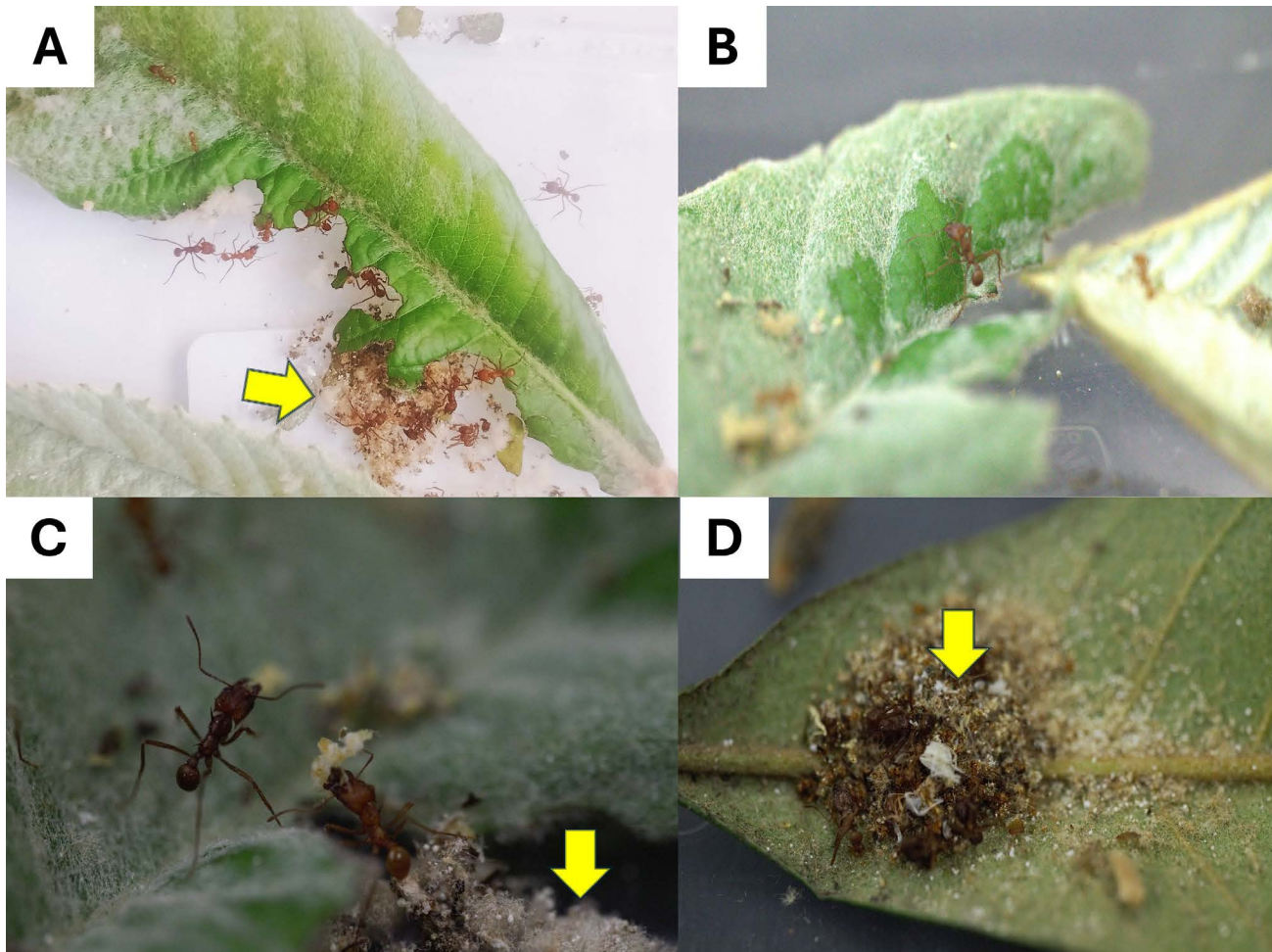
**Figure 2.** Leaf discs of *P. padifolia*, *P. mexicana*, and *E. japonica* (mean  $\pm$  SE) were collected in 24 h by the workers of *A. mexicana*.

**Table 1.** Values of hardness, thickness, moisture, presence or absence of trichomes in the studied species. In the table, the values (mean  $\pm$  SD) with different superscripts for hardness (kg/cm<sup>2</sup>), thickness (mm), and leaf moisture (%) show significant differences (Dunn or Tukey post hoc tests with  $\alpha < 0.05$ ).

Species	Hardness	Thickness	Moisture	Trichomes
<i>P. padifolia</i>	$8.7 \pm 2.44^c$	$0.12 \pm 0.02^a$	$69.1 \pm 8.54^a$	Absent
<i>E. japonica</i>	$18.4 \pm 8.57^a$	$0.10 \pm 0.10^b$	$56.3 \pm 8.54^c$	Present
<i>P. mexicana</i>	$10.9 \pm 3.94^b$	$0.10 \pm 0.09^b$	$64.14 \pm 8.19^b$	Present

**Table 2.** Qualitative phytochemical analysis in the methanolic extracts. The symbols (+), (++) and (+++) mean low, intermediate, or high presence in the leaf extract.

Secondary metabolites	Plant species		
	<i>P. padifolia</i>	<i>E. japonica</i>	<i>P. mexicana</i>
Alkaloids	++	+	+
Terpenes	+	+	+
Phenols	+	+++	++
Flavonoids	+	+++	+++
Tannins	+	++	++
Coumarins	+	+	+



**Figure 3:** Cleaning behavior of trichomes on *E. japonica* leaves by *A. mexicana* workers (A–B). Trichomes (marked with arrows) are more easily observed in the subcolony dump (C–D) of one of the experimental subcolonies.

sis (PCA). This unsupervised analysis revealed that *P. padifolia*, a preferred food plant, could be distinguished from non-preferred food plants in a multivariate analysis of leaf traits. The first two PCA components, with eigenvalues of 7.14 and 0.85, accounted for 89.4 and 10.6 % of the total explained variance, respectively. A high presence of alkaloids and the thickness value was associated with the preferred species, *P. padifolia*. In contrast, higher hardness, phenols, and trichomes were associated with the less-consumed plant, *E. japonica*. Finally, a high presence of tannins and flavonoids was a notable characteristic of *P. mexicana* leaves (Figure 4).

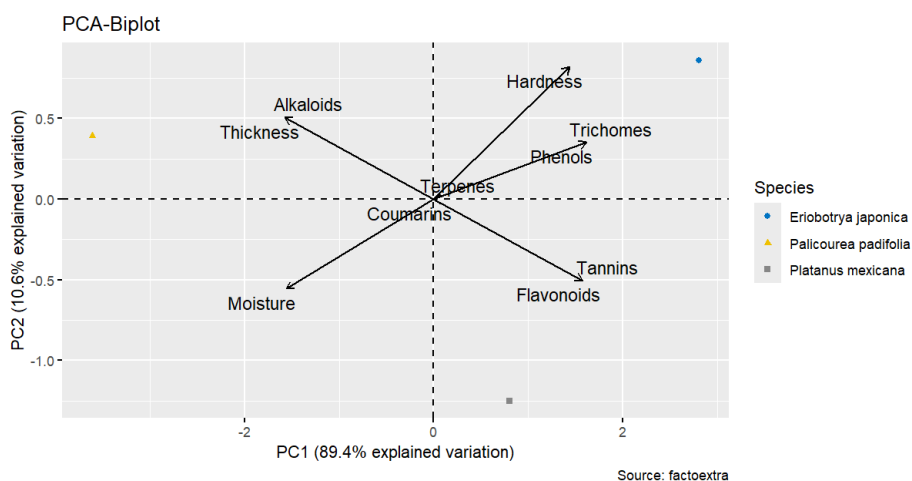
*Effect of methanolic extracts on ant survival.* The methanolic leaf extracts of *P. padifolia*, *P. mexicana*, and *E. japonica* showed no statistically significant differences in mortality compared to the negative control. *Palicourea padifolia* showed a mortality of  $10.0 \pm 2.88$  % (mean  $\pm$  SE), *P. mexicana* showed a mortality of  $27.5 \pm 12.99$  %, *E. japonica* presented  $15.0 \pm 7.07$  % mortality, and the negative control presented  $7.5 \pm 3.33$  % mortality at 48 hours. Only treatment with Spinetoram (positive control) showed a significant effect on workers cast mortality due to ingestion ( $H_{(4)} = 11.185$ ,  $P < 0.05$ ), with a mortality of  $100 \pm 0$  % (Figure 5).

*Symbiont fungus mycelial growth in PDA plus methanolic extracts.* Significant differences were observed with mycelial growth in the methanolic extracts after 35 days of measurement ( $F_{5,12} = 89.61$ ,  $P > 0.05$ ) (Figure 6). The PDA

medium enriched with methanolic extracts of *P. padifolia* (2 mg/ mL) promoted the growth of *L. gongylophorus* with a mycelial growth diameter of  $37.33 \pm 4.16$  mm (mean  $\pm$  SD) compared to the PDA positive control where it obtained a mycelial growth diameter of  $28 \pm 2$  mm. PDA media enriched with leaf extracts of *P. mexicana* and *E. japonica* had lower mycelial growth compared to the PDA with an average growth of  $22 \pm 2$  mm and  $20.66 \pm 2$  mm, respectively. The lowest mycelial growth was obtained in the negative controls of PDA with thiabendazole with an average growth of  $10.66 \pm 1.15$  mm and PDA with methyl thiophanate with  $0.6 \pm 1.15$  mm (Figure 6).

## Discussion

The results of this work provide important insights into the foraging behavior of *A. mexicana* on three selected species collected in the cloud forest of Veracruz State, which is fragmented and surrounded by agricultural fields, pastures, and coffee plantations with introduced species (Williams-Linera 2002, Williams-Linera *et al.* 2013). Currently, information on the foraging behavior of *A. mexicana* is limited and focused on shrubs from semi-arid ecosystems (Mintzer 1979, 1980) or perennial shrubs from the desert of the intertropical region of central Mexico (Zavala-Hurtado *et al.* 2000). Nowadays, the potential distribution of *A. mexicana* is concentrated in central Mexico, with the most favorable conditions in the Sierra Madre Occidental (pine-oak forests) and the dry forests of the Bajío region (Gómez-Díaz *et al.* 2023).



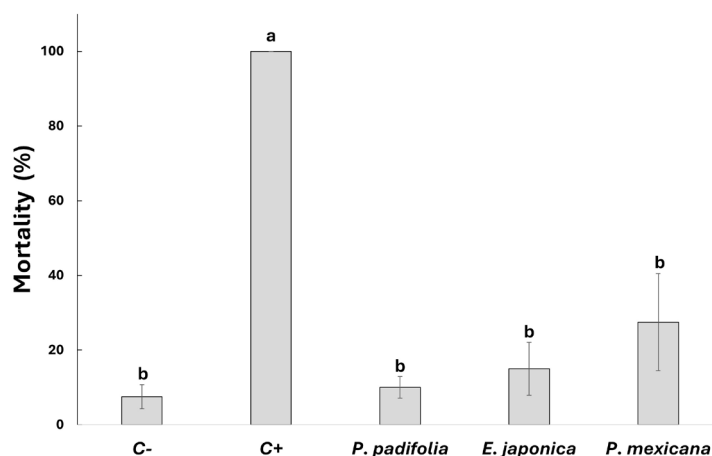
**Figure 4.** Principal Component Analysis (PCA) of mechanical and phytochemical leaf traits of three food plant species. Positively correlated variables were grouped, while negatively correlated variables were positioned on opposite sides of the plot origin. Variables that are far away from the origin are well represented on the factor map along the first two principal axes. Vectors of each variable ( $n = 10$ ) are shown with arrows and are superimposed onto the ordination plot.

Previous studies have shown that *A. mexicana* exhibits a preference for certain species within the families Zygophyllaceae, Fabaceae, Plantaginaceae, Malvaceae, Cannabaceae, Styraceae, and Rosaceae (Mintzer 1979, Zavala-Hurtado *et al.* 2000, Serratos-Tejeda *et al.* 2017, Infante-Rodríguez *et al.* 2020), and have shown rejection of some species from the families Rubiaceae, Rutaceae, and Myrtaceae (Infante-Rodríguez *et al.* 2020), where the rejected species contain compounds such as caffeine (alkaloid), quercetin (flavonoid) and chlorogenic acid (phenolic acid), suggesting that certain specialized metabolites in the leaves can affect the foraging and growth of their mutualistic fungus (Infante-Rodríguez *et al.* 2020). However, it is interesting to note that in the present study, *A. mexicana* showed greater leaf consumption on *P. padifolia* (Rubiaceae) compared to *E. japonica* (Rosaceae) and *P. mexicana* (Platanaceae). The main findings of the current study indicate that *A. mexicana* does not exclusively base its selection on plant species with a low concentration of specialized metabolites in the leaves. For instance, the leaves of *E. japonica* and *P. mexicana* exhibited elevated levels of hardness and the presence of trichomes. Leaf hardness seems

## Foraging by *Atta mexicana* and mutualistic fungus growth

**Table 3.** Eigenvalues, percentage of the total and cumulative variance, and correlation coefficients (r) between tested variables of three plant species and the first two PCA ordination axes (PC1-PC2), (\*) indicates high correlations.

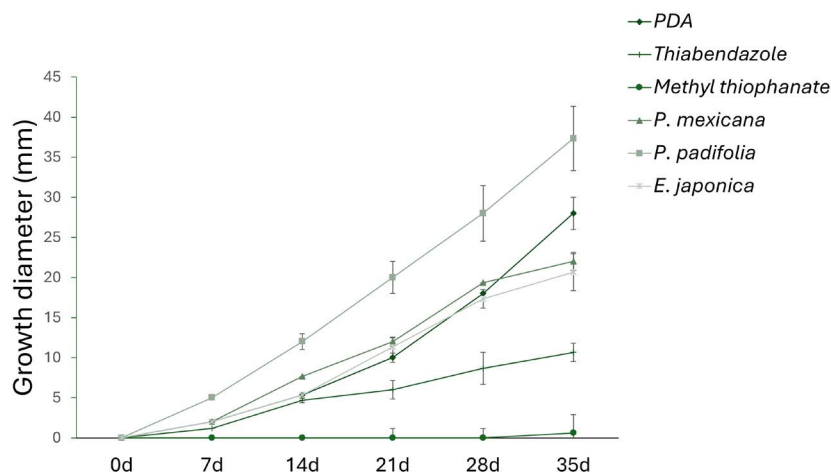
	Axis	
	PC1	PC2
Eigenvalues	7.14	0.85
% of the total variance	89.4 %	10.6 %
% of cumulative variance	89.4 %	100 %
Trichomes	0.98*	0.20
Alkaloids	-0.95*	0.31
Terpenes	0	0
Phenols	0.96*	0.26
Flavonoids	0.94*	-0.31
Tannins	0.95*	-0.31
Coumarins	0	0
Thickness	-0.95*	0.48
Hardness	0.87	0.48
Moisture	-0.95*	0.32



**Figure 5.** Mortality by ingestion (%) in *A. mexicana* at 48 h with the foliar methanolic extracts of the evaluated species, positive control (Spinetoram), and negative control (water). Bars represent the mean mortality (%) [ $\pm$ SE] in each treatment. Different letters above the bars indicate statistically significant difference at  $P < 0.05$ .

to be an important trait in the selection process of *Atta* species (Nichols-Orians & Schultz 1989). Defensive structures such as trichomes have also been documented to reduce the palatability of foraging material in *A. cephalotes* (Howard 1987) and *Acromirmex rugosus* (Tozin *et al.* 2017).

Trichomes are hairy structures of the plant epidermis that can reduce the effect of herbivore attacks (Styrsky *et al.* 2006). There is evidence that workers of *A. sexdens* remove trichomes from leaves before foraging on *Licania tomentosa* (Benth.) Fritsch (Kitayama *et al.* 2010) has shown that indeed mechanical removal of trichomes in *Virola sebifera* Aubl. increases foraging rates in this species (Carlos *et al.* 2018). In this work, it was observed that *A. mexicana* workers remove the trichomes of *E. japonica* leaves before cutting on their fragments, thereby influencing the rate of leaf consumption in this species. Given that trichomes can deter fungal interactions with the leaves (Łaźniewska *et al.*



**Figure 6.** Accumulated *in vitro* mycelial growth of *Leucoagaricus gongylophorus* at 35 d [mean ± SD] using PDA media (negative control), PDA enriched with the leaf methanolic extracts of *Palicourea padifolia*, *Platanus mexicana* and *Eriobotrya japonica* (at 2 mg/mL), thiabendazole and methylthiophanate (positive controls).

2012), it can be assumed that ants withdraw them to facilitate fungal colonization of the leaves by the *L. gongylophorus* garden. To our knowledge, this trichome-removal behavior has not been reported in *A. mexicana*. In contrast, *P. padifolia* had glabrous, soft, thick leaves with high moisture content. High leaf moisture content is an important trait sought by foragers of *Atta colombica* (Bowers & Porter 1981), *A. cephalotes* (Cherrett 1972), and *A. mexicana* (Mehlreter & Valenzuela 2012). In the leaf disc foraging experiment, we observed the same pattern, with *P. padifolia* the most accepted species, while *P. mexicana* and *E. japonica* were less consumed, although greater recruitment was observed in the leaf discs. *Atta cephalotes* and *A. colombica* workers operate on a narrow margin of energetic gain (Garrett *et al.* 2016). In controlled laboratory settings, *A. colombica* have been observed to adapt their foraging behavior in response to variations in characteristics of leaf fragments, such as size and shape (Dussutour *et al.* 2007) and distance traversed to access the plant resource (Travaglini *et al.* 2015), exhibiting a preference for smaller fragments (Dussutour *et al.* 2009). This phenomenon can be attributed to the observed increase in preference for leaf discs among the three species offered in the present study. When plant material is presented at a manipulable size, where they do not need to invest energy in additional cutting effort, workers focus on carrying the offered leaf discs less selectively.

The qualitative phytochemical analysis of the leaf extracts showed that *P. padifolia* has a low presence of terpenes or steroids, phenols, tannins, and coumarins in the leaves and an intermediate presence of alkaloids. The phytochemical composition of *P. padifolia* leaves has been sparsely reported. However, compounds such as indole alkaloids and flavonols have been previously described (Berger *et al.* 2015, 2016). Leaves of *P. padifolia* are rich in micronutrients such as Ca and Mg and its leaf methanolic extract contained mainly phenolic compounds including chlorogenic acid, scopoletin, *trans*-cinnamic acid, and (-)-epicatechin (Infante-Rodríguez *et al.* 2024b). Some *Palicourea* species' leaves contain sodium monofluoroacetate, a highly toxic compound that has been used as a pesticide (Cook *et al.* 2014, Lee *et al.* 2014). However, the effect of methanolic extracts on ant survival results does not support the hypothesis that the methanolic extracts of the three evaluated species contain insecticidal compounds that affect *A. mexicana* workers.

The ionic composition of growth media may be important in the development of several fungi (Jones & Jennins 1965), and we hypothesized that the low presence of specialized metabolites and the presence of some key minerals such as Ca and Mg found in *P. padifolia* leaves might improve the mycelial growth of *L. gongylophorus*. For instance, *in vitro* culture of this fungus is possible using a complete basic mineral medium (CMB) rich in glucose, peptone, yeast extract,  $\text{KH}_2\text{PO}_4$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{CaCl}_2$ , and agar (Infante-Rodríguez *et al.* 2024a). On the contrary, it has been reported that leaves of *E. japonica* (one of the low-preferred plants of this study) have a high concentration

of Na ( $97.96 \pm 64.9$  cmol/Kg) and also contain high levels of flavonoids (Wu *et al.* 2018), mainly rutin ( $558.38 \pm 569.83$   $\mu\text{g/g}$  of dry plant), kaempferol-3-O-glucoside ( $356.54 \pm 362.21$   $\mu\text{g/g}$ ), and quercetin-3-glucoside ( $309.86 \pm 69.31$   $\mu\text{g/g}$ ) in their leaves (Infante-Rodríguez *et al.* 2024c). Rieseberg & Soltis (1987) indicate that the leaves of *P. mexicana* are also a rich source of flavonoids. Flavonoids can inhibit the mycelial growth of a wide range of pathogenic organisms or have fungistatic effects on filamentous fungi (Al Aboody & Mickymaray 2020). Also, it has been suggested that increasing concentrations of NaCl in soil can affect the hyphal growth of some vesicular-arbuscular mycorrhizal fungi (McMillen *et al.* 1998). This information and the results obtained from the *L. gongylophorus in vitro* growth experiment showed that methanolic leaf extracts of less consumed species significantly affect the mycelial growth of this fungus.

In conclusion, the results of this study suggest that in the evaluated plant species, the hardness, the presence of trichomes, the high presence of flavonoids, and some elements found in the leaves could contribute to less consumption of *E. japonica* and *P. mexicana*. In the case of *E. japonica*, the trichomes were an obstacle for the workers to forage, but *A. mexicana* was able to remove the trichomes from the leaf and use moderately this foliar material. Conversely, the foraging preferences of *A. mexicana* were predominantly influenced by the low presence of specialized metabolites, the absence of trichomes, and the softness of the leaves. In a complementary manner, it was observed that leaf extracts did not present harmful effects on the ants; however, in the case of the *in vitro* culture of its mutualistic fungus, a growth-promoting effect was observed by the PDA medium enriched with *P. padifolia* extract. This outcome contrasted with the results obtained with the extract of *E. japonica* and *P. mexicana*, where moderate inhibitory activities were recorded in the *in vitro* culture of *L. gongylophorus*.

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### Literature cited

- Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**: 265-267. DOI: <https://doi.org/10.1093/jee/18.2.265a>
- Abril AB, Bucher EH. 2004. Nutritional sources of the fungus cultured by leaf-cutting ants. *Applied Soil Ecology* **26**: 243-247. DOI: <https://doi.org/10.1016/j.apsoil.2003.12.008>
- Al Aboody MS, Mickymaray S. 2020. Anti-Fungal efficacy and mechanisms of flavonoids. *Antibiotics* **9**: 45. DOI: <https://doi.org/10.3390/antibiotics9020045>
- AntWeb. Version 8.25.1. 2020. California Academy of Science. <https://www.antweb.org> (accessed April 1, 2024)
- Araújo S, Seibert J, Ruani A, Alcántara-de la Cruz R, Cruz A, Pereira A, Zandonai, D, Forim M, Silva MF, Bueno O, Fernandes J. 2022. The Symbiotic Fungus *Leucoagaricus gongylophorus* (Möller) Singer (Agaricales, Agaricaceae) as a Target Organism to Control Leaf-Cutting Ants. *Insects* **13**: 359. DOI: <https://doi.org/10.3390/insects13040359>
- Badawy MEI, Abdelgaleil SAM. 2014. Composition and antimicrobial activity of essential oils isolated from Egyptian plants against plant pathogenic bacteria and fungi. *Industrial Crops and Products* **52**: 776-782. DOI: <https://doi.org/10.1016/j.indcrop.2013.12.003>
- Barrera CA, Sosa-Calvo J, Schultz TR, Rabeling C, Bacci Jr M. 2022. Phylogenomic reconstruction reveals new insights into the evolution and biogeography of *Atta* leaf-cutting ants (Hymenoptera: Formicidae). *Systematic Entomology* **47**: 13-35. DOI: <https://doi.org/10.1111/syen.12513>
- Berger A, Kostyan MK, Klose SI, Gastegger M, Lorbeer E, Brecker L, Schinnerl J. 2015. Loganin and secologanin derived tryptamine-iridoid alkaloids from *Palicourea crocea* and *Palicourea padifolia* (Rubiaceae). *Phytochemistry* **116**: 162-169. DOI: <https://doi.org/10.1016/j.phytochem.2015.05.013>

- Berger A, Preinfalk A, Robien W, Brecker L, Valant-Vetschera K, Schinnerl J. 2016. New reports on flavonoids, benzoic-and chlorogenic acids as rare features in the *Psychotria* alliance (Rubiaceae). *Biochemical Systematics and Ecology* **66**: 145-153. DOI: <https://doi.org/10.1016/j.bse.2016.02.027>
- Boulogne I, Petit P, Ozier-Lafontaine H, Desfontaines L, Loranger-Merciris G. 2012. Insecticidal and antifungal chemicals produced by plants: a review. *Environmental Chemistry Letters* **10**: 325-347. DOI: <https://doi.org/10.1007/s10311-012-0359-1>
- Bowers MA, Porter SD. 1981. Effect of foraging distance on water content of substrates harvested by *Atta colombica* (Guérin). *Ecology* **62**: 273-275. DOI: <https://doi.org/10.2307/1936686>
- Bueno FC, Godoy MP, Leite AC, Bueno OC, Pagnocca FC, Fernandes JB, Hebling MJA, Bacci Jr M, Vieira PC, Silva MFGF. 2005. Toxicity of *Cedrela fissilis* to *Atta sexdens rubropilosa* (Hymenoptera: Formicidae) and its symbiotic fungus. *Sociobiology* **45**: 389-399.
- Branstetter MG, Jesovnik A, Sosa-Calvo J, Lloyd MW, Faircloth BC, Brady SG, Schultz TR. 2017. Dry habitats were crucibles of domestication in the evolution of agriculture in ants. *Proceedings of the Royal Society B: Biological Sciences* **284**: 20170095. DOI: <https://doi.org/10.1098/rspb.2017.0095>
- Bicalho KU, Terezan AP, Martins DC, Freitas TG, Fernandes JB, da Silva MF, das GF, Vieira PC, Pagnocca FC, Bueno OC. 2012. Evaluation of the Toxicity of *Virola sebifera* Crude Extracts, Fractions and Isolated Compounds on the Nest of Leaf-Cutting Ants. *Psyche: A Journal of Entomology* **2012**: 1-7. DOI: <https://doi.org/10.1155/2012/785424>
- Carlos AA, da Silva Malaquias K, Consolmagno RC, Sarria ALF, Fernandes J B, Bueno OC. 2018. *Virola sebifera* Aubl. (Myristicaceae) leaf chemical composition and implications on leaf-cutter ant foraging choice. *Arthropod-Plant Interactions* **12**: 361-368. DOI: <https://doi.org/10.1007/s11829-017-9582-9>
- Cherrett JM. 1972. Some factors involved in the selection of vegetable substrate by *Atta cephalotes* (L.) (Hymenoptera: Formicidae) in tropical rain forest. *The Journal of Animal Ecology* **41**: 647. DOI: <https://doi.org/10.2307/3200>
- Cook D, Lee ST, Taylor CM, Bassuner B, Riet-Correa F, Pfister JA, Gardner DR. 2014. Detection of toxic monofluoroacetate in *Palicourea* species. *Toxicon* **80**: 9-16. DOI: <https://doi.org/10.1016/j.toxicon.2013.12.003>
- Currie CR. 2001. A Community of ants, fungi, and bacteria: A multilateral approach to studying symbiosis. *Annual Review of Microbiology* **55**: 357-380. DOI: <https://doi.org/10.1146/annurev.micro.55.1.357>
- De Mendiburu F. 2010. *Agricolae*: Statistical procedures for agricultural research. R package ver. 1.0-9. <http://CRAN.Rproject.org/package=agricolae> (accessed July 1, 2023).
- Dejean A, Azémar F, Naskrecki P, Tindo M, Rossi V, Faucher C, Gryta H. 2023a. Mutualistic interactions between ants and fungi: A review. *Ecology and Evolution* **13**: e10386. DOI: <https://doi.org/10.1002/ece3.10386>
- Dejean A, Naskrecki P, Faucher C, Azémar F, Tindo M, Manzi S, Gryta H. 2023b. An Old World leaf-cutting, fungus-growing ant: A case of convergent evolution. *Ecology and Evolution* **13**: e9904. DOI: <https://doi.org/10.1002/ece3.9904>
- Dirzo R. 1987. Estudios sobre interacciones planta–herbívoro en Los Tuxtlas, Veracruz. *Revista de Biología Tropical* **35**:119-131.
- Domínguez XA. 1973. *Métodos de investigación fitoquímica*. DF, México: Limusa.
- Dussutour A, Beshers S, Deneubourg JL, Fourcassie V. 2007. Crowding increases foraging efficiency in the leaf-cutting ant *Atta colombica*. *Insectes Sociaux* **54**: 158-165. DOI: <https://doi.org/10.1007/s00040-007-0926-9>
- Dussutour A, Deneubourg JL, Beshers S, Fourcassie V. 2009. Individual and collective problem-solving in a foraging context in the leaf-cutting ant *Atta colombica*. *Animal Cognition* **12**: 21-30. DOI: <https://doi.org/10.1007/s10071-008-0165-0>
- Fürstenberg-Hägg J, Zagrobelny M, Bak S. 2013. Plant defense against insect herbivores. *International Journal of Molecular Sciences* **14**: 10242-10297. DOI: <https://doi.org/10.3390/ijms140510242>
- Garrett RW, Carlson KA, Goggans MS, Nesson MH, Shepard CA, Schofield R MS. 2016. Leaf processing behavior in *Atta* leafcutter ants: 90% of leaf cutting takes place inside the nest, and ants select pieces that require less cutting. *Royal Society Open Science* **3**: 150111. DOI: <https://doi.org/10.1098/rsos.150111>

- Gómez-Díaz JA, Baena ML, González-Zamora A, Delfin-Alfonso CA. 2023. Potential present and future distributions of the genus *Atta* of Mexico. *Plos One* **18**: e0292072. DOI: <https://doi.org/10.1371/journal.pone.0292072>
- Haines BL. 1978. Element and energy flows through colonies of the leaf-cutting ant, *Atta colombica*, in Panama. *Biotropica* **10**: 270. DOI: <https://doi.org/10.2307/2387679>
- Howard JJ. 1987. Leaf cutting ant diet selection: the role of nutrients, water, and secondary chemistry. *Ecology* **68**: 503-515. DOI: <https://doi.org/10.2307/1938455>
- Hubbell SP, Howard JJ, Wiemer DF. 1984. Chemical leaf repellency to an attine ant: seasonal distribution among potential host plant species. *Ecology* **65**: 1067-1076. DOI: <https://doi.org/10.2307/1938314>
- Infante-Rodríguez DA, Monribot-Villanueva JL, Mehltreter K, Carrión GL, Lachaud JP, Velázquez-Narváez AC, Vásquez-Reyes VM, Valenzuela-González JE, Guerrero-Analco JA. 2020. Phytochemical characteristics of leaves determine foraging rate of the leaf-cutting ant *Atta mexicana* (Smith) (Hymenoptera: Formicidae). *Chemoecology* **30**: 147-159 DOI: <https://doi.org/10.1007/s00049-020-00306-4>
- Infante-Rodríguez DA, Landa-Cansigno C, Gutiérrez-Sánchez A, Murrieta-León DL, Reyes-López C, Castillejos-Pérez AB, Pucheta-Fiscal JE, Velázquez-Narváez AC, Monribot-Villanueva JL, Guerrero-Analco JA. 2022. Análisis fitoquímico y actividad antidiabética, antibacteriana y antifúngica de hojas de *Bursera simaruba* (Burseraceae). *Acta Botanica Mexicana* **129**: 2022. DOI: <https://doi.org/10.21829/abm129.2022.2109>
- Infante-Rodríguez, DA, Velázquez-Narváez AC, Monribot-Villanueva JL, Carrión G, Mehltreter K, Lachaud JP, Guerrero-Analco JA, Valenzuela González, JE. 2024a. *In vitro* culture of *Leucoagaricus gongylophorus* (Agaricaceae), symbiont fungus of the leaf-cutting ant *Atta mexicana* (Hymenoptera, Formicidae), using solid and plant-supplemented culture media. *Acta Botanica Mexicana* **131**: e2397. DOI: <https://doi.org/10.21829/abm131.2024.2397>
- Infante-Rodríguez, DA, Aguilar-Méndez MJ, Landa-Cansigno C, Juárez-Trujillo N, Vásquez-Morales SG, Valenzuela-González JE, Kiel-Martínez AL, Monribot-Villanueva JL, Guerrero-Analco JA. 2024b. Phenolic and volatile compounds, total protein content, antioxidant and antidiabetic activities of leaves of *Palicourea padifolia* (Rubiaceae). *Acta Botanica Mexicana* **131**: e2405. DOI: <https://doi.org/10.21829/abm131.2024.2405>
- Infante-Rodríguez DA, Aguilar-Méndez MJ, Landa-Cansigno C, Vásquez-Morales SG, Velázquez-Narváez, AC, Valenzuela-González JE, Kiel-Martínez AL, Monribot-Villanueva JL, Guerrero-Analco JA. 2024c. Phytochemical composition of *Eriobotrya japonica* (Rosaceae) leaves extracts from central Veracruz, Mexico, and its effect on  $\alpha$ -glucosidase enzyme inhibition. *Botanical Sciences* **102**: 1231-1250. DOI: <https://doi.org/10.17129/botsci.3487>
- Jin X, Shi C, Yu CY, Yamada T, Sacks EJ. 2017. Determination of Leaf Water Content by Visible and Near-Infrared Spectrometry and Multivariate Calibration in Miscanthus. *Frontiers in Plant Science* **8**: 2017. DOI: <https://doi.org/10.3389/fpls.2017.00721>
- Jones EBG, Jennings DH. 1965. The effect of cations on the growth of fungi. *New Phytologist* **64**: 86-100. DOI: <https://doi.org/10.1111/j.1469-8137.1965.tb05378.x>
- Kassambara A, Mundt F. 2020. Factoextra: Extract and visualize the results of multivariate data analyses. R package version 1.0.7. <https://CRAN.R-project.org/package=factoextra> (accessed July 1, 2023).
- Kitayama K, Hay J, Souto L, Ottoni R, Aquino P. 2010. Trichomes and *Atta sexdens* (Hymenoptera: Formicidae): a study of foraging behavior in the laboratory. *Sociobiology* **55**: 107-116.
- Łażniewska J, Macioszek VK, Kononowicz AK. 2012. Plant-fungus interface: The role of surface structures in plant resistance and susceptibility to pathogenic fungi. *Physiological and Molecular Plant Pathology* **78**: 24-30. DOI: <https://doi.org/10.1016/j.pmpp.2012.01.004>
- Leal-Dutra CA, Yuen LM, Guedes BAM, Contreras-Serrano M, Marques PE, Shik JZ. 2023. Evidence that the domesticated fungus *Leucoagaricus gongylophorus* recycles its cytoplasmic contents as nutritional rewards to feed its leafcutter ant farmers. *IMA Fungus* **14**: 19. DOI: <https://doi.org/10.1186/s43008-023-00126-5>
- Lee ST, Cook D, Pfister JA, Allen JG, Colegate SM, Riet-Correa F, Taylor CM. 2014. Monofluoroacetate-containing plants that are potentially toxic to livestock. *Journal of Agricultural and Food Chemistry* **62**: 7345-7354. DOI: <https://doi.org/10.1021/jf500563h>
- Little AEF, Murakami T, Mueller UG, Currie CR. 2006. Defending against parasites: fungus-growing ants combine

- specialized behaviours and microbial symbionts to protect their fungus gardens. *Biology Letters* **1**: 330-332. DOI: <https://doi.org/10.1098/rsbl.2005.0371>
- Littleddyke M, Cherrett J. 1976. Direct ingestion of plant sap from cut leaves by the leaf-cutting ants *Atta cephalotes* (L.) and *Acromyrmex octospinosus* (Reich) (Formicidae, Attini). *Bulletin of Entomological Research* **66**: 205-217. DOI: <https://doi.org/10.1017/S0007485300006647>
- Lobo Echeverri T, Salazar LC, Hernández A, Ortiz Reyes A. 2016. Efectos de *Capsicum baccatum* y *C. frutescens* sobre *Atta cephalotes* (Hymenoptera: Formicidae) y el hongo simbionte *Leucoagaricus gongylophorus*. *Revista Colombiana de Entomología* **42**: 137-145. DOI: <https://doi.org/10.25100/socolen.v42i2.6684>
- McMillen BG, Juniper S, Abbott LK. 1998. Inhibition of hyphal growth of a vesicular-arbuscular mycorrhizal fungus in soil containing sodium chloride limits the spread of infection from spores. *Soil Biology and Biochemistry* **30**: 1639-1646. DOI: <https://doi.org/10.4236/ojss.2017.711023>
- Maya-Yescas ME, Revah S, Le Borgne S, Valenzuela-González J, Palacios-González E, Terrés-Rojas E, Viguera-Ramírez G. 2021. Growth of *Leucoagaricus gongylophorus* Möller (Singer) and production of key enzymes in submerged and solid-state cultures with lignocellulosic substrates. *Biotechnology Letters* **43**: 845-854. DOI: <https://doi.org/10.1007/s10529-020-03057-y>
- Mehdiabadi NJ, Schultz TR. 2010. Natural history and phylogeny of the fungus-farming ants (Hymenoptera: Formicidae: Myrmicinae: Attini). *Myrmecological News* **13**: 37-55. DOI: [https://doi.org/10.25849/myrmecol.news\\_013:037](https://doi.org/10.25849/myrmecol.news_013:037)
- Mehlreter K, Valenzuela JE. 2012. Leafcutter ants as test organism for leaf quality of ferns. *Indian Fern Journal* **29**: 262-268.
- Mintzer A. 1979. Foraging activity of the Mexican leafcutting ant *Atta mexicana* (F. Smith), in a Sonoran desert habitat (Hymenoptera, Formicidae). *Insectes Sociaux* **26**: 364-372. DOI: <https://doi.org/10.1007/BF02223555>
- Mintzer A. 1980. Simultaneous Use of a foraging trail by two leafcutter ant species in the Sonoran Desert. *Journal of the New York Entomological Society* **88**: 102-105.
- Mintzer AC. 1995. Diet of the leafcutting ant, *Atta mexicana* (Hymenoptera: Formicidae), in a Sonoran desert habitat. *Journal of the Arizona-Nevada Academy of Science* **28**: 33-40.
- Mueller UG, Rabeling C. 2008. A breakthrough innovation in animal evolution. *Proceedings of the National Academy of Sciences of the United States of America*. 105: 5787-5788. DOI: <https://doi.org/10.1073/pnas.0801464105>
- Mundim FM, Costa AN, Vasconcelos HL. 2009. Leaf nutrient content and host plant selection by leaf-cutter ants, *Atta laevigata*, in a Neotropical savanna. *Entomologia Experimentalis et Applicata* **130**: 47-54. DOI: <https://doi.org/10.1111/j.1570-7458.2008.00789.x>
- Murguía-González J, Leyva O, Galindo ME, Landero-Torres I, Llarena-Hernández RC, García-Martínez MA. 2018. New records of ants in Oaxaca, Mexico: their agricultural importance in sugarcane crops. *Agrociencia* **52**: 379-391.
- Nichols-Orians C, Schultz J. 1989. Leaf toughness affects leaf harvesting by the leaf cutter ant, *Atta cephalotes* (L.) (Hymenoptera: Formicidae). *Biotropica* **21**: 80-83. DOI: <https://doi.org/10.2307/2388446>
- Perkovich C, Ward D. 2020. Protein:Carbohydrate Ratios in the diet of gypsy moth *Lymantria dispar* affect its ability to tolerate tannins. *Journal of Chemical Ecology* **46**: 299-307. DOI: <https://doi.org/10.1007/s10886-020-01161-x>
- R Core Team. 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. <https://www.r-project.org/> (accessed May 2, 2023).
- Rieseberg LH, Soltis DE. 1987. Flavonoids of fossil miocene *Platanus* and its extant relatives. *Biochemical Systematics and Ecology* **15**: 109-112. DOI: [https://doi.org/10.1016/0305-1978\(87\)90088-3](https://doi.org/10.1016/0305-1978(87)90088-3)
- Rivera A, Romero O, Hernández F, Badillo F, Aragón A. 2016. Secondary metabolites isolated from the bacterial complex of *Apis mellifera* and their action against *Atta mexicana*. *Entomology and Applied Science Letters* **3**: 10-16
- Rocha SL, Jorge VL, Della Lucia TMC, Barreto RW, Evans HC, Elliot SL. 2014. Quality control by leaf-cutting ants: Evidence from communities of endophytic fungi in foraged and rejected vegetation. *Arthropod-Plant Interactions* **8**: 485-493. DOI: <https://doi.org/10.1007/s11829-014-9329-9>

- Rockwood LL. 1976. Plant selection and foraging patterns in two species of leaf-cutting ants (*Atta*). *Ecology* **57**: 48-61. DOI: <https://doi.org/10.2307/1936397>
- Rønheide S, Boomsma JJ, Rosendahl S. 2004. Fungal enzymes transferred by leaf-cutting ants in their fungus gardens. *Mycological Research* **108**: 101-106. DOI: <https://doi.org/10.1017/S0953756203008931>
- Schultz TR, Brady SG. 2008. Major evolutionary transitions in ant agriculture. *Proceedings of the National Academy of Sciences of the United States of America* **105**: 5435-5440. DOI: <https://doi.org/10.1073/pnas.0711024105>
- Silva A, Bacci M, Pagnocca FC, Bueno OC, Hebling MJA. 2006. Starch metabolism in *Leucoagaricus gongylophorus*, the symbiotic fungus of leaf-cutting ants. *Microbiological Research* **161**: 299-303. DOI: <https://doi.org/10.1016/j.micres.2005.11.001>
- Smith F. 1858. Catalogue of hymenopterous insects in the collection of the British Museum. Part VI. Formicidae. London: British Museum. pp. 1-216.
- Styrsky JD, Kaplan I, Eubanks MD. 2006. Plant trichomes indirectly enhance tritrophic interactions involving a generalist predator, the red imported fire ant. *Biological Control* **36**: 375-384. DOI: <https://doi.org/10.1016/j.biocontrol.2005.10.003>
- Serratos-Tejeda S, García AA, Torres BCP, Olguín JFL. 2017. Alternativa agroecológica para el manejo de *Atta mexicana* en Puebla, México. *Southwestern Entomologist* **42**: 261-273. DOI: <https://doi.org/10.3958/059.042.0123>
- Swanson AC, Schwendenmann L, Allen MF, Aronson EL, Artavia-León A, Dierick D, Fernandez-Bou AS, Harmon TC, Murillo-Cruz C, Oberbauer SF, Pinto-Tomás AA, Rundel PW, Zelikova TJ. 2019. Welcome to the *Atta* world: A framework for understanding the effects of leaf-cutter ants on ecosystem functions. *Functional Ecology* **33**: 1386-1399. DOI: <https://doi.org/10.1111/1365-2435.13319>
- Tozin LRDS, Mayo Marques MO, Maria Rodrigues T. 2017. Herbivory by leaf-cutter ants changes the glandular trichomes density and the volatile components in an aromatic plant model. *AoB Plants* **9**: plx057. DOI: <https://doi.org/10.1093/aobpla/plx057>
- Travaglini R, Forti LC, da Silva Camargo R. 2015. Foraging behavior of leaf cutting ants: How do workers search for their food? *Sociobiology* **62**: 347-350. DOI: <https://doi.org/10.13102/sociobiology.v62i3.714>
- Valdés-Rodríguez OA, Pérez-Vázquez A, Palacios-Wassenaar OM. 2015. Insectos plaga en cultivo asociado de *Ricinus communis* y *Moringa oleifera* en el centro de Veracruz, México. *Revista Mexicana de Ciencias Agrícolas* **6**: 2233-2239. DOI: <https://doi.org/10.29312/remexca.v0i11.805>
- Vasconcelos HL, Cherrett JM. 1995. Changes in leaf-cutting ant populations (Formicidae: Attini) after the clearing of mature forest in Brazilian Amazonia. *Studies on Neotropical Fauna and Environment* **30**: 107-113. DOI: <https://doi.org/10.1080/01650529509360947>
- Van Bael S A, Estrada C, Weislo W T. 2011. Fungal-fungal interactions in leaf-cutting ant agriculture. *Psyche: A Journal of Entomology* **2011**: 1-9. DOI: <https://doi.org/10.1155/2011/617478>
- Vigueras G, Paredes-Hernández D, Revah S, Valenzuela-González J, Olivares-Hernández R, Le Borgne S. 2017. Growth and enzymatic activity of *Leucoagaricus gongylophorus*, a mutualistic fungus isolated from the leaf-cutting ant *Atta mexicana*, on cellulose and lignocellulosic biomass. *Letters in Applied Microbiology* **65**: 173-181. DOI: <https://doi.org/10.1111/lam.12759>
- Williams-Linera G. 2002. Tree species richness complementarity, disturbance and fragmentation in a Mexican tropical montane cloud forest. *Biodiversity and Conservation* **11**: 1825-1843. DOI: <https://doi.org/10.1023/A:1020346519085>
- Williams-Linera G, Toledo-Garibaldi M, Gallardo-Hernández C. 2013. How heterogeneous are the cloud forest communities in the mountains of central Veracruz, México? *Plant Ecology* **214**: 685-701. DOI: <https://doi.org/10.1007/s11258-013-0199-5>
- Wu Y, Jian T, Lv H, Ding X, Zuo Y, Ren B, Chen J, Li W. 2018. Antitussive and expectorant properties of growing and fallen leaves of loquat (*Eriobotrya japonica*). *Revista Brasileira de Farmacognosia* **28**: 239-242. DOI: <https://doi.org/10.1016/j.bjp.2018.02.006>
- Zárate-Castrejón JL, Martínez-Martínez TO, Jiménez-Quiroz E, Pecina-Quintero V, Bujanos-Muñiz R. 2020. Un-

usual damage to cladodes of xocconostle (*Opuntia* sp.) by leafcutter ant, *Atta mexicana* (F. Smith). *Southwestern Entomologist* **45**: 305-307. DOI: <https://doi.org/10.3958/059.045.0134>

Zavala-Hurtado JA, Valverde PL, del Carmen Herrera-Fuentes M, Díaz-Solís A. 2000. Influence of leaf-cutting ants (*Atta mexicana*) on performance and dispersion patterns of perennial desert shrubs in an inter-tropical region of Central Mexico. *Journal of Arid Environments* **46**: 93-102. DOI: <https://doi.org/10.1006/jare.2000.0655>

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