



Beyond predator bodyguards: ant attendance at post-floral pericarpial nectaries increases fruit production, improves fruit morphology, and reduces nectar thief visitation in *Declieuxia fruticosa* (Rubiaceae)

Taís Alves da Cruz¹ · Gabriela Fraga Porto^{1,2} · Bruno de Sousa-Lopes¹ · Kleber Del-Claro¹

Received: 28 June 2025 / Accepted: 26 February 2026 / Published online: 23 March 2026
© The Author(s) 2026

Abstract

Declieuxia fruticosa produces branches with inflorescences that simultaneously bear floral buds, distylous flowers, and fruits with post-floral pericarpial nectaries (PPNs). These branches attract ants, pollinators, herbivores, and nectar thieves. Ants feeding on PPNs may act as plant bodyguards against herbivores, but they can also influence floral visitors, potentially generating both costs and benefits for plant reproduction. However, the net effects of these multitrophic interactions on plant fitness remain unclear. We tested the hypothesis that ant presence at PPNs enhances fruit production and improves fruit morphological traits in *D. fruticosa* by repelling herbivores and nectar thieves, while not interfering with legitimate pollinators. Field experiments and observations were conducted in Uberlândia, MG, Brazil, manipulating ant presence and absence. We quantified fruit number, weight, width, and length, and evaluated stigma receptivity, pollen availability, and the assemblage of floral visitors and herbivores. Our results demonstrate that ant presence at PPNs significantly increases fruit set and all evaluated morphological traits. Fruits produced in the presence of ants were, on average, 25% larger and 2.4 times heavier than those from ant-excluded plants. Moreover, ant presence was associated with a 2.9-fold reduction in the frequency of nectar thieves and a 65% increase in visitation frequency by *Apis mellifera*, the main pollinator. These findings indicate that ant-mediated protection against herbivores and floral exploiters, without substantial interference in pollination, directly enhances plant fitness. Overall, our study highlights the role of ants in shaping plant reproductive success through direct and indirect multitrophic interactions.

Keywords *Apis mellifera* · Brazil · Cerrado · Distylous species · Facultative mutualism · Multitrophic interactions

Introduction

Interactions between plants and insects are among the most frequent ecological relationships in terrestrial ecosystems and play a central role in shaping plant fitness and community structure (Strong et al. 1984; Agrawal and Maron 2022). Although herbivory is the most common form of interaction and often imposes substantial fitness costs on plants, it has also acted as a major selective force driving the evolution of a wide range of defensive strategies (Marquis 1992; Fine et al. 2006). Among these strategies, indirect defenses are particularly notable, as plants reduce herbivore pressure by attracting, nourishing, or sheltering natural enemies such as predators or parasitoids (Heil 2008). These defenses may involve the emission of herbivore-induced volatile compounds or the presence of specialized structures, including domatia and extrafloral nectaries (Bronstein

Communicated by Carol Frost.

✉ Kleber Del-Claro
delclaro@ufu.br
Taís Alves da Cruz
tais_alvescruz@outlook.com
Gabriela Fraga Porto
gabrielafraga@usp.br
Bruno de Sousa-Lopes
brunolopesprof@gmail.com

- ¹ Laboratório de Ecologia Comportamental e de Interações, Instituto de Biologia, Universidade Federal de Uberlândia, Uberlândia, MG, Brazil
- ² Programa de Pós-Graduação em Entomologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto – FFCLRP, Universidade de São Paulo – USP, Ribeirão Preto, Brazil

et al. 2006; Kaplan et al. 2008). Although indirect mutualisms are thought to be widespread, they are still rarely tested experimentally, and their net effects on plant reproductive success remain poorly quantified under natural conditions.

Among indirect mutualisms, interactions between ants and plants bearing extrafloral or post-floral pericarpial nectar sources are especially frequent and ecologically relevant. Nectar production plays distinct functional roles depending on its origin (Koptur 1992; Heil 2015). While floral nectaries directly contribute to pollination by attracting floral visitors, extrafloral nectaries occur on vegetative or reproductive organs and are not involved in pollination, instead attracting ants and other arthropods that may reduce herbivory (Del-Claro et al. 2013; Calixto et al. 2018; Barbosa et al. 2025). In some species, floral nectaries remain active after anthesis and continue secreting nectar during fruit development; in these cases, they are referred to as post-floral pericarpial nectaries (Sanz-Veiga et al. 2017). Although structurally derived from floral nectaries, these glands function ecologically in a manner similar to extrafloral nectaries during the reproductive phase (Del-Claro et al. 2013; Sanz-Veiga et al. 2017; Sousa-Lopes et al. 2020).

Ants attracted to these nectar sources may reduce herbivory on leaves, flowers, and fruits, thereby enhancing plant reproductive performance (Nascimento and Del-Claro 2010; Lange and Del-Claro 2014; Porto et al. 2023). However, the outcomes of ant–plant interactions are context dependent and may range from mutualistic benefits to negative effects, such as interference with floral visitors or facilitation of sap-feeding herbivores (Ness 2006; Assunção et al. 2014; Costa-Silva et al. 2025). In addition to their potential effects on pollinators, ants may also influence plant reproductive success by interacting with other floral visitors (Calixto et al. 2024), including nectar thieves. Nectar thieves exploit floral rewards without contributing to pollination and often remain on flowers for extended periods, potentially reducing resource availability for legitimate pollinators. By deterring or excluding such visitors, ants may act not only as defenders against herbivores but also as filters that limit exploitation of floral resources during the reproductive phase (Aguirre-Jaimes et al. 2018).

In the Cerrado biome, ant–plant interactions are particularly frequent due to the high diversity of plant species bearing extrafloral and post-floral pericarpial nectaries (Del-Claro et al. 2013; Calixto et al. 2018; Barbosa et al. 2025). The Rubiaceae family is well represented in this biome and includes several species that develop pericarpial nectaries during fruit development (Delprete and Jardim 2012; Falcão et al. 2014). Among these species, *Declieuxia fruticosa* (Wild. ex Ruiz and Pav.) Kuntze is a characteristic savanna plant whose inflorescences simultaneously bear flower buds, distylous flowers, and fruits with active post-floral

pericarpial nectaries, allowing the coexistence of floral visitors, herbivores, and ants (Oliveira and Pie 1998; Cardoso et al. 2023; Sousa-Lopes et al. 2020). The species is heterostylous, with brevistylous and longistylous floral morphs that promote cross-pollination (Matias et al. 2016). Field observations suggest that ants typically remain on the outer portions of the calyx and fruits without obstructing access to the floral tube, indicating that they may reduce herbivory without negatively affecting pollination (Sousa-Lopes et al. 2020). Moreover, ant presence may shorten pollinator visit duration, potentially increasing pollen flow among conspecific individuals and favoring cross-pollination (Sousa-Lopes et al. 2020).

Despite these observations, the extent to which ants influence both fruit production and fruit morphological traits in plant species bearing post-floral pericarpial nectaries remains poorly understood. In particular, little attention has been given to the possibility that ants may enhance plant reproductive success not only by reducing herbivory, but also by limiting the activity of nectar thieves without deterring effective pollinators. If ant-mediated reductions in herbivory and nectar theft increase resource availability during reproductive development, fruits may become not only more numerous but also larger and heavier; however, these combined effects have received little empirical testing.

Here, we first quantified pollen availability, stigma receptivity, and the assemblage of floral visitors and herbivores associated with *D. fruticosa* to characterize the ecological context of this interaction. We then experimentally evaluated the role of ants in modulating plant reproductive success. We hypothesized that ant attendance at post-floral pericarpial nectaries (i) increases fruit production and promotes the development of larger fruits, as measured by fruit weight, width, and length, and (ii) does not negatively affect legitimate pollinators while contributing to plant defense by reducing the activity of herbivores and nectar thieves during the reproductive phase.

Materials and methods

Study area and plant species

The study was conducted in the privately owned ecological reserve known as Clube Caça e Pesca Itororó (CCPIU) (18° 58' 56.65" S; 48° 17' 45.62" W), located in the southern part of the municipality of Uberlândia, Minas Gerais, in the Triângulo Mineiro region of Brazil. The reserve covers an area of approximately 640 hectares, of which 127 hectares are designated as a Vegetation Reserve (RV), comprising two main phytophysiognomies of the Cerrado biome: cerrado sensu stricto and vereda (Bacci et al. 2016; Appolinario

and Schiavini 2002). The local climate is classified as tropical wet with a dry winter (Aw) according to the Köppen classification system.

The plant species used for the experiments was *D. fruticosa*, which can grow as a shrub or subshrub, ranging from 7 to 150 cm in height, and typically occurs in savanna formations, with flowers available throughout the entire rainy season (Delprete 2010; Matias et al. 2016). As previously noted, this plant has EFNs, which are nectar-producing structures not directly involved in pollination and that persist as post-floral pericarpial nectaries on the fruits (Sousa-Lopes et al. 2020). Its flowers exhibit distyly, the most common form of heterostyly, a floral polymorphism characterized by the spatial separation of the pistil (female part) and the androecium (male part), which prevents self-pollination and self-fertilization (Fig. 1) (Matias et al. 2016).

Assessment of pollen availability and stigma receptivity

To determine the timing of pollen availability, we followed the methods and schedules used for the same species by Sousa-Lopes et al. (2020). Ten individuals were selected,

five of each floral morphotype. A total of 25 flowers were observed at 30-min intervals from 06:00 to 13:00 over three days. The presence or absence of available pollen was assessed 10 min after anthesis by direct observation and using a cotton swab (see Ferreira and Torezan-Silingardi 2013). To determine the timing of stigma receptivity, another 25 flowers were evaluated at 30-min intervals using the method proposed by Dafni and Maués (1998). Flowers were immersed in a tube containing 3% hydrogen peroxide (10-volume hydrogen peroxide), and the formation of bubbles on the stigma indicated its receptivity. This test was also conducted 10 min after anthesis.

Floral visitors and herbivores

To identify the main floral visitors and herbivores associated with *D. fruticosa*, we performed 20-min ad libitum observations (Altmann 1974) on each of the 80 individuals used in the ant-exclusion experiment (see below). Observations took place from 08:00 to 12:00, coinciding with the peak periods of pollen availability, stigma receptivity, and ant foraging activity. Each plant was observed once, totaling 1,600 min of direct observation. All insects interacting

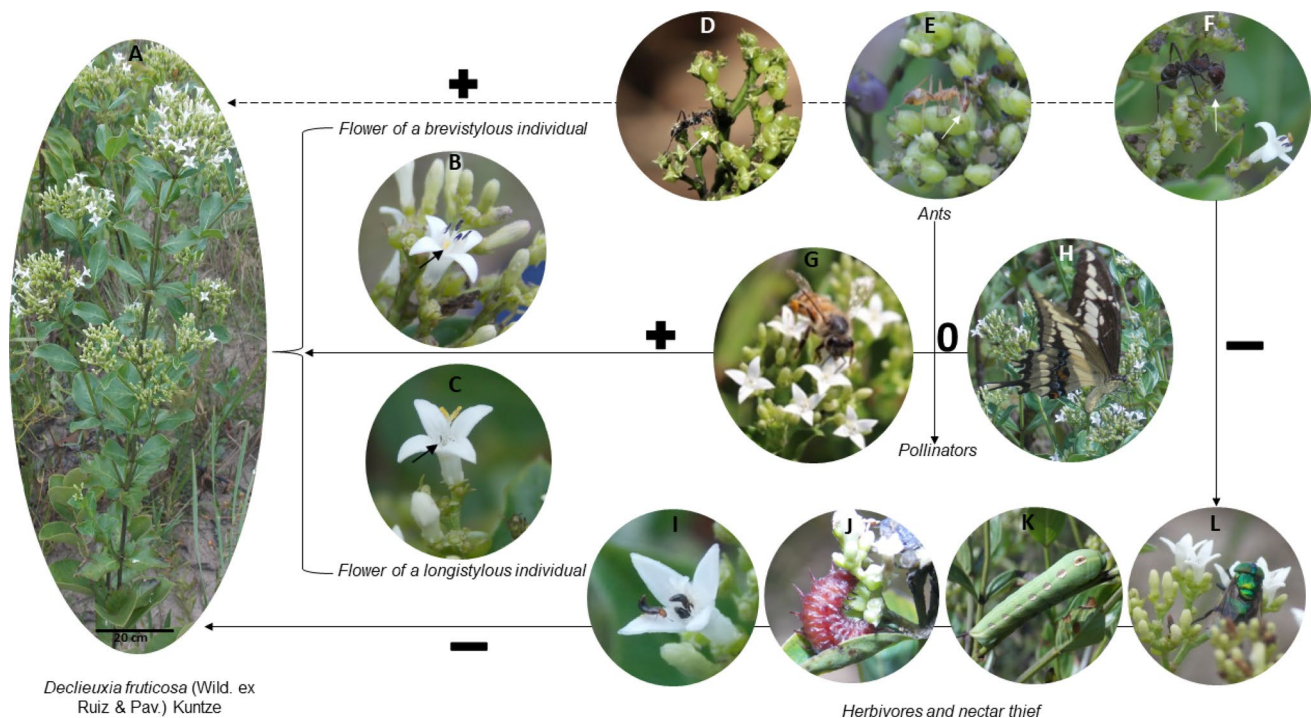


Fig. 1 Study system. **A** General architecture of *Declieuxia fruticosa*. **B** Brevistylous flower, with stamens positioned above the stigma. **C** Longistylous flower, with stamens positioned below the stigma. **D–F** Ant species (*Camponotus crassus*, *Camponotus* sp., and *Ectatomma* sp., respectively) foraging on *D. fruticosa* inflorescences. **G, H** Floral visitors, *Apis mellifera* and *Heraclides* sp., respectively, visiting *D. fruticosa* flowers. **I–K** *Staphylinidae* sp., *Lepidoptera* sp., and *Xylophanes tersa* feeding on reproductive and vegetative structures of *D.*

fruticosa, respectively. **L** *Chrysomya megacephala*, one of the main nectar thieves of *D. fruticosa*. The black arrows within the images indicate the stigma (**B**) and the stamens (**C**). The white arrows within the images indicate the pericarpial nectaries (**D–F**). The black dashed line indicates an interaction with indirect effects (ant–plant) and the solid line indicates a direct effect (ant–herbivore, ant–pollinator, and pollinator–plant). The + sign means the interaction is positive, – negative, and 0 neutral

with flowers or vegetative structures were recorded and, whenever possible, collected with an entomological net or photographed for later identification at the Behavioral Ecology and Interactions Laboratory (LECI/UFU). All collected visitors were identified using taxonomic keys (Silveira et al. 2002; Oliveira et al. 2013; Baccaro et al. 2015) and through comparisons with reference collections previously verified by specialized taxonomists. Specimens were identified to the lowest possible taxonomic level, and all vouchers were deposited in the entomological collection of the Federal University of Uberlândia (UFU). Insects were categorized as floral visitors when they contacted reproductive floral structures, and as herbivores when feeding damage on flowers, fruits, or leaves was observed.

Fruit formation and morphological traits in the presence and absence of ants

To test whether ant presence influences fruit set and fruit traits in *D. fruticosa*, we conducted a field exclusion experiment with 80 individuals, comprising 40 of each floral morphotype (brevistylous and longistylous), spaced at least three meters apart. For each morphotype, 20 individuals were assigned to the control group, allowing unrestricted ant access, while the remaining 20 had ant access excluded by applying Tanglefoot around the main stem at a height of 20 cm. To ensure effective exclusion, branches and trunks from neighboring plants that could facilitate ant access were also removed. Fruit production was monitored from October 2022 to January 2023, corresponding to the rainy season and the peak in flowering and insect activity in the study area. Two flowering branches were marked on each plant, totaling 160 branches monitored to quantify fruit set.

To evaluate the influence of ant presence on reproductive success, we analyzed both the number of fruits produced per branch (as a proxy for fitness) and fruit morphological traits, including fruit weight, length, and width. For this, 10 individuals from each floral morphotype were selected (five from each treatment group), and five mature fruits were collected per plant. All fruits were processed at LECI/UFU, where their dimensions were measured using a precision scale and digital caliper. Fruits and seeds were also visually inspected for evidence of herbivory.

Data analyses

We tested whether ant exclusion affected fruit production and fruit morphology in *Declieuxia fruticosa* using R v4.5.1 (R Core Team 2025). All analyses were conducted separately for the two floral morphs (brevistylous and longistylous). Fruit production (number of fruits per branch) was modelled as a function of ant treatment (control vs. ant

exclusion) using generalized linear models with a negative binomial distribution, implemented in the MASS package (Ripley et al. 2013), after detecting overdispersion in Poisson models. Model fit was evaluated using residual diagnostics from the DHARMA package (Hartig 2025).

Fruit morphological traits (weight, width, and length) were analysed using Gaussian linear models (LMs), with ant treatment (control vs. ant exclusion) included as the predictor variable in each model. The two fruit morphological traits correspond to correlated dimensions of the same organ and are therefore not statistically independent. However, because fruits of *Declieuxia fruticosa* are not strictly isodimensional, variation along longitudinal and lateral axes may reflect distinct functional and developmental responses. We therefore analysed each trait separately to identify which specific components of fruit morphology responded to ant exclusion, rather than combining them into a single multivariate index.

All analyses were interpreted with emphasis on effect sizes and confidence intervals. Estimated marginal means ($\pm$ SE) were obtained using the emmeans package (Lenth 2025), and figures were produced using ggplot2 (Wickham 2016).

Results

Pollen availability and stigma receptivity

Anthesis in *D. fruticosa* occurred early in the morning for both floral morphotypes. Brevistylous flowers began opening at 07:40 ($n=4$) and longistylous at 07:50 ($n=2$), with 94% of the observed flowers (47/50) fully open by 09:10. Stigma receptivity was detected primarily between 08:00 and 09:00 ($n=39$), although a few stigmas remained receptive until 09:30. The interval between anthesis and stigma receptivity was approximately 40–60 min. Pollen release began at around 08:10, peaked at 09:00, and progressively declined with each floral visit. By 12:00, anthers were entirely depleted of pollen.

Floral visitors and herbivores

Ant presence differed strongly between treatments, confirming that the exclusion protocol was effective. During focal observations, no ants were recorded on exclusion branches, whereas control branches were consistently patrolled. Mean ant abundance was 7.6 individuals on brevistylous controls and 11.0 on longistylous control plants per 1600 min of direct observation (Table 1). Most ants belonged to *Camponotus crassus* Mayr, 1862, with occasional records of other Formicidae.

Table 1 Summary of floral visitors recorded per 1600 min of direct observation for each treatment group. “Control” branches allowed ant access, whereas “Exclusion” branches prevented ants from accessing flowers. Values are totals and means $\pm$ standard errors (SE)

Treatment	Total ants	Mean ants (SE)	Total <i>Apis</i>	Mean <i>Apis</i> (SE)	Total other visitors	Mean other visitors (SE)	Total pollinators	Mean pollinators (SE)	Total nectar thieves	Mean nectar thieves (SE)
Brevistylous control	144	7.58 $\pm$ 1.43	200	10.53 $\pm$ 2.76	58	3.05 $\pm$ 0.71	238	12.53 $\pm$ 2.32	56	2.95 $\pm$ 1.09
Brevistylous exclusion	0	0	146	7.68 $\pm$ 2.10	72	3.79 $\pm$ 0.75	218	11.47 $\pm$ 2.29	92	4.84 $\pm$ 0.99
Longistylous control	265	11.04 $\pm$ 1.33	245	10.21 $\pm$ 2.68	77	3.21 $\pm$ 0.64	321	13.38 $\pm$ 2.78	91	3.79 $\pm$ 1.14
Longistylous exclusion	0	0	123	6.15 $\pm$ 1.41	46	2.3 $\pm$ 0.48	169	8.40 $\pm$ 1.63	90	4.5 $\pm$ 1.23

Apis mellifera Linnaeus, 1758 was the main pollinator of both floral morphs and exhibited higher visitation rates on control branches. Mean visitation rates were 10.5 and 10.2 on brevistylous and longistylous control branches, respectively, compared with 7.7 and 6.2 visits on exclusion branches per 1600 min of direct observation. Other floral visitors (Diptera, *Trigona* spp., Vespidae, Lepidoptera and occasional Hemiptera) showed weaker responses to the exclusion treatment, with mean visitation rates ranging from 2.3 to 3.8 across morphs and treatments per 1600 min of direct observation (Table 1). When *Apis* and other visitors were considered together, total pollinator visitation was consistently higher in the presence of ants; brevistylous plants received a mean of 12.5 visits on control branches and 11.5 on exclusion branches, whereas longistylous plants received 13.4 and 8.4 visits per 1600 min of direct observation, respectively.

In contrast, nectar thieves were more frequent in treatments without ants. Mean visitation reached 4.8 on brevistylous exclusion branches, compared with 3.0 when ants were present, and 4.5 on longistylous exclusion branches, compared with 3.8 on controls per 1600 min of direct observation. Despite frequent ant patrolling, no aggressive interactions between ants and floral visitors were observed during 1600 min of focal watches.

Herbivores comprised Staphylinidae, Thysanoptera and Lepidoptera larvae feeding on reproductive structures, while *Xylophanes tersa* (Linnaeus, 1771) caterpillars fed on leaves exclusively on ant-excluded plants (Fig. 1). Some specimens could only be identified to higher taxonomic levels due to early developmental stages or incomplete morphological characters. A full list of taxa is provided in Porto (2025), and supplementary figures in Sousa-Lopes et al. (2020) illustrate characteristic insects associated with *D. fruticosa*.

Fruit production and morphological traits

Ant presence significantly increased fruit production in both floral morphs (Fig. 2). Values reported correspond to model-estimated marginal means $\pm$ standard error (SE). In brevistylous plants, branches with ant access produced more fruits per branch than ant-excluded branches (120.0 ± 12.9

vs. 79.8 ± 8.2 ; Fig. 2A; $z = -2.76$, $p = 0.006$). A similar pattern was observed in longistylous plants, where control branches produced 119.0 ± 15.7 fruits per branch compared with 75.0 ± 10.2 fruits on ant-excluded branches (Fig. 2B; $z = -2.42$, $p = 0.015$).

Ant exclusion also significantly reduced fruit weight in both floral morphs (Fig. 3). In brevistylous plants, fruits formed in the presence of ants were heavier than those from ant-excluded branches (0.060 ± 0.003 g vs. 0.031 ± 0.003 g; Fig. 3A; $t = -7.79$, $p < 0.001$). Likewise, in longistylous plants, fruit weight decreased from 0.073 ± 0.003 g on control branches to 0.030 ± 0.003 g under ant exclusion (Fig. 3B; $t = -11.96$, $p < 0.001$).

Fruit size dimensions were also consistently reduced by ant exclusion (Fig. 4). In brevistylous plants, fruits produced on control branches were wider and longer than those from ant-excluded branches (Fig. 4A, B). Fruit width declined from 4.31 ± 0.12 mm to 3.43 ± 0.12 mm ($t = -5.40$, $p < 0.001$), and length from 5.99 ± 0.09 mm to 4.75 ± 0.09 mm ($t = -9.28$, $p < 0.001$).

Similarly, in longistylous plants, fruits formed in the presence of ants showed greater width and length than those from ant-excluded branches (Fig. 4C, D). Fruit width decreased from 4.64 ± 0.10 mm to 3.34 ± 0.10 mm ($t = -8.87$, $p < 0.001$) and length from 6.35 ± 0.11 mm to 4.59 ± 0.11 mm ($t = -11.45$, $p < 0.001$).

Discussion

Our results demonstrate that ant attendance at post-floral pericarpial nectaries significantly enhances reproductive success in both floral morphotypes of *Declieuxia fruticosa*, increasing fruit production as well as fruit size and weight. These findings support our primary hypothesis that ants act as effective indirect defenders during fruit development. In addition, they corroborate the hypothesis that ant attendance does not negatively affect legitimate pollinators and may instead contribute to plant fitness by reducing the activity of nectar thieves. Together, these results highlight the multifaceted role of ants in mediating plant reproductive outcomes through both herbivore deterrence and regulation of floral resource exploitation.

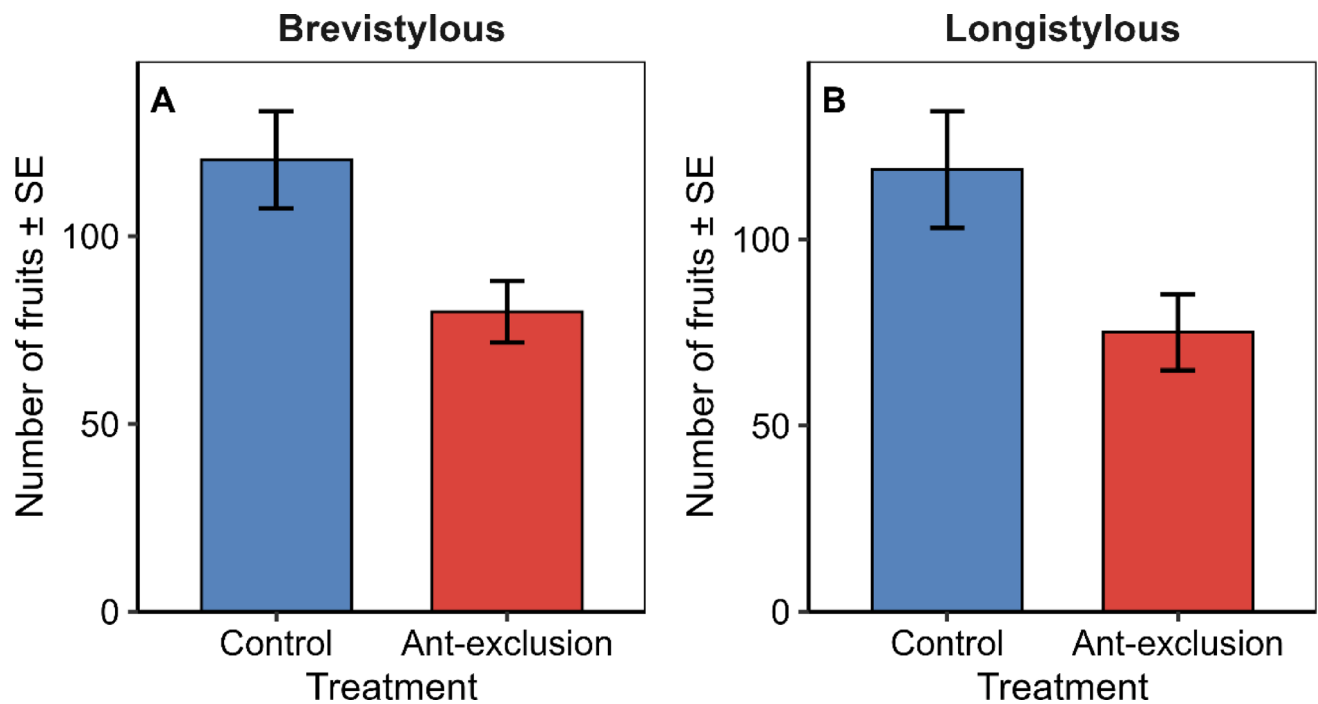


Fig. 2 Fruit production (model-estimated marginal means $\pm$ SE) of brevistylous (A) and longistylous (B) *Declieuxia fruticosa* plants with ants present (control) and absent (ant exclusion). Differences between treatments were significant ($p < 0.05$)

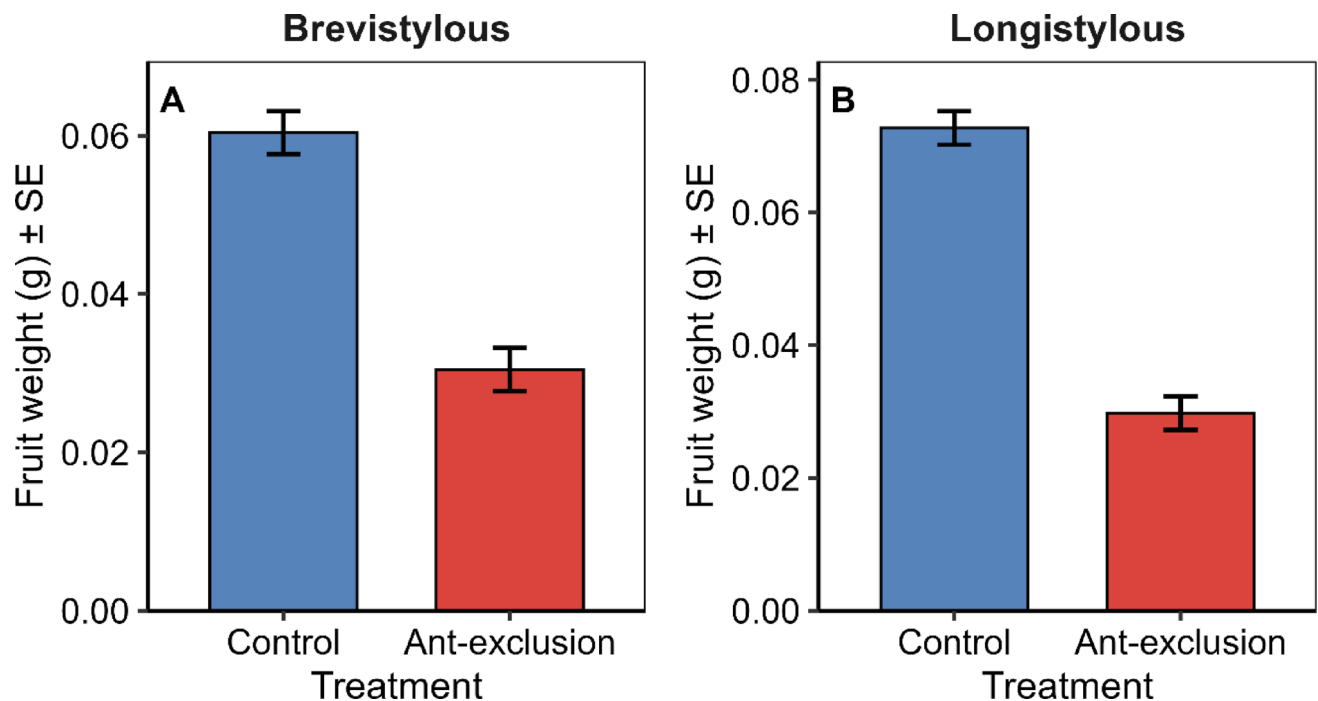
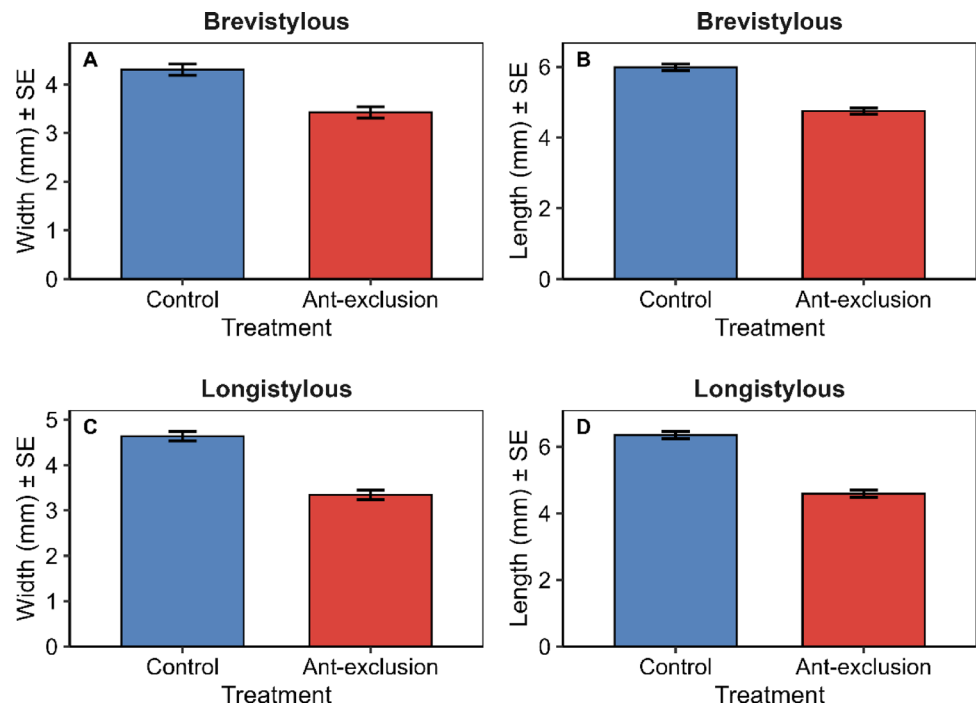


Fig. 3 Fruit weight (model-estimated marginal means $\pm$ SE) of brevistylous (A) and longistylous (B) *Declieuxia fruticosa* fruits formed in the presence of ants (control) and in the absence of ants (ant exclusion). Differences between treatments were significant ($p < 0.05$)

The positive effects of ants on fruit set and fruit morphological traits may be partly mediated by ant effects on herbivores. In this study, the lepidopteran larvae of *Xylophanes tersa* were recorded exclusively on ant-excluded plants,

suggesting that ant presence limits herbivore access to both reproductive and vegetative tissues. Reduced herbivory during fruit development may increase resource availability for fruit filling, resulting not only in a greater number of fruits

Fig. 4 Fruit morphological traits (model-estimated marginal means $\pm$ SE) of brevistylous (A, B) and longistylous (C, D) *Declieuxia fruticosa* fruits formed in the presence of ants (control) and in the absence of ants (ant exclusion). Plants with ants produced fruits with greater width (A, C; mm), and length (B, D; mm) than ant-excluded plants ($p < 0.05$)



but also in heavier and larger fruits. Similar patterns have been reported in other systems, where ant-mediated indirect defenses translate into both quantitative and qualitative gains in plant reproductive output, particularly when protection is concentrated on reproductive structures (Villamil et al. 2022; Nogueira et al. 2024).

In contrast to previous studies in Rubiaceae where the protective role of ants was weak or inconsistent (Del-Claro et al. 2013; Sousa-Lopes et al. 2020), our experimental manipulation revealed clear benefits of ant attendance. Fruits produced in the presence of ants were, on average, 25% larger and 2.4 times heavier than those from ant-excluded plants. These differences likely reflect the context dependency of ant-plant interactions, driven largely by variation in ant species composition and foraging behavior. In our study system, the dominant ant species, *Camponotus crassus*, exhibited rapid and transient foraging behavior, remaining on individual inflorescences for only short periods (see Lange et al. 2019). Such behavior appears sufficient to deter herbivores and nectar thieves while minimizing interference with legitimate pollinators. By contrast, other ant species previously reported for *D. fruticosa*, such as *Ectatomma tuberculatum* (Olivier, 1792), tend to remain longer on plant structures and may exert stronger behavioral effects on pollinators (Sousa-Lopes et al. 2020), potentially shifting the balance between costs and benefits. As this latter species was not recorded in the present study, our results also suggest that changes in ant assemblages over time may influence interaction outcomes, highlighting the importance of considering temporal dynamics in future investigations.

Importantly, our results provide no evidence that ant presence negatively affects legitimate pollinators. Consistent with this pattern, Cardoso et al. (2023) showed that *C. crassus* foraging on *Palicourea rigida* did not alter fruit production when hummingbirds visited the plants. Notably, this lack of effect persisted even when larger and potentially more aggressive ant species, such as *Ectatomma tuberculatum*, were also present, suggesting that pollinator activity was not disrupted by ant assemblages. In the present study, visitation by the main pollinator, *Apis mellifera*, was not reduced in the presence of ants, and no aggressive interactions between ants and this species were observed during focal observations. On the contrary, ant presence was associated with a 65% increase in visitation frequency by *A. mellifera*. This pattern likely reflects an indirect effect of ant attendance mediated by changes in the floral visitor assemblage: nectar thieves and other less efficient visitors typically spend longer periods at flowers and are therefore more exposed to agonistic encounters with ants, whereas honey bees perform short, rapid visits that minimize contact with ants and reduce foraging costs. By maximizing pollen intake rates and preferentially exploiting patches with lower interspecific competition, *A. mellifera* may benefit from foraging on plants with ants, which are avoided by more disturbance-sensitive visitors (see Santos and Leal 2019). In addition, the relatively aggressive behavior of honey bees may further reduce their sensitivity to ant presence, allowing them to exploit floral resources that are less accessible to other visitors. Similar patterns have been reported for *Melastoma malabathricum* L. flowers pollinated by large carpenter bees

of *Xylocopa* spp., where outcomes were attributed to the filtering effect of ants on the flower visitor community: ants deter less effective pollinators and indirectly favor efficient pollinators through reduced resource depletion (González et al. 2013).

In addition to the absence of negative effects on pollinators, ant presence may even enhance pollination dynamics. Previous studies suggest that ants can shorten pollinator visit duration (Aguirre-Jaimes et al. 2018), a behavioral change that may increase pollen flow among individuals and promote cross-pollination in heterostylous systems. In *D. fruticosa*, Sousa-Lopes et al. (2020) reported that floral visitors make shorter visits in the presence of ants, a pattern that may contribute to maintaining the balanced 1:1 proportion of brevistylous and longistylous morphotypes observed in natural populations.

Beyond herbivore deterrence, our study supports the hypothesis that ants may also enhance plant fitness by limiting nectar theft. Nectar thieves exploit floral rewards without contributing to pollination and often remain on flowers for extended periods, potentially reducing nectar availability for effective pollinators (Aguirre-Jaimes et al. 2018). In our experiment, nectar thieves were more frequent on ant-excluded branches, indicating that ant patrolling functions as a behavioral filter that deters these exploiters. Notably, ant presence was associated with a 2.9-fold reduction in the frequency of nectar thieves on ant-excluded plants. By selectively discouraging visitors that do not contribute to pollination, ants may indirectly increase the efficiency of plant–pollinator interactions, reinforcing the net positive effect of ant presence on reproductive success (Calixto et al. 2024).

Similar outcomes have been reported in other tropical systems, where the benefits of ant protection outweigh potential costs to pollination. For instance, studies on *Euterpe oleracea* Mart. showed that although ants altered floral visitor behavior, fruit production remained unaffected due to the net protective effect against floral herbivores (Belém et al. 2020). Together with our findings, these studies emphasize that the ecological outcomes of ant–plant interactions depend not only on ant presence per se, but also on the identity, behavior, temporal dynamics of both ants and floral visitors, and location of extrafloral nectaries (Silva et al. 2025).

Although we did not directly quantify rates of herbivory or nectar removal, the strong and consistent differences between ant-access and ant-exclusion treatments provide compelling evidence that ants play a central protective role during the reproductive phase of *D. fruticosa*. The combined increase in fruit number and fruit size observed in the presence of ants suggests meaningful gains in plant fitness, likely translating into greater seed output and offspring

recruitment. Moreover, previous studies conducted in the same study area documented a balanced population structure between brevistylous and longistylous morphotypes of *D. fruticosa*, indicating a stable and effective pollination system in which ant presence does not disrupt reproductive dynamics (Sousa-Lopes et al. 2020).

Therefore, our study highlights the importance of ants as key mediators of plant reproductive success in tropical savanna ecosystems. By simultaneously reducing herbivory and nectar theft without deterring legitimate pollinators, ants associated with post-floral pericarpial nectaries can generate strong net fitness benefits for plants. These findings reinforce the view that ant–plant mutualisms are highly context dependent and underscore the need for integrative approaches that consider multiple interaction partners, behavioral mechanisms, and temporal variation to fully understand the ecological and evolutionary consequences of indirect plant defenses.

Acknowledgements TAC thanks FAPEMIG for the scholarship that made this work possible, and KDC thanks CNPq for grant 403647/2021-5 and for the PQ fellowship.

Author contributions TAC—field work; data collection; analysis; wrote the first version of the manuscript; GFP—data analysis; wrote the first version of the manuscript; prepared the figures; BSL—data analysis; wrote the first version of the manuscript; prepared the figures; KDC—field work; data collection; analysis; wrote the first version of the manuscript; funding; All authors reviewed the manuscript.

Funding The Article Processing Charge (APC) for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614).

Data availability We will put all the data available in Figshare or Driid after paper final acceptance.

Declarations

Conflict of interest There are no conflicts of interest to be reported.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Agrawal AA, Maron JL (2022) Long-term impacts of insect herbivores on plant populations and communities. *J Ecol*. <https://doi.org/10.1111/1365-2745.13996>
- Aguirre-Jaimes A, Dáttilo W, Rodríguez-Morales D, Canchola-Orozco S, Coccoletzi E, Coates R, Ángeles G (2018) Foraging ants on the extrafloral nectaries repel nectar thieves but not the effective pollinator of *Vigna luteola* (Fabaceae) in a Mexican coastal sand dune. *Sociobiology* 65:621–629. <https://doi.org/10.13102/sociobiology.v65i4.3466>
- Altman J (1974) Observational study of behaviors: sampling methods. *Behaviour* 49:227–267. <https://doi.org/10.1163/156853974x00534>
- Appolinario VAR, Schiavini I (2002) Levantamento Fitossociológico de espécies arbóreas de cerrado (strictu sensu) em Uberlândia—Minas Gerais. *Bol Herb Ezechias Paulo Heringer* 10:57–75
- Assunção MA, Torezan-Silingardi HM, Del-Claro K (2014) Do ant visitors to extrafloral nectaries of plants repel pollinators and cause an indirect cost of mutualism? *Flora* 209:244–249
- Baccaro FB, Feitosa RM, Fernández F, Fernandes I, Souza JLP, Izzo T (2015) *Guia para os Gêneros de Formigas do Brasil*. Editora INPA, Manaus, Brazil, p 388
- Bacci LF, Versiane AFA, Romero R (2016) Melastomataceae da Reserva do Clube Caça e Pesca Itororó Uberlândia, Minas Gerais, Brasil. *Rapid Color Guide*. The Field Museum of Chicago. <http://fieldguides.fieldmuseum.org/guides/guide/392>
- Barbosa VDC, Bächtold A, Del-Claro K, Alves-Silva E (2025) Extrafloral nectary plants in Brazilian biomes: dominance of Cerrado and Fabaceae. *Flora* 323:152668. <https://doi.org/10.1016/j.flora.2024.152668>
- Belém SO, Guia BP, Campbell A, Maués MM, Viana JH (2020) Effects of ants (Hymenoptera: Formicidae) on flying insect visitor behaviour and fruit production in açai palm (*Euterpe oleracea* Martius). *Austral Entomol* 59(3):612–618. <https://doi.org/10.1111/aen.12458>
- Bronstein JL, Alarcón R, Geber M (2006) The evolution of plant-insect mutualism. *New Phytol* 172:412–428. <https://doi.org/10.1111/j.1469-8137.2006.01864.x>
- Calixto ES, Lange D, Del-Claro K (2018) Protection mutualism: an overview of ant-plant interactions mediated by extrafloral nectaries. *Oecologia Aust* 22:410–425. <https://doi.org/10.4257/oeco.2018.2204.05>
- Calixto ES, de Oliveira Pimenta IC, Lange D, Marquis RJ, Torezan-Silingardi HM, Del-Claro K (2024) Emerging trends in ant-pollinator conflict in extrafloral nectary-bearing plants. *Plants* 13(5):651. <https://doi.org/10.3390/plants13050651>
- Cardoso PB, Calixto ES, Torezan-Silingardi HM, Del-Claro K (2023) Context-dependent ant-pollinator mutualism impacts fruit set in a hummingbird-pollinated plant. *Plants* 12(21):3688. <https://doi.org/10.3390/plants12213688>
- Costa-Silva VM, Porto GF, Vázquez-González C, Calixto ES, Moreira X, Del-Claro K (2025) Complex interactions in ant-plant mutualisms: the role of multiple ant food resources in mediating plant defence, pollination and fitness in a tropical shrub. *Ecol Entomol*. <https://doi.org/10.1111/een.70004>
- da Silva AV, Nogueira A, Bronstein JL, Rey PJ, Leal LC (2025) Ants on flowers: protective ants impose a low but variable cost to pollination, moderated by location of extrafloral nectaries and type of flower visitor. *J Ecol* 113(8):2187–2198. <https://doi.org/10.1111/1365-2745.70087>
- Dafni A, Maués MM (1998) A rapid and simple procedure to determine stigma receptivity. *Sex Plant Reprod* 11:177–180. <https://doi.org/10.1007/s004970050138>
- Del-Claro K, Guillermo-Ferreira R, Zardini H, Almeida EM, Torezan-Silingardi HM (2013) Ants visiting the post-floral secretions of pericarpial nectaries in *Palicourea rigida* (Rubiaceae) provide protection against leaf herbivores but not against seed parasites. *Sociobiology* 60(3):217–221. <https://doi.org/10.13102/sociobiology.v60i3.217-221>
- Delprete PG, Jardim JG (2012) Systematics, taxonomy and floristics of Brazilian Rubiaceae: an overview about the current status and future challenges. *Rodriguésia* 63:101–128. <https://doi.org/10.1590/S2175-78602012000100009>
- Delprete PG (2010) Flora dos estados de Goiás e Tocantins. Gráfica e Editora Vieira Goiânia, Goiânia, p 1610
- Falcão JCF, Dáttilo W, Izzo TJ (2014) Temporal variation in extrafloral nectar secretion in different ontogenic stages of the fruits of *Alibertia verrucosa* S. Moore (Rubiaceae) in a Neotropical savanna. *J Plant Interact* 9(1):137–142. <https://doi.org/10.1080/17429145.2013.782513>
- Ferreira CA, Torezan-Silingardi HM (2013) Implications of the floral herbivory on Malpighiaceae plant fitness: visual aspect of the flower affects the attractiveness to pollinators. *Sociobiology* 60(3):323–328. <https://doi.org/10.13102/sociobiology.v60i3.323-328>
- Fine PVA, Miller ZJ, Mesones I, Irazuzta S, Appel HM, Stevens MHH, Sääksjärvi I, Schultz JC, Coley PD (2006) The growth–defense trade-off and habitat specialization by plants in Amazonian forests. *Ecology* 87:150–162
- González FG, Santamaría L, Corlett RT, Rodríguez-Gironés MA (2013) Flowers attract weaver ants that deter less effective pollinators. *J Ecol* 101(1):78–85. <https://doi.org/10.1111/1365-2745.12006>
- Hartig, F. DHARMA: residual diagnostics for hierarchical (multi-level/mixed) regression models (Version 0.4.1) [R Package]. <https://cran.r-project.org/package=DHARMA>. Accessed 30 Jan 2025
- Heil M (2008) Indirect defence via tritrophic interactions. *New Phytol* 178:41–61
- Heil M (2015) Extrafloral nectar at the plant-insect interface: a spotlight on chemical ecology, phenotypic plasticity, and food webs. *Annu Rev Entomol* 60:213–232. <https://doi.org/10.1146/annurev-ento-010814-020753>
- Kaplan I, Halitschke R, Kessler A, Sardanelli S, Denno RF (2008) Constitutive and induced defenses to herbivory in above and belowground plant tissues. *Ecology* 89:392–406. <https://doi.org/10.1890/07-0471.1>
- Koptur S (1992) Extrafloral nectary-mediated interactions between insects and plants. In: Bernays EA (ed) *Insect-plant interactions*, vol 4. CRC Press, Boca Raton, Florida, pp 81–129
- Lange D, Del-Claro K (2014) Ant-plant interaction in a tropical savanna: may the network structure vary over time and influence on the outcomes of associations? *PLoS ONE* 9(8):e105574. <https://doi.org/10.1371/journal.pone.0105574>
- Lange D, Calixto ES, Rosa BB, Sales TA, Del-Claro K (2019) Natural history and ecology of foraging of the *Camponotus crassus* Mayr, 1862 (Hymenoptera: Formicidae). *J Nat Hist* 53(27–28):1737–1749. <https://doi.org/10.1080/00222933.2019.1660430>
- Lenth R (2025) emmeans: Estimated marginal means, aka least-squares means. <https://cran.r-project.org/web/packages/emmeans/html>. Accessed 30 Jan 2025
- Marquis RJ (1992) Selective impact of herbivores. In: Fritz RS, Simms EL (eds) *Ecology and plant resistance to herbivores and pathogens*. University of Chicago Press, Chicago, pp 301–325
- Matias R, Oliveira AS, Furtado MT, Sá T, Rodrigues EB, Oliveira PE, Consolaro H (2016) Sistema reprodutivo atípico de duas espécies de Rubiaceae: distília com autoincompatibilidade parcial no morfo brevistilo? *Rodriguésia* 67:357–368. <https://doi.org/10.1590/02175-7860201667207>

- Nascimento EA, Del-Claro K (2010) Ant visitation to extrafloral nectaries decreases herbivory and increases fruit set in *Chamaecrista debilis* (Fabaceae) in a Neotropical savanna. *Flora* 205(11):754–756. <https://doi.org/10.1016/j.flora.2009.12.040>
- Ness JH (2006) A mutualism's indirect costs: the most aggressive plant bodyguards also deter pollinators. *Oikos* 113:506–514
- Nogueira RR, de Sousa-Lopes B, Del-Claro K (2024) Effects of foliar herbivory on the vegetative and reproductive traits of *Malpighiaceae* from Cerrado: when the ontogeny of leaves and flowers matters. *Arthropod-Plant Interact.* <https://doi.org/10.1007/s11829-024-10109-x>
- Oliveira OS, Pie MR (1998) Interaction between ants and plants bearing extrafloral nectaries in cerrado vegetation. *An Soc Entomol Brasil* 27:161–176. <https://doi.org/10.1590/S0301-80591998000200001>
- Oliveira FF, Richers BTT, Silva JR, Farias RC, Matos TAL (2013) Guia Ilustrado das Abelhas Sem-Ferrão das Reservas Amanã e Mamirauá, Brasil (Hymenoptera, Apidae, Meliponini). IDS N, Manaus, Brazil
- Porto GF (2025) Ant presence increases fruit set and improves morphological traits in the distylous pericarpial nectary-bearing *Declieuxia fruticosa* (Rubiaceae) in the Brazilian Savanna (study data). <https://doi.org/10.5281/zenodo.17551311>
- Porto GF, Pezsonia JH, Del-Claro K (2023) Extrafloral nectary-bearing plants recover ant association benefits faster and more effectively after frost-fire events than frost. *Plants* 12(20):3592. <https://doi.org/10.3390/plants12203592>
- R Core Team (2025) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
- Ripley B, Venables B, Bates DM, Hornik K, Gebhardt A, Firth D, Ripley MB (2013) Package 'MASS.' CRAN R 538:113–120
- Santos ATF, Leal LC (2019) My plant, my rules: bodyguard ants of plants with extrafloral nectaries affect patterns of pollinator visits but not pollination success. *Biol J Lin Soc* 126(1):158–167. <https://doi.org/10.1093/biolinnean/bly165>
- Sanz-Veiga PA, Ré Jorge L, Benitez-Vieyra S, Amorim FW (2017) Pericarpial nectary-visiting ants do not provide fruit protection against pre-dispersal seed predators regardless of ant species composition and resource availability. *PLoS ONE* 12(12):e0188445. <https://doi.org/10.1371/journal.pone.0188445>
- Silveira FA, Melo GAR, Almeida EAB (2002) Abelhas Brasileiras: Sistemática e Identificação. Fundação Araucária, Belo Horizonte, Brazil
- Sousa-Lopes B, Calixto ES, Torezan-Silingardi HM, Del-Claro K (2020) Effects of ants on pollinator performance in a distylous pericarpial nectary-bearing Rubiaceae in Brazilian Cerrado. *Sociobiology* 67(2):173–185. <https://doi.org/10.13102/sociobiology.v67i2.4846>
- Strong DR, Lawton JH, Southwood TRE (1984) Insect on plants: community patterns and mechanisms. Backwell Science, London
- Villamil N, Boege K, Stone GN (2022) Defensive mutualists affect outcross pollen transfer and male fitness in their host plant. *Oikos* 9:e08788. <https://doi.org/10.1111/oik.08788>
- Wickham H (2016) ggplot2: elegant graphics for data analysis. Springer-Verlag, New York

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.