CAROLINA CALDERÓN ARROYO

PLANTS FOR FITNESS ENHANCEMENT OF A COFFEE LEAF MINER PARASITOID

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Magister Scientiae*.

Orientadora: Madelaine Venzon

Coorientadores: Pedro Henrique Brum Togni
Angelo Pallini

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Para quem com as suas ações e serviços contribuem com um sistema alimentar mais justo e sustentável, seja desde o campo ou desde as universidades. Com especial dedicatória para as e os colegas do Laboratório de Entomologia da EPAMIG que orientam a suas pesquisas nesse sentido dentro do maravilhoso mundo do controle biológico conservativo.

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RESUMO

CALDERÓN-ARROYO, Carolina, M.Sc., Universidade Federal de Viçosa, dezembro de 2021. **Plantas para melhorar o desempenho de um parasitoide do bicho-mineiro do cafeeiro**. Orientadora: Madelaine Venzon. Coorientadores: Pedro Henrique Togni e Angelo Pallini.

O controle biológico conservativo visa potencializar o controle de pragas através da conservação dos inimigos naturais no campo e uma das estratégias utilizadas é a introdução ou manejo de plantas que fornecem alimento para os inimigos naturais. Neste trabalho, o efeito de recursos alimentares sobre o desempenho de um parasitoide do bicho-mineiro do cafeeiro, *Proacrias coffeae*, foi avaliado. Para avaliar o efeito do néctar na sobrevivência de *P. coffeae* usamos três plantas com flores: *Bidens pilosa*, *Galinsoga parviflora* e *Varronia curassavica*; e o néctar extrafloral de uma leguminosa arbustiva, *Senna cernua*. Os parasitoides aumentaram sua sobrevivência ao se alimentar do néctar floral de *V. curassavica* e do néctar extrafloral de *S. cernua*. Não foram encontradas diferenças significativas na sobrevivência do parasitoide ao se alimentarem de néctar floral de *B. pilosa* e *G. parviflora*. Após resultados promissores com o néctar extrafloral de *S. cernua* na sobrevivência de *P. coffeae*, avaliamos seu efeito na carga de ovos. Os parasitoides aumentaram sua carga de ovos quando expostos ao néctar extrafloral e com o aumento da idade. Nossos resultados mostram o potencial de *V. curassavica* e *S. cernua* em fornecer recursos nutricionais ao parasitoide *P. coffeae* e aumentar seu desempenho por um incremento de sua sobrevivência quando confinados com o recurso. Em termos de controle biológico conservativo, isso significa que introduzir no agroecossistema do café recursos alimentares para *P. coffeae* pode aumentar seu sucesso como agentes de biocontrole, não apenas por aumentar sua sobrevivência, mas também seu potencial reprodutivo.

Palavras-chave: Controle biológico conservativo. *Proacrias coffeae*. Néctar. Alimento alternativo.

ABSTRACT

CALDERÓN-ARROYO, Carolina, M.Sc., Universidade Federal de Viçosa, December, 2021. **Plants for fitness enhancement of a coffee leaf miner parasitoid**. Advisor: Madelaine Venzon. Co-advisors: Pedro Henrique Togni and Angelo Pallini.

Conservative biological control aims to enhance the pest control through the conservation of natural enemies in the field, one of the strategies used is to introduce or manage plants that provide food for natural enemies. The effect of feeding resources on the fitness of a coffee leaf miner parasitoid *Proacrias coffeae* was assessed. To evaluate the effect of nectar on *P. coffeae* survival we used three flowering plants: *Bidens pilosa*, *Galinsoga parviflora* and *Varronia curassavica*; and we also tested the extrafloral nectar of a leguminous shrub *Senna cernua*. The parasitoids increase their survival when feeding on the floral nectar of *V. curassavica* and on the extrafloral nectar of *S. cernua*. No significant differences were found in their survival when feeding on *B. pilosa* and *G. parviflora* floral nectar. After promising results with the extrafloral nectar of *S. cernua* in the parasitoids survival, we evaluated its effect on the egg load of *P. coffeae*. The parasitoids increased their egg load when exposed to the extrafloral nectar and with increasing age. Our results show the potential of *V. curassavica* and *S. cernua* for providing nutritional resources to the parasitoid *P. coffeae* and therefore enhance their fitness by an increment of their survival when they were confine with the resource. In terms of conservative biological control, that means that introducing in the coffee agroecosystem feeding resources for *P. coffeae* can increase their success as biocontrol agents, not just by enhancing their survival but also their reproductive potential.

Keywords: Conservative biological control. *Proacrias coffeae*. Nectar. Plant provided food.

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INTRODUCTION

The maintenance and/or augmentation of populations of natural enemies in crop fields are the main objective of the conservation biological control, which consist in the manipulation of the habitat to provide the necessary resources to the optimal natural enemy's fitness (Shields et al., 2019). Such manipulation can be performed by the diversification of the vegetation or the maintenance of natural vegetation, as well as other strategies like the provision of supplementary food sources and the use of pest control products that doesn't harm natural enemies. Those strategies may supply different resources and conditions for the natural enemies, such as alternative hosts and prey, shelter, food sources, among others (Venzon & Sujii, 2019).

The maintenance of non-crop plants to attract and maintain natural enemies has a low acceptation among farmers for being considered as damaging weeds (Landis et al., 2000; Shields et al., 2019; Venzon et al., 2019). That perception needs to be change given the complexity of the relations between such plants with other organisms, some of the non-crop plants have direct effects on the agroecosystem functioning and may provide other ecosystem services (Petit et al., 2011). Fiedler et al. (2008) recommended the use of plants for conservation biological control programs that may be able to supply other ecosystem services, besides the pest control, such as pollination, nutrient cycling, human health, cultural values, among others.

There are several criteria for choosing plants to be introduced in the agroecosystem aiming to enhance natural enemies' populations (Venzon, 2021). It is also necessary to consider the possible positive interactions that the plants may have with the pest that needs to be controlled (Landis et al., 2000; Wäckers & Van Rijn, 2012). To select plant species with potential to be used for conservative biological control purpose, several studies have been conducted to assess the effect of floral and extrafloral resources of non-crop plants on the fitness of natural enemies. (Wäckers & Van Rijn, 2012: Venzon et al., 2006; Rezende et al., 2014)

Feeding on flower resources may affect several aspects of parasitoid biology, such as their longevity, egg viability, foraging decisions, and time of fly initiation (Olson et al., 2005). Nectar feeding may have an indirect effect in parasitoid fecundity through an increase in their longevity, giving a higher amount of time to find their host and to oviposit (Strand & Casas, 2008). Also, it may have a direct effect by enhancing the parasitoid egg load (i.e., the total number of mature eggs in the ovaries) (Papaj, 2000). Adult parasitoids feeding is particularly important to carry on this process in synovigenic parasitoids. Synovigenic parasitoids emerge as adult with a few mature eggs and continue maturing them over the days, while pro-ovigenic parasitoids emerge with all their mature eggs. There are some clues to determine if a parasitoid is synovigenic, like an increase in egg load over time, or a constant quantity of eggs in parasitoids that are ovipositing (Jervis et al., 2001). In this sense, provide the crops field with feeding resources for the parasitoids may increase their chances to oviposit and hence perform a better pest ´s biocontrol in the field.

Coffee leaf miner, *Leucoptera coffeella* Guérin-Méneville (Lepidoptera: Lyonetiidae) is one of the key coffee pests in Brazil. At high population levels, it may cause defoliation up to 70%, which decreases photosynthesis and results in up to 50% decrease in coffee yield (Reis & Souza, 1996). Rezende et al. (2021) report an increment in the coffee leaf miner parasitism and a reduction of the damage in coffee plants near *Inga edulis* Martius (Fabaceae) plants, which possess extrafloral nectaries. One of the possible mechanisms involved in that phenomena was the increment in the longevity of coffee leaf miner parasitoids after feeding on the inga's extrafloral nectar.

The increase in the coffee productivity in Brazil has been supported by an intensive model of production, which is mainly done in full sun with higher yields than shaded coffee in the short term, but with a greater dependence on agrochemicals and depleting the soil more quickly (Watson & Achinelli, 2008). In addition, the full sun production system results in a decrease in the biodiversity, which is needed to sustain important ecosystem services, such as pollination, climate regulation, nutrient cycling, and pest control (Jha et al., 2014), leaving the use of insecticides as the main pest control strategy (Reis et al., 2002).

For coffee leaf miner the most used measure is chemical control, with a small percentage of the farmers combining it with cultural management (Leite et al., 2020). Although several parasitoid species have been reported in coffee plantations, their use in applied biological control hasn't been well explored yet (Dantas et al., 2020). There are several studies reporting the occurrence of coffee leaf miner parasitoids in Brazil, whose species are found in the Braconidae and Eulophidae families (Hymenoptera) (Perioto et al., 2004; Melo et al., 2007; Miranda, 2009; Amaral et al., 2010; Fernandes, 2013; Rezende et al., 2014; Tango et al., 2014; Marques, 2017).

For the state of Minas Gerais, Pereira et al. (2007) and Rezende et al. (2014) reported the parasitoid *Horismenus* sp. (Hymenoptera: Eulophidae) as the more abundant one, while other studies report greater abundance of *Orgilus niger* Penteado-Dias (Hymenoptera: Braconidae) and *Stiropius reticulatus* Penteado-Dias (Hymenoptera: Braconidae) (Amaral et al., 2010; Fernandes, 2013; Marques, 2017). This variation may occur due to different climatic conditions and biological factors in the coffee agroecosystem, such as the occurrence of alternative hosts or food sources and shelter for the adult parasitoid wasps, besides others so far not studied factors as the biology of the parasitoids (Fernandes, 2013).

The percentage of parasitism on coffee leaf miner that occurs naturally may also vary a lot according to the agroecosystem conditions, getting up to 34,17% in the most diversified systems (Fernandes, 2013). That kind of agroecosystems may count with more resources to maintain natural enemies' populations (Amaral et al., 2010; Carvalho et al., 2019; Rezende, 2014; Venzon, 2021).

In the Neotropical region there are few studies on the interactions among plants and parasitoid insects. In the case of coffee leaf miner parasitoids, there are studies about the positive effect of the diversity increment in the production system on the parasitism (Fernandes, 2013; Rezende et al., 2014; Marques, 2017), whilst, except for Rezende (2014), those studies do not elucidate the functional role of any specific plant neither the involved mechanisms in the positive answer.

Coffee plantations host an abundant number of non-crop plants, from which *Bidens pilosa* L. (Asteraceae), and *Galinsoga parviflora* Cav. (Asteraceae) are among the most common ones (Silva et al., 2009). Several non-crop plants growing in coffee fields have been studied for their medicinal properties and traditional uses, indicating that non-crop plants may serve for multiple purposes (Bartolome et al., 2013; Sayed et al., 2016; Ali et al., 2017). Besides, other plants can be intercropped with coffee, such as leguminous plants that present both floral and extrafloral nectar and provide other ecosystemic services as nitrogen fixation (Rezende et al., 2021b). The presence of *Inga edulis* plants in coffee crops increased the coffee yield, maybe because of the enhance of natural enemies' populations and their fitness along with other factors (Rezende et al., 2021a).

Other attributes like medicinal and insecticidal properties of aromatic plants can be considered to add value to plants candidates to associate with crops (Fiedler et al., 2008), which is the case of *Varronia curassavica* Jacq. (Boraginaceae), whose leave´s essential oil has shown selective toxic effect on important pests, *Myzus persicae* Sulzer (Hemiptera: Aphididae) and *Tetranychus urticae* Koch (Acari: Tetranychidae) without affecting the survival of the predator *Ceraeochrysa cubana* Hagen (Neuroptera: Chrysopidae) (Andrade et al., 2021). *V. curassavica* has also been popularly used against muscle pain, bruises, and inflammatory processes, uses that are already approve by the National Health Surveillance Agency of Brazil (ANVISA) (Bristot et al., 2021).

Here, I evaluated the potential of two Asteraceae non-crop plants naturally encountered in coffee fields, one leguminous and one medicinal plant to provide food resources to a coffee leaf miner parasitoid. I studied whether feeding on floral and extrafloral nectar by *Proacrias coffeae* Ihering (Hymenoptera: Eulophidae), would improve its fitness. I expect that feeding on the plant´s resources would increase the survival and the egg load of the parasitoid. This species has been registered as one of the most frequently found parasitizing coffee leaf miner (Martins, 2021; Melo et al., 2007; Miranda, 2019; Silva et al., 2011).

MATERIAL AND METHODS

2.1 Coffee leaf miner parasitoids

Coffee leaf miner parasitoids were initially obtained from intact mined coffee leaves from Catuaí coffee variety, sampled in the experimental field Diogo Alves de Mello of the Universidade Federal de Viçosa (20°46'01.8"S 42°52'10.0"W), located in the Brazilian Atlantic Rainforest Region. The leaves were collected once a week from June of 2020 until August 2021. The parasitoid that emerged where separated into morphospecies and them identify with the help of taxonomic keys and the guidance from Dr. Valmir Costa from the Instituto Biológico de São Paulo. From all species emerged, *Proacrias coffeae* was the most frequently encountered and easier to handle and to rear. The genus *Proacrias* could be identified by a set of morphological traits, being one of the most outstanding the "propodeum with modified median carina: either broadened and dorsally flattened or split posteriorly" (Burks 2003) (Figure 1).

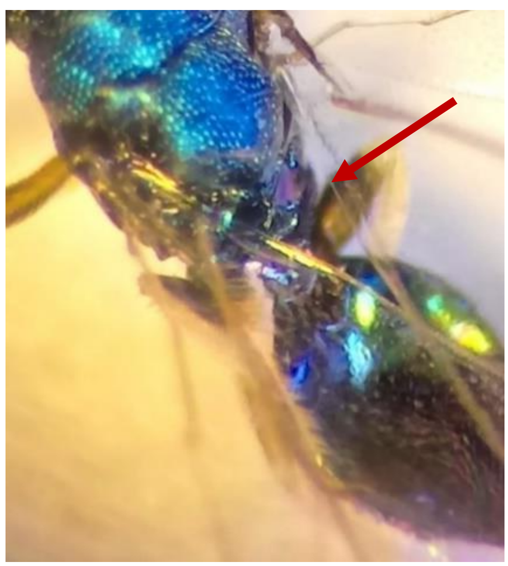

Figure 1. Dorsal view of a section of a *Proacrias coffeae* female, showing the modified median carina of the propodeum (pointed with the red arrow).

2.2 Rearing of *Proacrias coffeae*

After emergence, the parasitoids were identified and sexed and then both sexes were placed in a plastic glass with honey to feed and copulate, for 24 hours. Males can be differentiated from females because they have the abdomen narrower than the females with a distinguish translucid region in the proximal region of the gaster (Figure 2).

Figure 2. Ventral view of a *Proacrias coffeae* male showing the translucid region in the proximal region of the gaster.

After 24 hours, females were place individually in plastic glasses with a drop of honey in the wall of the glass and three mined coffee leaves that had their petioles inserted in water inside a pot to maintain turgidity. The rearing was maintained with mined coffee leaves that were collected weekly in the experimental field. Collected leaves were checked to assure that the coffee leaf miner larvae have not been predated, as the predators should break the surface of the mine to get to the larvae just intact mines were collected.

The leaves that were placed in the glasses with the female parasitoid were replaced every three days and then placed in a rearing cage until the parasitoid pupated inside the mines (about 15 days). After that time, every mine was opened to look and collect the parasitoid pupae. Pupae were place in plastic vials that were check every day until the emergence of the parasitoids.

2.3 Survival experiments

To assess the effect of the plant resources (floral and extrafloral nectar) on *P. coffeae* survival, a laboratory experiment was performed. The choice of plants for the experiments was based on their abundance in coffee plantations, such as *Bidens pilosa* and *Galinsonga parviflora* (Silva et al., 2009), which are both annual Asteraceae herbs that may provide floral resources throughout the year (Budumajji & Solomon Raju 2018; Moreira & Bragança 2011). The leguminous plant, *Senna cernua* Balb. H.S Irwin & Barneby (Caesalpinioideae), was chosen because this kind of plants provides other ecosystem services such as nitrogen fixation, and possess extrafloral nectaries, which are found in the base of the petiole (Oliveira & Garcia 2021). The medicinal plant, *Varronia curassavica*, was chosen due to the presence of essential oils with both medicinal and insecticidal properties, and previous report about natural enemy attraction (Venzon et al., 2019).

For *B. pilosa*, *G. parviflora* and *V. curassavica*, the flowers were collected from plants naturally growing in the green areas of the Livestock Research Enterprise of Minas Gerais (EPAMIG), cut off from the plant and place in a plastic glass with their peduncles inserted in a vial with water, since it was easier to handle than the entire plant and there is evidence that the presentation method of the plant does not altered the results of longevity experiments (Wade & Wratten 2007). Flowers were replaced during the experiment when they became senescent, approximately every one or two days. One newly emerged *P. coffeae* female was placed for each plastic glass which was considered the experimental unit (Figure 3), and other female was placed in a glass with the vial containing only water to serve as a control. The parasitoids were observed daily to assess their survival and the number of days they survived were registered. For *B. Pilosa* 20 *P. coffeae* fed on the floral nectar and 22 were controls; for *G. parviflora* 22 parasitoids fed on its floral nectar and 22 serve as controls; 22 *P. coffeae* fed on *V. curassavica* and 22 were controls.

Figure 3. Experimental unit for the plants where floral nectar was assessed, here for the *V. curassavica* treatment.

In the case of *S. cernua*, in which the resource assessed was the extrafloral nectar, the experimental unit consist of a clip cage (3 cm diameter), with one female *P. coffeae* inside, placed in a part of the plant containing an extra floral nectary, and with the controls placed in a part of the plant without the extra floral nectary (Figure 4). The parasitoids were observed daily to assess their survival and the number of days they survived were registered. 23 parasitoids fed on the extrafloral nectar and 23 serve as a control. The plants were kept in pots in the laboratory.

Figure 4. Clip cage used to confine each *P. coffeae* on *S. cernua*. Blue arrows showing active extrafloral nectaries in the plant apex.

2.4 Egg load experiment

We assess the effect of *P. coffeae* feeding on extrafloral nectar of *S. cernua* by measuring the egg load in a laboratory experiment. The plant choice for the experiment was based on the promising results found in the *P. coffeae* survival experiment. The experimental unit consist in a clip cage (3 cm diameter) with one newly emerged *P. coffeae* female inside, placed in a part of the plant containing an extrafloral nectary and with the controls placed in a part of the plant without an extrafloral nectary (Figure 4). Females of *P. coffeae* were dissected after 2 days (n=11), 4 days (n=9), 6 days (n=9), 8 days (n=5) and 10 days (n=9). To dissect the wasp, each individual was placed into the freezer for 3 minutes and then placed in an excavated plate with phosphate buffered saline solution. Then, the ovaries were removed with fine needles and eggs were counted under the stereoscope at a magnification of 40x (Figure 5).

Figure 5 . Dissected ovaries of a six-day old *P. coffeae* female.

2.5 Statistical analysis

Survival of *P. coffeae* parasitoids when exposed to floral and extrafloral, nectar was estimated by a Kaplan-Meier survival analysis, each treatment was contrasted with its control. The effect of the extrafloral nectar of *S. cernua* on *P. coffeae* egg load and the parasitoids age on their egg load was estimated by a Generalized Linear Model with Poisson distribution, and the significance by a deviance analysis. All the analyses were performed using the R software version i386 4.1.1.

RESULTS

3.1 Survival experiments

Proacrias coffeae adult females survived longer when feeding on floral nectar of *V. curassavica,* 10.32 ± 2.23 days (mean ± SE), than in the control treatment, with water only, 3.41 ± 0.21 ($p<0.05$; Figure 5.a). *P. coffeae* fed on extrafloral nectar from *S. cernua*, also lived longer 24.22 ± 2.78 days than the controls 6.34 ± 1.36 days ($p<0.05$; Figure 5.b). The parasitoids that fed on *G. parviflora* survived 4.50 ± 0.29 days and 4.27 ± 0.26 for the controls ($p>0.05$; Figure 5.c). In the case of *B. pilosa* the parasitoids survived 4.85 ± 0.50 days feeding on it and 3.80 ± 0.32 days for the controls ($p=0.05$, Figure 5.d).

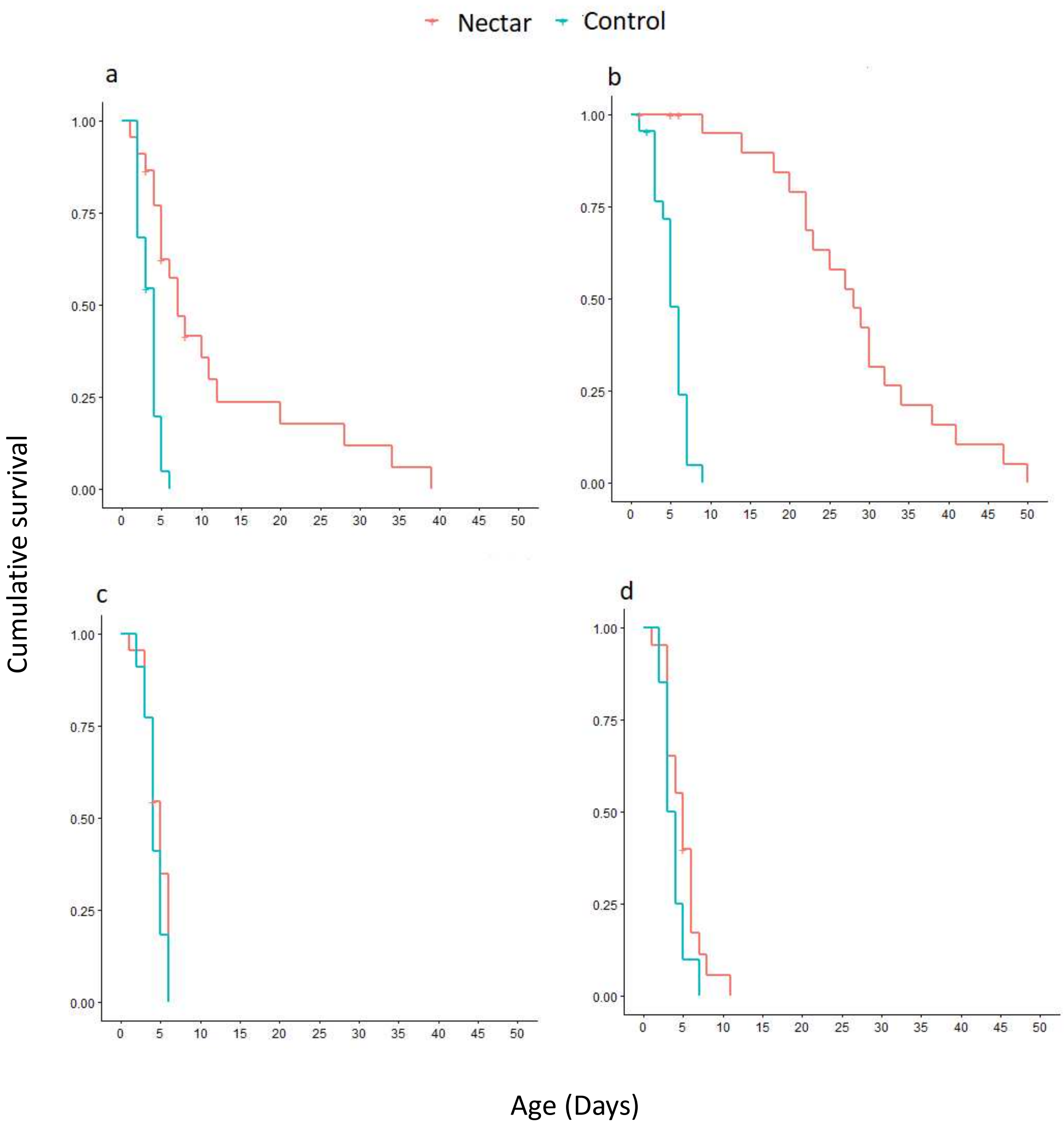

Figure 6. Kaplan-Meier estimates of survivorship functions of the coffee leaf miner parasitoid *P. coffeae* fed on the nectar of: (a) *Varronia curassavica* ($p<0.05$); (b) *Senna cernua* ($p<0.05$); (c) *Galinsoga parviflora* ($p>0.05$); (d) *Bidens pilosa* ($p=0.05$).

3.2 Egg load experiment

The age of *P. coffeae* females and the feeding on *S. cernua* extrafloral nectar independently affected the parasitoid egg load (X_2= 4.0423, df= 4, p= 0.400309). At day 2 the parasitoids matured 4,45 ± 0,57 eggs with food and 2,50 ± 0,36 eggs without food; at day 4 the parasitoids matured 6,11 ± 0,48 eggs with food and 4,44 ± 0,65 eggs without food; at day 6 they matured 6,22 ± 0,41 eggs with food and 6,25 ± 0,82; at day 8 they matured 5,40 ± 0,54 eggs with food and 4,00 ± 1,25 eggs without food and at day 10 they matured 6,50 ± 0,53 eggs with food and 4,43 ± 0,67 eggs without food. The age of *P. coffeae* increase their egg load (X_2= 17.882, df= 4, p= 0.001301, Figure 6). Also, feeding on *S. cernua* extrafloral nectar increase *P. coffeae* egg load (X_2= 10.009, df= 1, p= 0.001558; Figure 7). The parasitoids produced in average 5,74 ± 0,37 when feeding on the extrafloral nectar and 4,32 ± 0,60 for the control treatment.

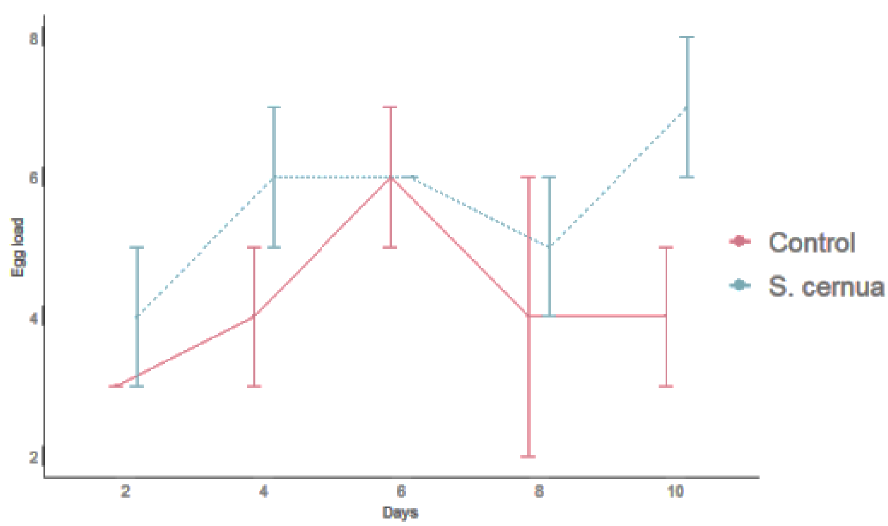

Figure 7. Effect *of P. coffeae* age on egg load (X2= 17.882, df= 4, p= 0.001301).

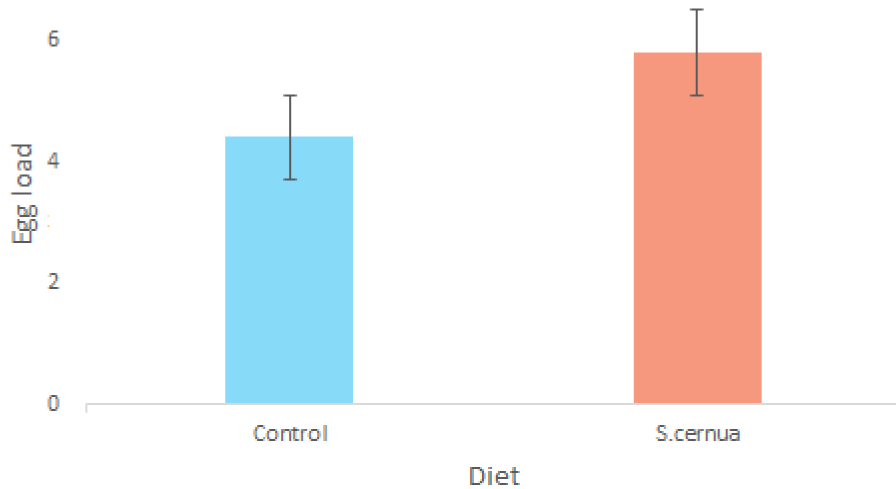

Figure 8. Effect of *S. cernua* extrafloral nectar on *P. coffeae* egg load (X2= 10.009, df= 1, p= 0.001558).

DISCUSSION

Proacrias coffeae could feed and survive longer when confine with the resources of *V. curassavica* and *S. cernua.* The parasitoids that were confine with *B. pilosa* and *G. parviflora* did not show an increment in their survival. However, *B. pilosa* did show a trend of increasing the parasitoids survival. Thus, not all flowering plants are suitable for provide food for coffee leaf miner parasitoids. These results show the importance of selecting the appropriate plant resources aiming to increase natural enemies' fitness, instead of just increasing plant diversity.

The differential results regarding to the tested flowers, might be related to their morphological constrains. Stang et al. (2006) mention some constrains that a potential floral visitor may face to access the floral nectar, in general, the authors found that the number of visitor species decrease whether the nectar holder increase in depth and/or decrease in width. In here, we evaluated flowering plants with different flower morphology, and found that the parasitoids couldn't feed in both plants of the Asteraceae family.

The foraging behavior of two eulophid parasitoids, *Edovum puttleri* Grissell (Hymenoptera: Eulophidae) and *Pediobius foveolatus* Crawford (Hymenoptera

Eulophidae), that are similar in size to *P. coffeae*, was observed by Patt et al. (1997). The authors found that these parasitoids could easily access the extrafloral nectar of *Phaseolus vulgaris* L. (Fabaceae), but not the floral nectar of the Asteraceae plants (*Ageratum houstonianum* Mill., *Achillea millefolium* L., *Galinsoga parviflora* Cav.; and *Matricaria chamomila*) due to the head size of the parasitoids. Although different families of parasitoids have been observed in Asteraceae flowers, the nectar accessibility was not confirmed (Jervis et al., 1993). We did not perform any measurement in our study, but we assume that the corolla tubes of both Asteraceae plants were too long for the *P. coffeae* mouth parts and/or the corolla opening too short to let the parasitoid enter and reach the nectar. *B. pilosa* and *G. parviflora* belong to the Heliantheae tribe which is one of the most diverse in the Asteraceae, however, late-branching tribes such as this tend to have longer corolla tubes (Torres & Galetto, 2002).

In general, *Bidens* sp. flower could favor bigger visitors (Schmalhofer, 2001), and this maybe the case for *Galinsoga,* since their flower share similar morphology. *Galinsoga* sp. and related Asteraceae have been observed to be more visited by flies, maybe due to their flower's colors (white-yellow) (Hernández-Villa et al., 2020). Nave et al. (2016) evaluate the theoretical accessibility of four parasitoids of *Prays oleae* Bernard (Lepidoptera: Praydidae), to the nectar of 21 flowering plants, and found that the Eulophidae species were not able to access the nectar of all four Asteraceae that were evaluated. They found that even the Encyrtidae parasitoid was too big to enter the corolla florets.

Many parasitoids can feed on the pollen of Asteraceae florets (Jervis et al., 1993), which could explain the trend of the *B. pilosa* treatment to increase the survival of *P. coffeae*, pollen is less sugar rich than floral nectar and represent a source of proteins and amino acids (Wäckers, 2005). Even though *P. coffeae* were able to feed on pollen, its effect on insects' survival haven't been as studied as the effect on reproduction, so other experiments would be needed to evaluate this hypothesis. Although we believe that the response of the feeding on the nectar of the two Asteraceae in *P. coffeae* fitness can be explained by their flower's morphology, other factor may influence the response. Repellency of some plants may cause a different response in the effect on the parasitoid's longevity when plants with similar morphological traits are offered (Lavandero et al., 2006).

The quality of the nectar may also influence the effect of the feeding resources in the longevity of parasitoids. Sucrose rich nectar seems to have a stronger effect on parasitoid survival than hexose rich nectar (Vattala et al., 2006). In Asteraceae most species have hexose predominance in their sugar composition (Torres & Galetto, 2002). Given that information, Asteraceae in general, would be less suitable feeding resources to small parasitoids like *P. coffeae*, in terms of either accessibility or nectar nutritional quality.

Unlike the Asteraceae, feeding on flowers of *V. curassavica* increased *P. coffeae* survival. *V. curassavica* has been reported to be visited by beneficial insects, such as, pollinators, predators, and parasitoids (Martins, 2017). This plant has the capacity of flowering through the entire year with an increment in the warmer months (Brandão et al., 2015), which makes them a good alternative to offer feeding resources to natural enemies. Besides that, their floral morphology allows small parasitoids to access its nectar, given that they have wide corollas.

There is no information about the sugar composition of neither the floral nectar of *V. curassavica* nor the extrafloral nectar of *S. cernua.* However, these two plants show a positive effect on *P. coffeae* survival, most likely due to the easy access that these parasitoids could have on their feeding resources. Parasitoids' mouth parts are not specialized to feed on floral nectar of flowers with long corollas, and are more benefited from sources of expose nectar, like open and short corollas, like in *V. curassavica* (Patt et al., 1997; Géneau et al., 2012). This has been shown as well for other eulophid parasitoids as *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae), which is capable of foraging on extrafloral nectaries and exposed nectaries (Patt & Rohriq, 2017).

Survival of *P. coffeae* increased when feeding on extrafloral nectar of *S. cernua*. In the case of extrafloral nectar, the parasitoids must deal with no morphological constrain, because the nectar is totally expose. Rezende (2014) show that *P. coffeae* can feed on *Inga* sp. extrafloral nectar and that leads to an increase of its survival as well as for other coffee leaf miner parasitoids. Although totally expose, the extrafloral nectar must meet another requirement to be suitable for parasitoids exploitation in the field that is their attractiveness. Damien et al. (2019) found that for the aphid's parasitoid *Aphidius rhopalosiphi* DeStefani-Peres (Hymenoptera: Braconidae), there was a preference for attractive flowers when compared to flowers with more accessible nectar. And in the case of extrafloral nectar, as it doesn't have any visual attractiveness, its odor must be enough for parasitoids to find it.

Some studies have reported the influence of extrafloral nectar on other parasitoid species fitness. Benelli et al. (2017) reported an increment in the longevity of *Diaeretiella rapae* McIntosh (Hymenoptera: Braconidae) when feeding on *Vicia faba* L. (Fabaceae) extrafloral nectar, greater than when feeding on honey. Géneau et al. (2012) assessed the effect of both floral and extrafloral nectar in two parasitoids of lepidopteran pests and found that both can increase the fitness of the parasitoids. These authors discuss the importance of extrafloral nectar in terms of temporal availability and the potential selective use for lepidopteran parasitic wasp since their concentration constrains the nectar uptake for lepidopteran insects.

This constrain of lepidopteran insect in the use of extrafloral nectar could be considered when designing coffee leaf miner conservation biological control programs. Although it wasn't measured in the present study, we can expect a similar response from this lepidopteran pest. Care must be taken, both with plant introduction for conservation biological control programs and experimental settings, because this response may vary according to the environmental conditions, as high relative humidity could change the concentration of the extrafloral nectar decreasing its viscosity and make it suitable for lepidopteran insects (Winkler et al., 2009).

The increase in *P. coffeae* longevity when feeding on nectar from *V. curassavica* and *S. cernua*, can benefit their parasitism ability giving to the parasitoids more time to foraging for host and to mature their eggs. We showed that *Proacrias coffeae* females emerge with a few mature eggs and their egg load increases over time. According to Jervis et al. (2001), this is a characteristic that belongs to synovigenic parasitoids, what give them the advantage of better responding to variations in their host availability, even though unable them to lay a great quantity of eggs in early their life (Jervis & Ferns, 2004). Synovigenic parasitoids have a greater lifespan than pro-ovigenic ones, although lifespan as well as egg load also depend on feeding resources availability (Jervis et al., 2001).

Adult parasitoids allocate the resources for egg manufacture from teneral reserve (acquire during larval feeding) and dietary components (Jervis et al., 2008). Synovigenic parasitoids acquire nutrients that can be allocated for maintenance or for oogenesis and egg maturation, and scarcity of food can lead to a tradeoff between those two processes, and some parasitoids can even reabsorb their eggs to survive to food depletion conditions (Wang et al., 2014). In terms of conservation biological control that means that introducing in the coffee agroecosystem feeding resources for *P. coffeae* can increase their success as biocontrol agents, not just by enhancing their survival but also their reproductive potential. Considering that parasitoids must deal with the decision of foraging for host or food, having the food near to the host can help solve this issue, and allow the permanence of the parasitoids in the agroecosystem (Damien et al., 2019).

Here we found that diet can increase egg load of *P. coffeae*, the parasitoids show a greater number of mature eggs when feeding on *S. cernua* extrafloral nectar. Extrafloral nectaries have the advantage of producing nectar through all the year, not depending on flowering seasons, and have no morphological constrains for parasitoids feeding. Thus, leguminous plants as *S. cernua* can be a suitable option to diversify coffee plantations (Venzon et al., 2021).

After these results, we suggest an initial screening of the plants to be considered in future experiments on *P. coffeae* fitness and similar parasitoids based on their floral morphology (particularly the position of the nectaries in flowering plants). Finding nectar resources that could be as attractive as beneficial for parasitoids can be a challenging task, Chen et al. (2020) discusses that even when introducing plants that have shown is positive effect on parasitoid's fitness in the laboratory it won't ensure nectar feeding, because the potential value of the resource may not correlate with its attractiveness. For *V. curassavica* Martins (2021) suggest that coffee diversified systems with this plant along with *Inga edulis* and *Senna macranthera* DC. ex Collad. H.S.Irwin and Barneby (Fabaceae) can increased coffee leaf miner parasitoids populations, resulting in an effective control of coffee leaf miner.

Studies on *S. cernua* attractiveness need to be performed in order to assess their potential in the coffee field, as well as studies on their effect on coffee leaf miner fitness, given that the insect pest itself can fed on nectar. For *P. coffeae* can be interesting to assess the effect of other feeding resources, as plants have different compositions in their floral and extrafloral nectar, and different types of sugar can benefit differentially the parasitoids' fitness (Wang et al., 2014). Also, we didn't observed host feeding behavior for these parasitoids, although it may be important to acquire lipids for egg manufacture since the hymenopteran parasitoids lack lipogenesis (Visser & Ellers, 2008).

Together, our results show the potential of *V. curassavica* and *S. cernua* of providing nutritional resources to the parasitoid *P. coffeae* and therefore enhance their fitness by an increment of their survival in the case of both plants, and on the parasitoid egg load in the case of *S. cernua* extrafloral nectar. It is possible that same occurs for *V. curassavica*, but for logistic reasons, we did not teste it in our study. This finding leaves a lead on the kind of plants that can be used when aiming to control the coffee leaf miner, flowering plants with exposed nectaries and/or with extrafloral nectaries must be prioritized.

CONCLUSIONS

The fitness of *Proacrias coffeae* can be enhance in the presence of feeding resources as floral and extrafloral nectar. The observed response may be influence mainly for the floral morphology, we suggest that expose nectaries and extrafloral nectar may be more suitable for small parasitoids as *P. coffeae.* Evaluation of the diameter of the flower´s corollas and the parasitoids heads need to be performed*. Varronia curassavica* and *Senna cernua* were found to be promising candidates to be include in conservation biological control programs for coffee leaf miner management.

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