Effect of the floral nectar of *Varronia curassavica* Jacq. (Boraginaceae) on the survival of the coffee leaf miner parasitoid *Proacrias coffeae* Ihering (Hymenoptera: Eulophidae).

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RESUMO EXPANDIDO

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Abstract: The enhancement of pest control through the conservation of natural enemies in agricultural fields is called conservation biological control. One of the strategies used in this system is to introduce or manage plants that can provide food for natural enemies. We assessed the effect of feeding resources on the fitness of a coffee leaf miner parasitoid, *Proacrias coffeae* Ihering (Hymenoptera: Eulophidae), by evaluating the effect the floral nectar of *Varronia curassavica* on the survival of *P. coffeae*. When feeding on the floral nectar of *V. curassavica* the parasitoids increased their survival. Our results show the potential of *V. curassavica* as nutritional resource to enhance the fitness of the parasitoid *P. coffeae*, by increasing their survival. Based on the research study, it is evident that the introduction of feeding resources for *P. coffeae* in the coffee agroecosystem will increase their fitness as a potential biocontrol agent.
Keywords: plant-provided food; nectar; conservation biological control.

Introduction

Conservation biological control has been developed consistently in the tropics over the last decades (Venzon et al., 2019). Most of the advances have been possible due to successful experiences in understanding how natural enemy species or assemblages interact with local resources provided by companion and non-crop plants within or nearby the main crop (Venzon et al., 2019). This is directly related to the overarching goal of conservation biological control based on strategies for management practices that favor natural enemy attraction by increasing their fitness and impact on pest population control (Shields et al., 2019). Such manipulation can be performed by the diversification of the vegetation to increase the provision of alternative and supplementary food sources, which increases the availability of resources and creates suitable conditions for the natural enemies to establish within the crop (Venzon e Suji 2019). Several studies found that increased plant diversity in coffee crops increased the parasitism rate of the coffee leaf miner *Leucoptera coffeella* Guérin-Méneville (Lepidoptera: Lyonetiidae) (Fernandez, 2013; Marques 2017; Rezende, 2014; Rezende et al., 2021). The coffee leaf miner is one of the key coffee pests in Brazil. The most commonly used measure to manage this pest is chemical control (Leite et al., 2020). Although several parasitoid species have been reported in coffee crops (Ferreira et al., 2013; Melo et al, 2007; Ramiro et al., 2007; Resende et al., 2007; Tango et al., 2014) their use in augmentative and conservation biological control strategies would be benefited by selecting plants that increase

parasitoids' fitness, whether parasitoids were mass released or naturally attracted to the crop (Togni et al., 2019). The natural parasitism of the coffee leaf miner may reach 34.17% in diversified agroecosystems (Shields et al., 2019). This can be explained by the fact that in diversified agroecosystems there are more resources to attract, maintain, and reward natural enemy populations (Amaral et al., 2010; Venzon, 2021)

The presence of *Varronia curassavica* Jacq. (Boraginaceae), *Inga edulis* Mart. (Fabaceae), and *Senna macranthera* (DC. ex Collad.) H.S. Irwin & Barneby (Fabaceae), and non-crop plants kept the coffee leaf miner populations under the economic threshold level, mainly due to the increase in the pest parasitism rate [17]. However, except for Rezende et al. (2014) those studies do not elucidate the functional role of any specific plant or the related mechanisms that could explain the benefits of plant diversity in coffee crops. Here, we evaluated the potential of *V. curassavica*, to provide food resources in order to increase the fitness of coffee leaf miner parasitoids, using *P. coffeae* a as a model species. We anticipate that feeding on plant nectar will increase parasitoid survival *V. curassavica*.

Methodology

Coffee leaf miner parasitoids were initially obtained from intact mined coffee leaves from Catuaí coffee variety with plants at complete maturity (nine years old). The leaves were collected in a small plot in the experimental field "Diogo Alves de Mello" of the Universidade Federal de Viçosa (2048045" S; 4256015" W), state of Minas Gerais, Brazil. The region is located in the Brazilian Atlantic Rainforest biome, which presents an average temperature ranging from 12 to 22 C and annual rainfall ranging from 1300 to 1900 mm (Alvares et al., 2013).

In the laboratory, the leaves were placed individually in 350 mL plastic pots with their petioles inserted in vials with water to maintain their turgidity. The leaves were kept in the laboratory under ambient conditions (at 19 to 26 C and 40 to 90% RH). The pots were checked daily until the emergence of the parasitoids. After emergence, the parasitoids were kept in 10 mL vials with ethanol 70%. *Proacrias coffeae* was the most common and easiest to rear of all the species collected.

Immediately after emergence, the parasitoids were identified and sexed. Couples were kept inside a 350 mL plastic pot with a drop of honey for 24 h. Following that, females were individually placed in 350 mL plastic glasses with a drop of honey in the glass wall and three mined coffee leaves. The replaced leaves were kept in other 350 mL plastic glasses, and after 15 days, all leaves were opened to collect the parasitoid pupae. The pupae were placed inside 10 mL plastic vials that were checked every day until the emergence of the parasitoids. Medium-sized mines (about 1 cm diameter) were picked to maximize the occurrence of the first and second instars of the coffee leaf miner, which were assumed to be the preferred stages for the parasitoid's oviposition (Navarro-Gutiérrez et al., 2009).

To assess the effect of the floral nectar of *V. curassavica* on *P. coffeae* survival, a non-choice experiment was performed in the laboratory. The flowers were collected

from plants naturally growing in the green areas of the Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG), state of Minas Gerais, Brazil. They were cut off from different plants and immediately transferred to a 350 mL plastic pot with their peduncles inserted in a 10 mL vial with water. For the control group, one female of the same age was kept inside a 350 mL plastic pot containing only water in cotton inside a 10 mL vial. The parasitoids were observed daily to assess their survival, with 22 observations for the treatment and the control.

The survival of P. coffeae parasitoids when exposed to floral and extrafloral nectar were estimated by a Kaplan-Meier survival analysis. Each treatment was separately contrasted with its control. The analyses were performed using the R software (Core Team 2017).

Results and discussion

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 ($p < 0.0001$; Figure 1).

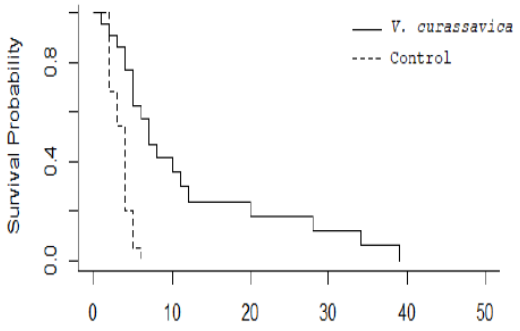

Figure 1. Kaplan-Meier estimates of survivorship functions of the coffee leaf miner parasitoid *P. coffeae* fed on the nectar of *Varronia curassavica* ($p<0.0001$).

We suggest that these results may be due to a combined effect on the parasitoid's capacity of accessing flower resources (Nave et al., 2016) and the quality of the nectar from this plant. *V. curassavica* has been reported to be visited by several beneficial insects, such as pollinators, predators, and parasitoids (Martins 2017). It has the capacity to flower through the entire year with an increment in the warmer months (Hoeltgebaum et al., 2018) which makes them a good alternative to offering feeding resources to natural enemies. Therefore, this plant could be used to improve biological control services as well as other ecosystem services such as pollination, which is desirable for biodiversity and ecosystem services in tropical regions, such as Brazil (Ramos et al., 2020). The floral morphology of this plant allows small parasitoids to access the nectar, given that they have wide corollas. The mouth parts of parasitoids are not specialized to feed on the floral nectar of flowers with long corollas and benefit more from exposed nectar sources, such as open and short corollas, as observed in *V. curassavica* (Géneau et al., 2012).

Conclusion

Our findings show that *V. curassavica* has the potential to provide nutritional resources to the parasitoid *P. coffeae*, thereby increasing their fitness by increasing survival. This finding leaves a lead on the kind of plants that can be used when aiming to control the coffee leaf miner in tropical agroecosystems. We suggest that native flowering plants with exposed nectaries should be prioritized.

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