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Plant-provided food for predators of key coffee pests

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Thesis submitted to the Entomology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Madelaine Venzon

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ABSTRACT

SILVEIRA, Pedro Inocencio, D.Sc., Universidade Federal de Viçosa, July, 2025. **Plant-provided food for predators of key coffee pests**. Adviser: Madelaine Venzon.

The intense population growth strongly affects the landscapes and pressures food production. Brazil is the bigger coffee producer and exporter in the world and chemical control of pests is the most used method to control unwanted insects. In this scenario, insecticides can create pest resistant populations, outbreaks of secondary ones and harm human and environment health. This context accentuate the need to integrate alternative methods in order to control pests in a safer way. In this thesis I investigated if plant-provided resources, such as nectar and pollen, selectively benefits natural enemies without benefiting key coffee pests. In Chapter I, I examined if *Senna cernua* extrafloral nectar improves *Chrysoperla externa* and *Harmonia axyridis* fitness. I performed survival experiments inside a green house to immature, where larvae from both species were fed with the extrafloral nectar at the plants. For the adults, I realized experiments on the green house and also in laboratory to analyze the effect of nectar on survival and reproduction of these important beneficial insects. The extrafloral nectar of *S. cernua* increased significantly the survival of *C. externa* and *H. axyridis*, both larvae and adults. *C. externa* females could even oviposit feeding only on this nectar. In Chapter II, I inspected if *Crambe abyssinica* flowers provides resources capable of benefit the same two predators from Chapter I, without favoring *Leucoptera coffeella adults*, the coffee leaf miner. I carried out laboratory experiments to examine if *C. abyssinica* floral resources (nectar or pollen), affects survival of larvae and survival or reproduction of the adults natural enemies. I also inspected if *L. coffeella* adults can feed on floral nectar and increase its survival or reproduction. Lastly, I investigated if honeydew produced by the green aphid *Myzus persicae*, a species hosted by *C. abyssinica*, affects the coffee leaf miner survival or reproduction. Larvae from *C. externa* and *H. axyridis* could feed on floral resources but just adults of *C. externa* could benefit from it, increasing its survival. *L. coffeella* adults did not feed on *C. abyssinica* floral nectar and also did not feed on honeydew produced by the aphids. Finally, in Chapter III, in laboratory, I tested whether ladybug species can prey and feed upon *L. coffella* and *Hypothenemus hampei* (coffee berry borer) life stages. For the first time, a predatory ladybug species was registered feeding on the coffee leaf miner pre-pupae. Similarly, for the first time ever, two new ladybug species were recorded entering the coffee berry borer galleries and preying on immature stages of this pest. In this regard, this study shows that is possible to integrate sustainable approaches to control pests. Both *S. cernua* and *C. abyssinica* should be introduced on coffee crops in order to increase biological control of *L. coffeella* and *H. hampei*, attracting and keeping natural enemies in the field over longer periods of time. This study presents a valuable contribution to regenerative coffee farming.

Keywords: conservation biological control; *Hypothenemus hampei*; *Leucoptera coffeella*; coccinellids

RESUMO

SILVEIRA, Pedro Inocencio, D.Sc., Universidade Federal de Viçosa, julho de 2025. **Alimentos fornecidos por plantas para predadores de pragas-chave do cafeeiro**. Orientadora: Madelaine Venzon.

O intenso crescimento populacional afeta fortemente as paisagens e pressiona a produção de alimentos. O Brasil é o maior produtor e exportador de café do mundo e o controle químico de pragas é o método mais utilizado para controlar insetos indesejados. Nesse cenário, os inseticidas podem criar populações resistentes a pragas, surtos de pragas secundárias e prejudicar a saúde humana e ambiental. Esse contexto acentua a necessidade de integrar métodos alternativos para controlar pragas de forma mais segura. Nesta tese, investiguei se recursos fornecidos pelas plantas, como néctar e pólen, beneficiam seletivamente os inimigos naturais sem beneficiar as principais pragas do café. No Capítulo I, examinei se o néctar extrafloral de *Senna cernua* melhora o fitness de *Chrysoperla externa* e *Harmonia axyridis*. Realizei experimentos de sobrevivência dentro de uma estufa para as fases imaturas, onde larvas de ambas as espécies foram alimentadas com o néctar extrafloral nas plantas. Para os adultos, realizei experimentos na estufa e também em laboratório para analisar o efeito do néctar na sobrevivência e reprodução desses importantes insetos benéficos. O néctar extrafloral de *S. cernua* aumentou significativamente a sobrevivência de *C. externa* e *H. axyridis*, tanto larvas quanto adultos. Fêmeas de *C. externa* conseguem até ovipositar alimentando-se apenas deste néctar. No Capítulo II, inspecionei se as flores de *Crambe abyssinica* fornecem recursos capazes de beneficiar os mesmos dois predadores do Capítulo I, sem favorecer os adultos de *Leucoptera coffeella*, o bicho-mineiro do cafeeiro. Realizei experimentos de laboratório para examinar se os recursos florais de *C. abyssinica* (néctar ou pólen) afetam a sobrevivência das larvas e a sobrevivência ou reprodução dos inimigos naturais adultos. Também inspecionei se adultos de *L. coffeella* podem se alimentar de néctar floral e aumentar sua sobrevivência ou reprodução. Por fim, investiguei se o honeydew produzido pelo pulgão verde *Myzus persicae*, uma espécie hospedada por *C. abyssinica*, afeta a sobrevivência ou reprodução do bicho-mineiro do cafeeiro. Larvas de *C. externa* e *H. axyridis* podem se alimentar de recursos florais, aumentando sua longevidade. Em relação aos adultos, apenas *C. externa* se beneficiam das flores, aumentando sua sobrevivência. Adultos de *L. coffeella* não se alimentaram de néctar floral de *C. abyssinica* e também não se alimentaram do honeydew produzido pelos pulgões. Finalmente, no Capítulo III, em laboratório, testei se espécies de joaninhas podem predar e se alimentar de estágios de vida de *L. coffella* e *Hypothenemus hampei* (broca do cafeeiro). Pela primeira vez, uma espécie de joaninha predadora foi registrada alimentando-se de pré-pupas do bicho-mineiro do cafeeiro. De maneira semelhante, pela primeira vez, duas novas espécies de joaninhas foram registradas entrando nas galerias da broca do café e predando estágios imaturos desta praga. Nesse sentido, este estudo mostra que é possível integrar técnicas sustentáveis para o controle de pragas. Tanto *S. cernua* quanto *C. abyssinica* devem ser introduzidos nas lavouras de café para aumentar o controle biológico de *L. coffeella* e *H. hampei*, atraindo e mantendo inimigos naturais no campo por períodos mais longos. Este estudo apresenta uma contribuição valiosa para a cafeicultura regenerativa.

Palavras-chave: controle biológico conservativo; *Hypothenemus hampei*; *Leucoptera coffeella*; coccinelídeos

Sumário

GENERAL INTRODUCTION

The constant population growth and demand for food production conducts to agricultural intensification which leads to environment homogenization, resulting in decrease of biodiversity and provision of ecosystem services (Tscharntke et al., 2005; Flohre et al., 2011). On the other side, landscape heterogeneity creates a more regenerative agriculture that makes use of ecosystem services in a sustainable way (Turner et al., 2013).

One of the major ecosystem services allied to sustainable and resilient agriculture is biological pest control (Emmerson et al., 2016). Brazil is the bigger coffee producer in the world and was responsible for 37% of all production last year (USDA, 2024). Nevertheless, various factors affect coffee production negatively, such as the attack of *Leucoptera coffeella* Guérin-Méneville (Lepidoptera: Lyonetiidae), the coffee leaf miner (CLM), and the attack of *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae), the coffee berry borer (CBB), both considered key coffee pests in Brazil, responsible for significant yield losses (Venzon, 2021).

CLM is native from the African continent and was first reported in 1850 in Brazil and afterwards to most American coffee-producing countries (Green, 1984). Its control is difficult because immature life stages feeds in the palisade parenchyma, protected inside the leaves (Pereira et al., 2007; Dantas et al., 2021). At high population densities, this pest can cause more than 70% defoliation, reducing coffee yields in 50% (Reis and Souza, 1996).

CBB is also a pest native from Africa and its control is complicated due to the fact that females pierce the coffee berries, oviposit in the endosperm, and the larvae complete their entire life cycle inside the fruits, reducing coffee yield and quality of grains (Damon, 2000; Infante, 2018). The losses generated by this pest, in Brazil, are estimated from more than US$300 million every year (Oliveira et al., 2013).

Yet, pesticides have been extensively used in an attempt to control these pests, many times creating resistant populations, harming human health and the environment (Johnson et al., 2020; Leite et al., 2020). This situation highlights the need to adopt alternative strategies to deal with these insects in a more environment-friendly approach. A very suitable way to do that is through conservation biological control.

Conservation biological control of pests is based on techniques such as habitat management and diversification of the environment with plants that provide alternative foods, oviposition sites or shelter to natural enemies, attracting them and improving their performance, without benefiting pests (Landis et al., 2000; Venzon et al., 2006; Begg et al., 2017). Additionally, these plants can not host key pests of the main crop and also can not compete severely for water and nutrients with coffee plants and eventually should not need effortful cultivation (Venzon and Sujii, 2009; Venzon et al., 2011).

In this thesis, I investigated if plant-provided resources selectively benefits natural enemies without benefiting coffee pests. In Chapter I, I examined if *Senna cernua* (Fabaceae) extrafloral nectar improves *Chrysoperla externa* (Neuroptera: Chrysopidae) and *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) fitness. I performed survival experiments on a green house to immature, where larvae from both species were fed with the extrafloral nectar. For the adults, I performed experiments on the green house and also in laboratory to analyze the effect of nectar on survival and reproduction of these natural enemies. In Chapter II, I inspected if *Crambe abyssinica* (Brassicaceae) flowers provides resources that benefits the same two predators above cited without favoring CLM adults. I carried out laboratory experiments to examine if *C. abyssinica* floral resources (nectar or pollen), affects survival of larvae and survival or reproduction of the adults natural enemies. I also inspected if CLM adults can feed on floral nectar and increase its survival or reproduction. Lastly, I investigated if honeydew produced by the green aphid *Myzus persicae* (Hemiptera: Aphididae), a species hosted by *C. abyssinica*, affects CLM survival or reproduction. Finally, in Chapter III, in laboratory, I tested whether ladybug species can prey and feed upon CLM and CBB life stages.

In this regard, this study shows that is possible to integrate sustainable approaches to control these two dangerous coffee pests in a safer way, both to humans and environment. This study can also be used as basis to new researches concerning diversification of coffee monocultures as well as it can help farmers to control CLM and CBB incorporating new techniques that contributes to regenerative agriculture.

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CHAPTER I

Does *Senna cernua* extrafloral nectar improve *Chrysoperla externa* and *Harmonia axyridis* fitness?

ABSTRACT

The strategic selection of candidate plants plays a crucial role in promoting balanced and diversified agricultural ecosystems, where certain plants not only attract natural enemies and pollinators but should also possess the ability to provide additional ecosystem services. *Senna spp.* are leguminous plants that fulfill these requisites due to their provision of extrafloral nectar. Attributable to the possibility to be associated among crops, we inspected if *Senna cernua* Balb. H.S Irwin & Barneby (Fabaceae) selectively provide vital resources for natural enemies. Firstly, we carried out survival experiments on immatures of *Chrysoperla externa* Hagen (Neuroptera: Chrysopidae) and *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in the presence or absence of extrafloral nectaries. We subsequently assessed the effects of extrafloral nectar on the survival and reproduction of adult individuals of the same species. The presence of extrafloral nectar of *S. cernua* increased survival of *C. externa* larvae by more than fourfold ($p < 0.0001$) and adult's by more than sixfold ($p < 0.0001$) in comparison with the control. Of the total, 30% of *C. externa* larvae pupated, and 13% reached adulthood solely on *S. cernua* extrafloral nectar. The mean number of eggs laid by females was 14, with an egg viability of 83% when nourished with this nectar. The same nectar increased the survival of *H. axyridis* larvae by nearly fivefold ($p < 0.0001$) and adult survival by fourfold ($p < 0.0001$) in comparison with the control groups. *S. cernua* is capable of benefit these two important generalist predators through the provision of extrafloral nectar as an alternative source of food, proving suitable to be introduced among crops.

Keywords: Conservation biological control, green lacewings, ladybugs, beneficial insects.

1. INTRODUCTION

Conservation biological control is an environment-friendly approach based on ecological techniques that support natural enemies in order to suppress pest populations (Begg et al., 2017; Shields et al., 2019; Venzon et al, 2019). One way to attract and keep beneficial insects in the area is through strategically diversification of vegetation, because they rely upon plant-derived food to supplement their diet during larval stage, others during adult stage or both or even during non-carnivorous life stage, like adult green lacewings (Venzon et al., 2006; Amaral et al., 2013). These plants must provide resources to natural enemies, including oviposition sites, shelter, and specially food, such as pollen and nectar, thereby contributing to pest control services (Landis et al., 2000; Gurr et al., 2003; Orr and Fox, 2012; Rusch et al., 2016).

Pollen, or pollen grain is the male gametophyte, formed in the anthers, used by plants to reproduce, in other words, without pollen, there are no seeds (Bedinger, 1992; McCormick, 2013). Pollen is mostly composed by nitrogenous compounds which protein levels vary from 2.5% from more than 60% (Roulston and Cane, 2000). Otherwise, floral nectar is a sugar-dominant solution (more than 90% of its dry weight) composed mainly of sucrose and its monomers glucose and fructose (Nepi et al., 2012). In a complementary way, floral nectar is reputed as the main available reward of plants to attract pollinators and boost its reproduction. While searching for nectar, pollinators, without intention, carry pollen grains and release them on stigma (Koptur, 1994; Fridman et al., 2012; Nepi et al., 2012).

Floral nectaries might be at the same level as the surface or be profoundly immersed inside the organ that bears them. If exposed, nectar can be gathered by many animals and can also evaporate quickly, but if the nectar is produced inside the corolla, or in a spur, it is preserved against evaporation and fewer animals would be able to collect it (Pacini et al., 2003). From the evolutionary point of view, the diversity in nectary location is shaped together with the large diversity of pollinators, their morphology and behavior as foragers (Bernardello, 2007).

On the other hand, extrafloral nectars are believed to be an indirect plant defense/resistance: a sugary meal, rich in carbohydrates with also some amino acids that attracts predators and parasitoids in return of protection against herbivory (Koptur, 1994; Wackers et al., 2001; Turlings and Wackers, 2004; Shenoy et al., 2012; Staab et al., 2017).

Extrafloral nectaries are nectar-secreting glands positioned outside the flowers and these structures have been seen on a broad diversity of plant species (over 93 families and 332 genera) (Koptur 1992; Marazzi et al., 2013). These nectaries normally produce nectar during longer periods (3-6 weeks) in comparison with floral nectaries (1-3 days) (Rogers, 1985; Koptur, 1992).

Indirect defenses of plants usually involve vegetative structures, such as extrafloral nectaries, that increase the abundance of polyphagous predators that control the damage caused by herbivores (Del-Claro et al. 2016; Moura et al. 2021). The susceptibility of a plant to herbivory can be affected by the characteristics of its plant neighbors. Neighboring plants can increase (associational susceptibility) or decrease (associational resistance or defense) the probability of a target plant being detected and consumed by an herbivore (Hay, 1986; Wahl and Hay, 1995). Plants bearing extrafloral nectaries have been shown to provide associational resistance to other plants close to them (Jezorek et al., 2011; Rezende et al., 2014; Rezende et al., 2021; Moura et al., 2022; Moura and Del-Claro, 2023).

Selecting appropriate companion plants within agricultural landscapes requires careful consideration to ensure that these plants effectively support natural enemies without benefiting pest populations (Venzon et al., 2006). Qualities such as survivability, simplicity of management, resource provision to beneficial insects are essential considerations to take. Furthermore, accurately interpreting the interactions among herbivores, natural enemies, and plants is crucial for the effective application of conservation biological control techniques (Venzon and Sujii, 2009; Venzon et al., 2011).

The use of plants that are able to provide more than one ecosystem service other than pest control must be considered, fostering even more the environment resilience (Fiedler et al., 2008; Blowers et al., 2017). Leguminous plants such as *Senna* spp. are well known for its capacity to provide soil nitrogen fixation besides producing extrafloral nectar that can attract beneficial insects to help in pest control (Franche et al., 2009; Rezende et al., 2021). *Senna. cernua* Balb. H.S Irwin & Barneby (Fabaceae) is native from South America (Brazil, Bolivia and Paraguay) (Royal Botanic Gardens) and bears extrafloral nectaries, on the base of its petioles that could be easily accessed by some insects (Oliveira and Garcia, 2021).

The green lacewing *Chrysoperla externa* Hagen (Neuroptera: Chrysopidae) is a widespread species native from the Neotropical region, encompassing Central and South Americas (Albuquerque et al., 1994). The larvae are generalist predators of a variety of soft-body arthropods (Carvalho and Souza, 2000) and the adults have glyciphagous/polliniphagous diets, feeding on pollen, nectar and honeydew (Albuquerque et al., 1994; Venzon et al., 2006). In turn, the multicolored ladybug *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) is native from the surroundings of Altai Mountains in Asia, both larvae and adults are generalist predators, preying on several aphid species but also other soft-body insects such as eggs and immature stages of Lepidoptera and Coleoptera, psyllids, mites (Iablokoff-Khnzorian, 1982; Cho et al., 1996; Stuart et al., 2002; Koch et al., 2003) and pollen (Berkvens et al., 2008). Although carnivorous, many predators rely on plant-derived food to supplement their diets, especially when preys are not abundant, and the nutritional value of these foods can vary with those situations and with insect species (Venzon et al, 2006).

Given to the role of extrafloral nectaries in crop plants, few studies have been conducted in agricultural settings (Jones et al., 2017). In this study, we evaluated the potential of *S. cernua* as a provider of extrafloral nectar. We tested the hypothesis that this nectar is beneficial to *C. externa* and *H. axyridis*, and that it can sustain these generalist predators, thereby enhancing the conservation biological control of pest populations. Specifically, we assessed the survival probability of larvae and adults of both species, as well as pre-oviposition period, oviposition rate and egg viability females in the presence and absence of extrafloral nectar from *S. cernua*.

2. MATERIAL AND METHODS

2.1. Green lacewing rearing

For the experiments, we used larvae and adults of *C. externa* from the rearing of the Laboratory of Entomology at Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG), located in Viçosa, state of Minas Gerais (MG), Brazil. The predator was reared following the methodology of Venzon et al., (2006). Adults were kept in PVC tube cages (15x15 cm) lined with paper towels. The cages were supported on plastic trays lined with paper towels and closed with PVC film. They are fed with a diet of yeast and honey (1:1) offered on a parafilm stripe hanged inside the cage. The water was provided in a glass bottle (10 mL) with cotton soaked. Diet and water were replaced twice a week. Paper towels and PVC film where females lay their eggs were replaced weekly. Eggs and larvae were individualized in plastic container (10.0 cm high and 4.0 cm of diameter). The larvae were fed with eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), added every three days, until pupation. The rearing is kept at 25 ± 2°C, 70 ± 10% RH and 12h photophase.

2.2. Ladybug rearing

We used larvae and adults of *H. axyridis* collected on maize crop at the Agronomy field of the Federal University of Viçosa (UFV), Viçosa, MG. The rearing was kept at the same place and conditions as described above for *C. externa* rearing. Adults were kept in plastic containers (15x10 cm), fed with a honey solution soaked cotton, aphids and eggs of *E. kuehniella,* replaced twice a week. A cabbage leaf with its petiole wrapped in wet cotton to keep it turgid was replaced weekly as an oviposition site. A crumpled piece of paper was also weekly replaced, serving as shelter to them. The eggs were then separated in masses and the larvae were individualized in J10 (10 mL) containers, fed with *E. kuehniella* eggs every three days until turning into pupae.

2.3.Survival and reproduction of *C. externa* on extrafloral nectaries of *S. cernua*

Here we analyzed the potential of the *S. cernua* in increasing the survival probability and reproduction of *C. externa* due to the provision of extrafloral nectar. Plants of the same age and similar height were used. In the first experiment, second instar larvae (to avoid the high natural mortality of first instar) were individually placed in small clip cages (3x3 cm) under two treatments: i) presence of one nectary in the plant; and ii) absence of the nectary (presence of plant stem and leaves). Their survival was observed daily and each plant housed one individual from treatment and one from control. In total, 23 repetitions of each treatment were done. Similar experiment was conducted with the adults. The larvae were fed with *E. kuehniella* eggs twice a week until pupation. After adult emergency, they were sexed under microscope regarding their difference on the end of their abdomen (Morse, 1931). Couples were caged in plastic containers (8x10 cm) attached to the plants, under two treatments: i) presence of two or three *S. cernua* EFN (extrafloral nectaries) in the plant; and ii) absence of the EFN in the plant (presence of plant stem and leaves). Male and female survival probability, pre-oviposition period, total and mean oviposition were evaluated. In total, 24 repetitions of each treatment were done. Due to the difficulty of opening the plastic containers without letting the adults to escape to analyze the egg viability, another experiment was conducted. After adults emerged, they were sexed like described above, and couples were caged in plastic containers (8x6 cm) under two treatments: i) presence of EFN of *S. cernua* and water-soaked cotton; and ii) just water-soaked cotton. The EFN was collected every two days from the plants with a brush and was rubbed around the sides of the plastic containers. A small green stripe of EVA (ethylene-vinyl-acetate) was glued in each container to encourage oviposition. When eggs were laid, their pedicels were cut and they were transferred individually to J10 containers until larvae emergence. In total, 10 repetitions (couples) of each treatment were done and 60 eggs were analyzed for their viability.

2.4.Survival and reproduction of *H. axyridis* on extrafloral nectaries of *S. cernua*

Here we analyzed the potential of the *S. cernua* EFN in increasing the survival probability and reproduction of *H. axyridis*. We utilized this ladybug species because it was the most abundant we found in the maize field and started rearing them. In the first experiment, second instar larvae (to avoid the high natural mortality of first instar) were placed in small cages (3x3 cm) attached to the plants under two treatments: i) presence of one nectary in the plant; and ii) absence of the nectary in the plant (presence of plant stem and leaves). Their survival was observed daily and each plant housed one individual from treatment and one from control. In total, 26 repetitions of each treatment were done. Similar experiment was conducted with the adults. To obtain the adults, the larvae were fed with *E. kuehniella* eggs twice a week until pupation. After adult emergency, they were sexed under microscope regarding their difference on prosternum pigmentation (McCornak et al., 2007). Couples were caged in plastic pots (8x10 cm) under the same two treatments just cited above on item 2.3 for the adults. In total, 30 repetitions of each treatment were done.

2.5.Composition of *S. cernua* extrafloral nectar sample using ^{1}H Nuclear Magnetic Resonance (NMR)

For the ^{1}H NMR analyses of protons, 20 mg of freshly-collected nectar dissolved in 0.700 mL of deuterated water (D_2O). The solution was transferred to a glass tube specifically designed for this technique. The analyses were performed on a Bruker 400 MHz AvanceCore spectrometer equipped with a 5 mm broadband inverse detection (BBI) probe with ATMA as well as a field gradient generator unit and temperature control.

3. DATA ANALYSES

Survival data were tested by survival analysis with the Kaplan-Meier estimator (Kaplan and Meier, 1958). The general similarity among the curves was tested with log-rank tests (Mantel, 1966). Oviposition was counted. All data analyses were performed using R (version 4.4.2) statistical software package (R Development Core Team, 2024).

4. RESULTS

4.1.Survival and reproduction of *C. externa* on extrafloral nectaries of *S. cernua*

The presence of *S. cernua* EFN increased the survival of *C. externa* larvae in comparison with the control (χ^2 = 49.98; df = 1; $p < 0.0001$; Fig. 1). The median time to achieve 0.5 of survival probability increased from 9 to 40 days. Adults that had open access to extrafloral nectaries also increased significantly their median time of survival probability, both females, from 3.5 to more than 30 days (χ^2 = 53.70; df = 1; $p < 0.0001$; Fig. 2 A) and males, from 4 to more than 30 days (χ^2 = 51.97; df = 1; $p < 0.0001$; Fig. 2 B). There was no difference between the sexes when adults were either deprived of nectar (χ^2 = 0.04; df = 1; $p = 0.90$; Fig. 2 C) or when with access to the food (χ^2 = 0.12; df = 1; $p = 0.70$; Fig. 2 D). Adults without nectar access did not reproduce and died. Females fed with nectar showed a mean pre-oviposition period of 6.79 days and laid 337 eggs in total. The mean number of eggs laid by female was 14.04 and the egg viability was 83%.

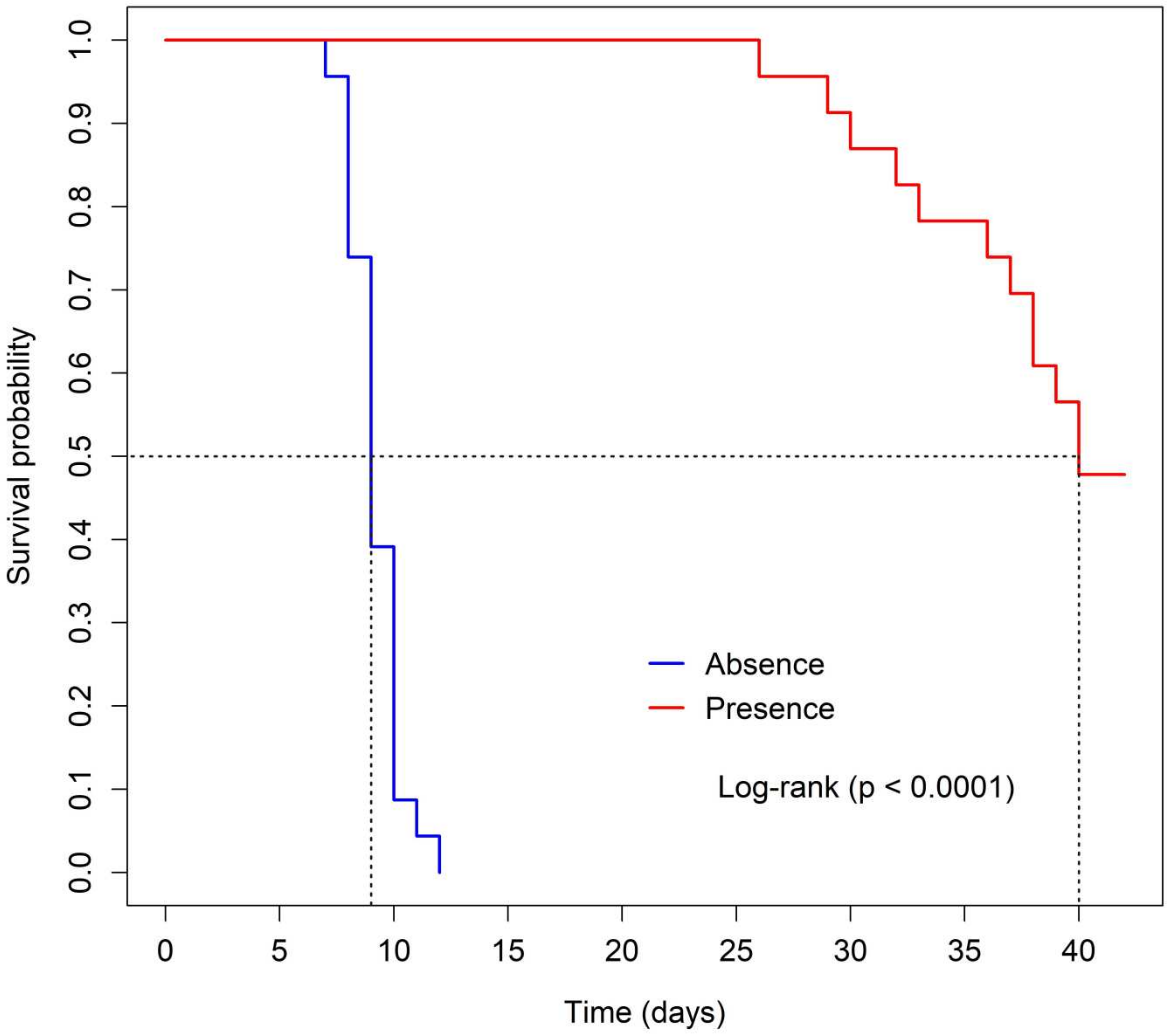

Figure 1. Survival probability of *C. externa* larvae in the presence and absence of *S. cernua* extrafloral nectaries ($\chi^2 = 49.98$; df = 1; $p < 0.0001$).

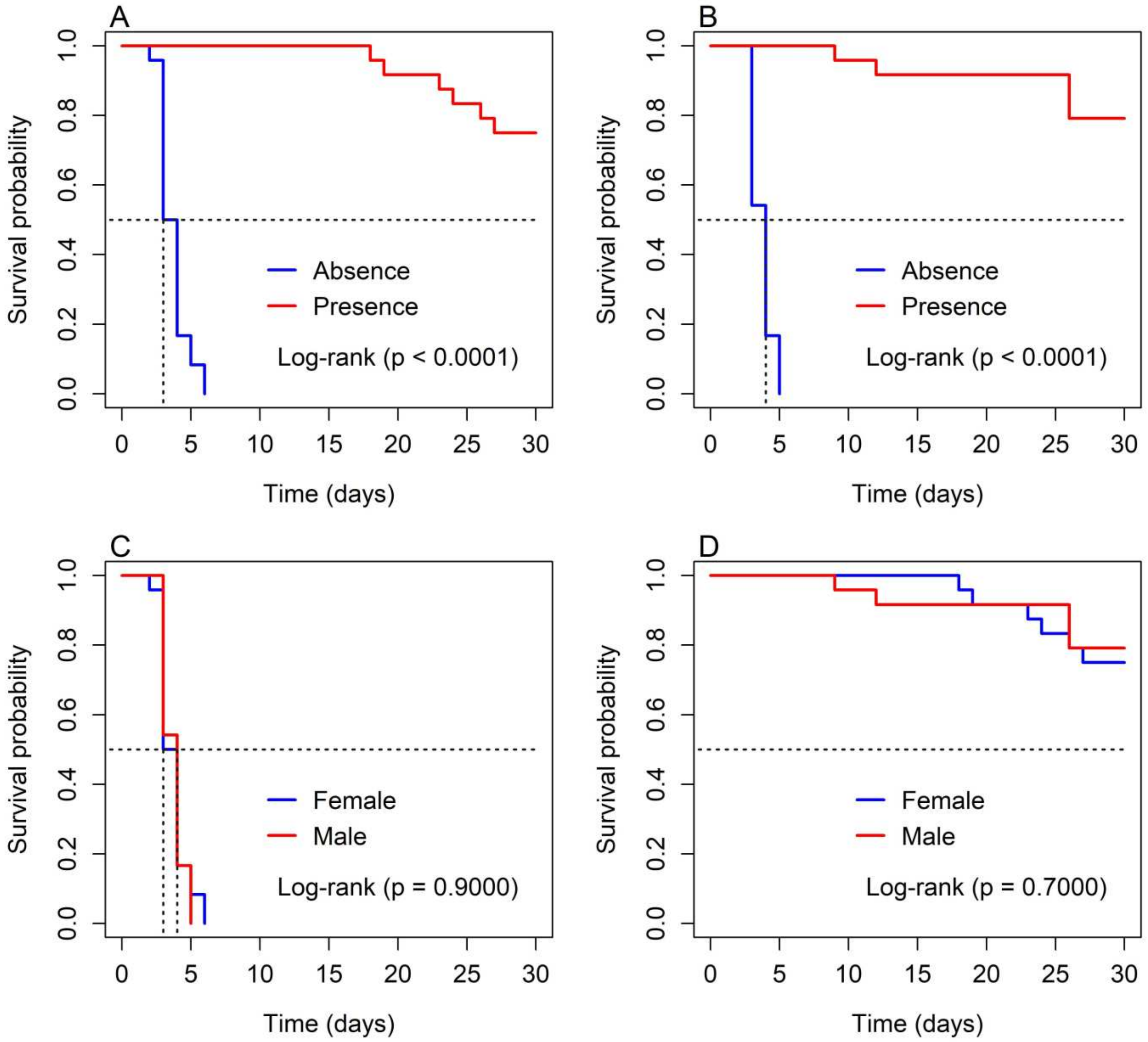

Figure 2. Survival probability of *C. externa* adults. **A** – female survival in the presence and absence of *S. cernua* extrafloral nectaries (χ^2 = 53.70; df = 1; p < 0.0001). **B** – male survival in the presence and absence of *S. cernua* extrafloral nectaries (χ^2 = 51.97; df = 1; p < 0.0001). **C** – female and male in the absence of extrafloral nectaries (χ^2 = 0.04; df = 1; p = 0.90). **D** – female and male in the presence of extrafloral nectaries (χ^2 = 0.12; df = 1; p = 0.70).

4.2.Survival and reproduction of *H. axyridis* on extrafloral nectaries of *S. cernua*

The presence of *S. cernua* EFN increased the survival of *H. axyridis* larvae in comparison with the control (χ^2 = 58.33; df = 1; p < 0.0001; Fig. 3). The median time to achieve 0.5 of survival probability increased from 4 to 19.5 days. Adults that had access to extrafloral nectaries also increased significantly their survival in comparison with the control, both females (χ^2 = 64.48; df = 1; p < 0.0001; Fig. 4 A) and males (χ^2 = 66.91; df = 1; p < 0.0001; Fig. 4 B), from a median time to achieve 0.5 of survival probability of 6 to more than 30 days (both sexes). There was no significant difference between survival of females and males when adults were deprived of nectar (χ^2 = 0.07; df = 1; p = 0.80; Fig. 4 C) or when they had open access to the EFN (χ^2 = 0.55; df = 1; p = 0.50; Fig. 4 D). Females did not oviposit in either treatment.

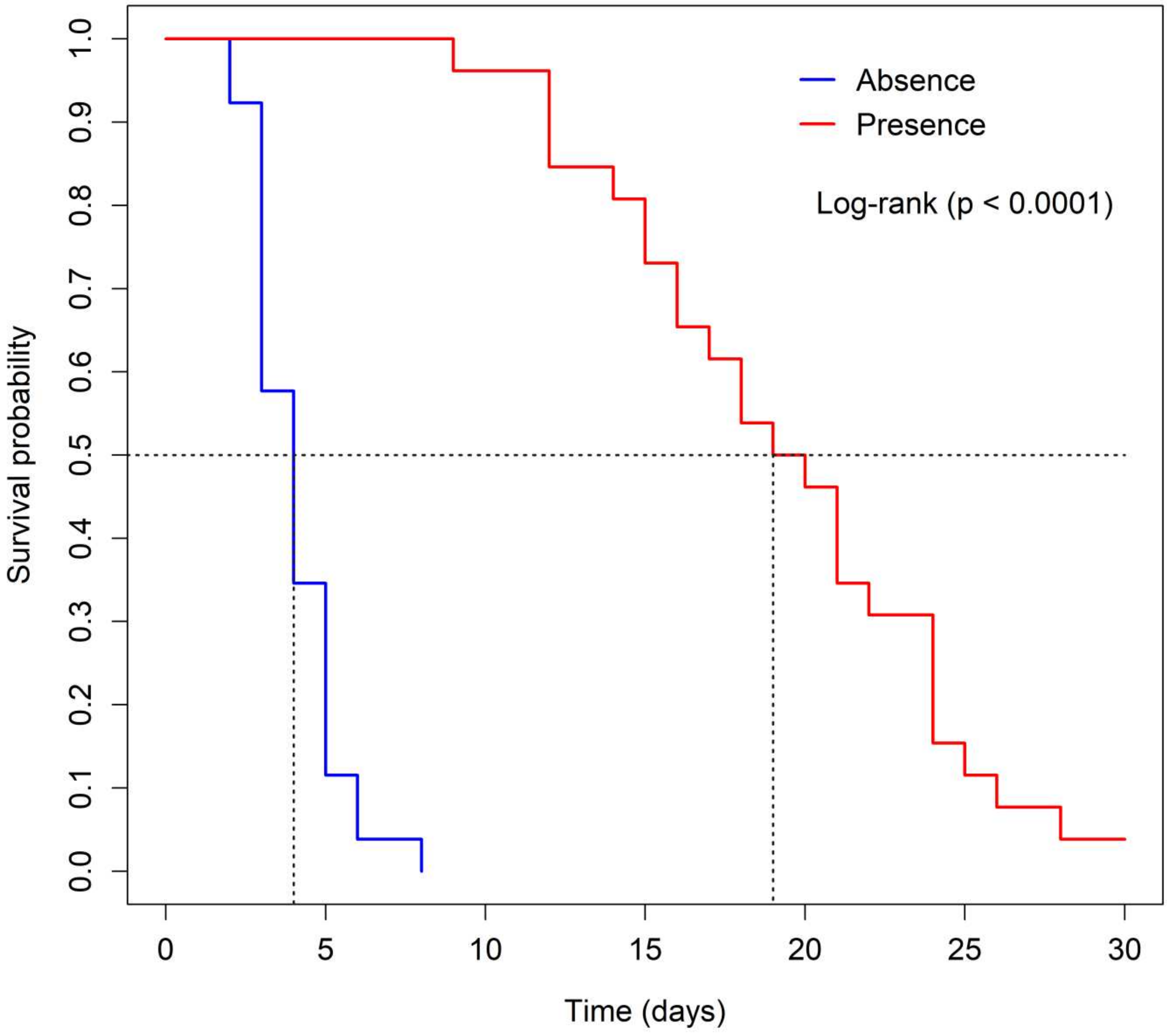

Figure 3. Survival probability of *H. axyridis* larvae in the presence and absence of *S. cernua* extrafloral nectaries (χ^2 = 58.33; df = 1; p < 0.0001).

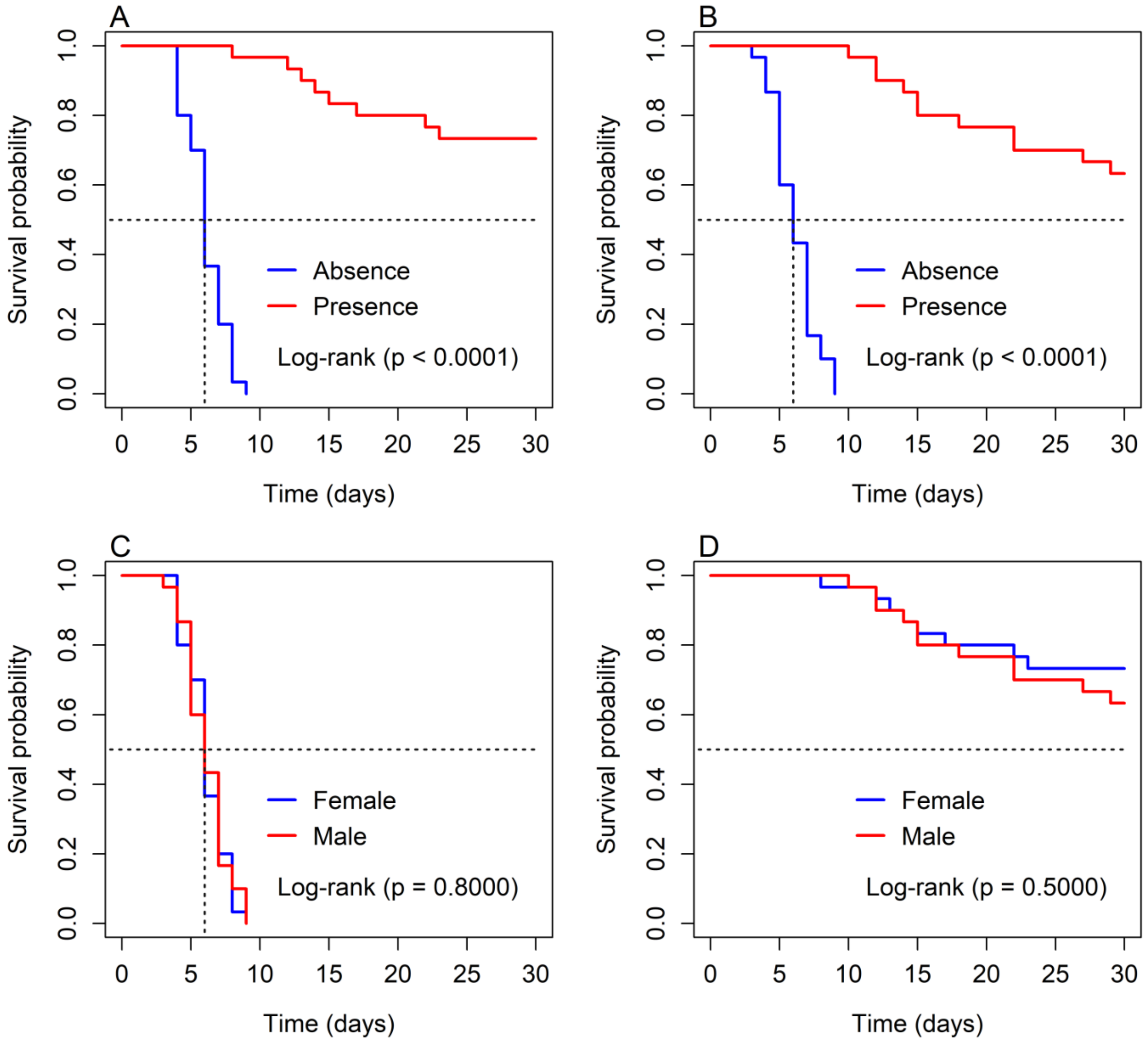

Figure 4. Survival probability of *H. axyridis* adults. **A** – female survival in the presence and absence of *S. cernua* extrafloral nectaries (χ^2 = 64.48; df = 1; p < 0.0001). **B** – male survival in the presence and absence of *S. cernua* extrafloral nectaries (χ^2 = 66.91; df = 1; p < 0.0001). **C** – female and male in the the absence of extrafloral nectaries (χ^2 = 0.07; df = 1; p = 0.80). **D** – female and male in the presence of extrafloral nectaries (χ^2 = 0.55; df = 1; p = 0.50).

4.3.*S. cernua* extrafloral nectar sample

The ^{1}H NMR spectrum signals of the nectar (Fig. 5) indicate that the main constituents are carbohydrates, due to the presence of signals in the region of 3.68 – 4.15 ppm, typical of -CH-OH (alcohols) and the signal at 5.33 ppm, typical of the hydrogen of the hemiacetal bonded to the anomeric carbon (CH-O-). These data are in agreement with Hölscher et al., (2008) and Pinto et al., (2025).

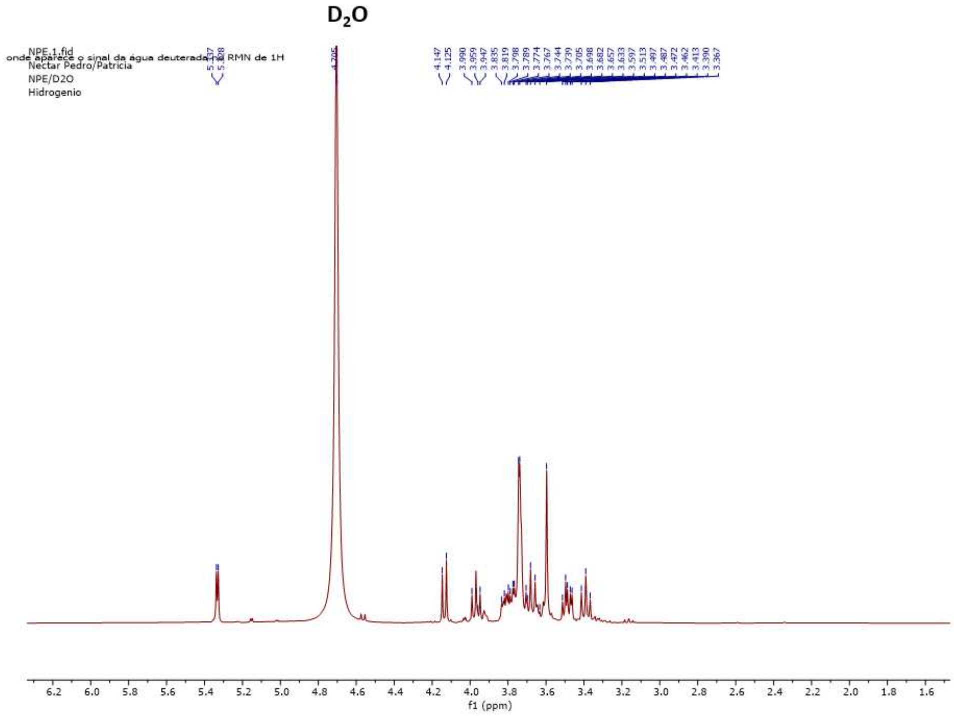

Figure 5. Nuclear Magnetic Resonance (^{1}H NMR) spectrum of *S. cernua* extrafloral nectar (D_2O).

Figure 6. Larva of *C. externa* (a) and adult (b) feeding on *S. cernua* extrafloral nectar; *C. externa* eggs deposited on the arena's voile (c); *H. axyridis* larva (d) and adults (e) feeding on *S. cernua* extrafloral nectar; (f) arena used for larvae experiments. All photos were taken by

Pedro Inocêncio Silveira at Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), Viçosa - MG, Brasil.

5. DISCUSSION

EFN trees are commonly found on tropical and subtropical habitats and produce extrafloral nectar, which is a solution with high levels of sucrose, glucose and fructose and lower amounts of amino acids (Shenoy et al., 2012; Staab et al., 2017). The extrafloral nectar of *S. cernua* significantly enhanced the fitness of the native *C. externa* but only the survival of the exotic ladybug *H. axyridis,* not its development neither reproduction. The Chrysopidae species is native from Latin (Central and South) America (Albuquerque et al., 1994) as well as *S. cernua* (Royal Botanic Gardens), and they could have already had contact at some point during their time co-evolving, this way, better increasing the green lacewing fitness. On the other hand, *H. axyridis* is native from Palaeartic region of Asia (Koch, 2003) and was introduced in South America in the 90's to promote the biological control of aphids on peaches orchards in Argentina (Saini, 2004). In Brazil, it was first seen in April 2002, in Curitiba, state of Parana, feeding on aphids of Lythraceae and Pinaceae trees (Almeida and Silva, 2002) and was first recorded in Viçosa in 2006, feeding mostly on aphids but also phyllids on roses, citrus, cabbage and leucaena plants (Rezende et al., 2010). For being native from Asia, the ladybug did not evolve with *S. cernua* and this could be the reason that the extrafloral nectar increased only its survival.

Besides the origin of predators, their feeding habits may also have influenced the suitability of *S. cernua* EFN. Both species larvae and *H. axyridis* adults are carnivorous but Chrysopidae adults are not. An increase of larvae survival was observed when both predators fed on EFN. One third of *C. externa* larvae completed their development and 13% pupate and turned into adults on a diet of exclusive EFN and none of *H. axyridis* developed to pupae. The EFN increased *C. externa* larvae survival in more than fourfold. The extrafloral nectar of *S. cernua* increased the survival probability of *C. externa* adults by more than sixfold. Besides, *C. externa* females could oviposit and generate a viable offspring feeding on this nectar. Calderón-Arroyo et al., (2023), testing the same nectar, found out that *S. cernua* extrafloral nectar ennhanced the survival and the egg load of the coffee leaf miner parasitoid *Proacrias coffeae* Ihering (Hymenoptera: Eulophidae). Similarly, extrafloral nectar produced by cotton plants was able to increase *Chrysoperla plorabunda* Fitch

(Neuroptera: Chrysopidae) larvae survival probability in sixfold in comparison with larvae in presence of just leaves or just water (Limburg and Rosenheim, 2001).

The averages that we found feeding *C. externa* adults with *S. cernua* extrafloral nectar are similar with other suitable diets found for *C. externa* adults by Venzon et al., (2006). The authors found a mean pre-oviposition period of 4.7 ± 0.82 and 5.3 ± 1.21 days when the green lacewings were fed with pigeon pea and sunn hemp pollens complemented with honey, respectively. We found a mean pre-oviposition period of 6.79 days, which is not disparate. The egg viabilities with these same diets were 81.8 ± 8.7% and 57.4 ± 24.28% respectively. We found a very close result of egg viability (83%) from those fed with pigeon pea pollen supplemented with honey. All diets offered by the authors made the adults survive from more than 30 days and we evaluated the adults only for 30 days. In the end of our experiment, around 80% of adults were still alive, feeding only on *S. cernua* extrafloral nectar. Venzon et al., (2006) also found that *C. externa* females did not oviposit when fed with only castor bean pollen or only honey but when fed with only *S. cernua* extraflorar nectar females laid a mean total number of 14 eggs. This oviposition number is lower than the other diets tested by the authors, but due to the difficulties in opening the adult's plastic containers without letting them to escape, some eggs hatched and neonate larvae possibly preyed upon new-laid eggs, consuming even their pedicels and hardening the count (this aspect warrants further laboratory investigation).

The extrafloral nectar of *S. cernua* increased the survival probability of *H. axyririds* larvae and adults by nearly fivefold and fourfold, respectively. However, *H. axyririds* did not develop neither reproduced feeding only on EFN. Likewise, Lundgren and Seagraves (2011), found out that the extrafloral nectar of faba bean increased in 50% the survival of *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) adults. Although, *H. axyridis* larvae did not turn into pupa and the adults could not oviposit, suggesting that the ladybug requires more protein-rich diets to better increase its fitness. As known, ladybeetles are carnivores in both immature and adult stages, feeding on scales, mites, white flies and mainly on aphids, often named the gardener's best friend (Sarwar, 2016; Costea et al., 2017). We could witness this latter information while initiating the coccinellid's rearing, because there was no oviposition until the moment we began adding aphids on their diet, even when initially fed with *E. kuehniella* eggs. Larvae of *H. axyridis* could successfully develop into adults feeding on *Toxoptera citricida* Kirkaldy (Hemiptera: Aphididae) and females could oviposit but none of the eggs hatched until pollen was added to their diet (Michaud, 2000). *H. axyridis* larvae were found to develop way faster and reached higher adult emergence when fed with *Chaitophorus populeti* Panzer (Hemiptera: Aphididae) in comparison with those fed on artificial diets or *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) eggs (Chen et al., 2012).

The extrafloral nectar secreted by *Humboldtia brunonis* Wallich (Fabaceae) is rich in sugars, with small quantities of amino acids (Shenoy et al., 2012). The extrafloral nectars of *Ailanthus altissima* Swingle (Simaroubaceae) and *Triadica cochinchinensis* Loureiro (Euphorbiaceae) were found to have about 1/10 of amino acids concentrations in opposition to 9/10 of sugar concentrations (Staab et al., 2017). *C. externa* adults feeds on pollen, nectar and honeydew (Albuquerque et al., 1994), that being so, in this stage, the green lacewing might be less exigent in protein rich diets and may be more exigent on carbohydrate rich diets, unlike *H. axyridis* adults, which are predators (Koch et al., 2003). A study showed that the number of eggs produced by *C. externa* females and their growth rate increased when honey was added on their diet of leguminous plant species pollen (Venzon et al., 2006). Similar results were found when castor bean pollen supplemented with honey increased the fecundity of *Chrysopa scelestes* Banks (Neuroptera: Chrysopidae) (Krishnamoorthy 1984, Gautam and Paul, 1988). The ^{1}H nuclear magnetic resonance of protons revealed that extrafloral nectar of *S. cernua* is also richer in carbohydrates than in amino acids, which could benefit *C. externa* more than *H. axyridis*.

A key factor to determine if a plant is suitable to be intercropped is that it can not benefit pests from the main crop. Small Lepidoptera that feed on sugar through narrow tube-like proboscis will feed on less concentrated (more dilute) nectars (Watt et al, 1974; Wäckers et al., 2005), which is the case of *Leucoptera coffeella* Guérin-Mèneville (Lepidoptera: Lyonetiidae). Thus, adults from this microlepidoptera cannot feed on such viscous and sticky extrafloral nectar produced by *S. cernua*. The diversification of coffee systems with *S. cernua* seems promising because it might provide associational resistance to target neighbor plants, soil nitrogen fixation, increase pollination and pest biological control besides other ecosystem services, all of this, without benefiting coffee pests.

A protective plant can give associational resistance to a target plant of interest that will suffer less herbivory (Moura and Del-Claro, 2023). *Inga* spp. trees, leguminous plants that bear extrafloral nectaries, were consorted within a coffee field, increased the fruit weight and decreased herbivory in the plants nearby in comparison with a monoculture (Rezende et al., 2014; Rezende et al., 2021). Associational resistance patterns were found on *Opuntia* individuals when associated with *Chamaecrista fasciculate* Michx (Fabaceae) that bears extrafloral nectaries (Jezorek et al., 2011). The authors observed that the cactus plants near to the leguminous plants were less attacked by the invasive moth *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae), had less damaged cladodes, bigger amount of new-emerged cladodes and higher ant abundance. Moura and Del-Claro (2023) also found associational resistance regarding *Smilax polyantha* Grisebach (Smilacaceae), often observed climbing on other plants in the Brazilian Cerrado. The authors found that *S. polyantha* supported by plants with extrafloral nectaries suffered three times less foliar herbivory and increased ant visitation on neighboring plants by two and a half times. That being so, *S. cernua* extrafloral nectaries might also give associational resistance to other crops once introduced among them.

These findings contribute to a clear understanding of conservation biological control strategies. By implementing practices that support ecosystem services, we can promote more sustainable agriculture. Non-prey resources, such as extrafloral nectars, help to attract and keep natural enemies in the field, in order to control a variety of pest populations more efficiently (Rezende et al., 2014; Batista et al., 2017), particularly with regard to generalist predators like green lacewings and ladybugs. From this perspective, this study demonstrates that extrafloral nectaries play an important role in agricultural landscapes. The increase on the survival and reproduction of *C. externa* and the increase on *H. axyridis* survival suggest that *S. cernua* produces a beneficial and nutritive meal that can be used as an alternative food source to complement the diets of these insects, especially during periods of prey scarcity.

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CHAPTER II

Does *Crambe abyssinica* flowers selectively benefit *Chrysoperla externa* and *Harmonia axyridis*? What about *Leucoptera coffeella?*

ABSTRACT

Crambe abyssinica Hochst (Brassicaceae) is an oilseed crop that might attract and keep beneficial insects in the field through the provision of pollen and floral nectar. We took a closer look to verify if *C. abyssinica* specifically provide essential resources for natural enemies. Initially, we performed survival experiments on immatures of *Chrysoperla externa* Hagen (Neuroptera: Chrysopidae) and *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in the presence of newly emerged floral stems plus water; in the presence of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs plus water; or just water. In sequence, we observed the effects of flowers on the survival and reproduction of adults from the same species. The same three treatments described above were conducted for *H. axyridis* adult's experiments, with one couple per replicate. For the *C. externa* adult's experiments, *E. kuehniella* eggs were replaced by a mixture of yeast and honey. Finally, we conducted experiments with *Leucoptera coffeella* Guérin-Mèneville (Lepidoptera: Lyonetiidae) adults. We observed the effects of *C. abyssinica* floral nectar on the survival and reproduction of this coffee pest and also the effects of honeydew produced by the green aphids *Myzus persicae* Sulzer (Hemiptera: Aphididae). Couples were placed under three treatments: just water; water plus newly emerged floral stems; or water plus aphid-infested newly emerged floral stems. The presence of *C. abyssinica* flowers increased survival probability of *C. externa* larvae and adults ($p < 0.0001$) and *H. axyridis* larvae ($p < 0.0001$) in comparison with individuals fed with only water. Only the ones fed with *E. kuehniella* eggs could complete their development. There was no significative difference between *H. axyridis* adults (both males and females) in presence of flowers or just water ($p = 0.2$). The majority of them fed with *E. kuehniella* eggs could survive for more than 30 days and only 4 females from this treatment oviposited. *C. abyssinica* flowers successfully increased the survival probability of *C. externa* adults. Only individuals fed with honey plus yeast could oviposit. There was no difference on the survival probability ($p = 0.22$ and $p = 0.24$, for females and males, respectively) and reproduction of *L. coffeella* in the presence or absence of *C. abyssinica* flowers and also in the presence of aphids. *C. abyssinica* is capable of benefit these two important generalist predators attributable to the provision of floral resources, such as pollen and nectar, especially in prey scarcity conditions. Neither *C. absyssinica* flowers nor the presence of *aphids* improved *L. coffeella* survival or reproduction, that being so, *C. abyssinica* is a promising candidate plant to be integrated in coffee agro ecosystems.

Keywords: Conservation biological control, green lacewings, ladybugs, beneficial insects.

1. INTRODUCTION

The United Nations recently reaffirmed its global commitment to sustainable development in Rio de Janeiro and established the 2030 Agenda, which outlines a series of objectives for governments and civil society to address through 17 key goals. Among these are: eradicating hunger and promoting sustainable agriculture, combating global climate change, and ensuring access to clean and affordable energy (United Nations, 2015). The global population is projected to grow from 7 billion to over 9 billion by 2050, and the increased demand for food production is expected to drive continued agricultural intensification (Emmerson et al., 2016).

Agricultural intensification leads to landscape simplification and homogenization, resulting in a decline in overall biodiversity and the provision of ecosystem services (Tscharntke et al., 2005; Flohre et al., 2011). In contrast, landscape heterogeneity plays a crucial role in creating and maintaining a healthier and more resilient environment, contributing to regenerative agriculture that utilizes ecosystem services in a more sustainable manner (Turner et al., 2013).

One of the most important ecosystem services supporting the sustainability of food production is biological pest control (Emmerson et al., 2016). This method offers an alternative to the widespread use of chemical pesticides, which are associated with risks to both the environment and human health (Ekström and Ekbom, 2011; Czaja et al., 2015). These circumstances underscore the need to incorporate ecologically-based approaches for pest management, while simultaneously attracting and retaining natural pest control agents in the field.

A wise way to make that approach is through conservation biological control. This method is based on ecological and behavioral processes, such as habitat management and environment diversification of the agro-ecosystem, sheltering and improving natural enemies' fitness (Venzon et al., 2006). The selection of plants that can provide protective sites or alternative sources of food, such as pollen and nectar, attracts and maintain these natural enemies in the field over longer periods of time, even in local scale (e.g. flowering stripes between the crop rows in a farm) or at larger scales (e.g. woodlands around multiple areas in the region), fostering their success as predators, parasitoids or pathogens (Begg et al., 2017; Shields et al., 2019).

Selecting the most suitable companion plants within an agro-ecosystem requires a certain level of knowledge. Key factors to consider include ease of cultivation, resilience, flowering at times that does not coincide with the main crop, and the provision of ecosystem services (Venzon et al., 2011). A thorough understanding of the trophic interactions within the environment and between its organisms is essential to ensure that the selected plants positively impact natural enemies without inadvertently benefiting pest populations (Chailleux et al., 2014; Begg et al., 2017).

Crambe abyssinica Hochst (Brassicaceae) is an oilseed plant which is mainly utilized as forage plant, in crop rotations or in between the main crop rows, preserving soil humidity and its chemical, physical and biological properties (Costa et al., 2019; Zorzenoni, 2019). It has an in-depth strong root that can reach more than one meter subsurface, granting a great adaptability to drought besides being a rustic plant which cultivation demands low inputs, conserving water and fertilizers (Merrill et al., 2005; Samarappuli et al., 2020).

The utilization of plant species that offers more than one ecosystem service is favorable to create a more sustainable environment (Ladino et al., 2019). *C. maritima* and *C. abyssinica* were found to reduce the phytophagous nematode *Heterodera glycines* Ichinohe (Heteroderidae) populations due to the presence of glucosinolates and isothiocyanates in the plants (Poromarto and Nelson, 2010; Nascimento et al., 2016).

C. abyssinica white flowers consists of 4 petals, 6 stamens and are grouped in raceme type inflorescences and the plant can bloom for almost 2 months from its cycle of 3 months, Yet, little is known about the relation of *C. abssynica* and biological control of insects, but at least five species of pollinator bees were found carrying its pollen (Simioni et al., 2015). Unlike nectar, which is mostly a sugary solution constituted by sucrose (one disaccharide), glucose and fructose (two hexoses) (Chalcoff et al, 2006), pollen is mainly a source of nitrogenous compounds extending its protein levels from 2.5% to more than 60% (Roulston and Cane, 2000), but it can also consist of some lipids and carbohydrates (Nepi and Franchi, 2000).

Pollen is the plant's male genetic information present in the stamen that is transferred to the female ovary inside the pistil. Its accessibility by insects is greatly associated with the floral architecture (shape, size, number and duration of flowering) and the differences in insect's mouthparts morphology (Wäckers, 2005; Li et al., 2007; Lázaro et al., 2016; Zhao et al., 2018). Floral nectar is the pioneer reward provided to pollinators in mostly angiosperms, and its composition, placement and amount is essential to determine plant-pollinator interactions (Parachnowitsch et al., 2019). In search for nectar, pollinators, unconscious, transport pollen grains and drops them on stigma (Koptur, 1994; Fridman et al., 2012; Nepi et al., 2012).

A variety of predaceous insects supplement their diet feeding on pollen and nectar, such as green lacewings (Bozsik, 1992; Venzon et al., 2006; Andrade et al., 2013; Herrera et al, 2021; González et al., 2022), coccinellids (Michaud and Jyoti, 2007; Lundgren, 2009; Zazycki et al., 2015; D'Ávila et al., 2016) and others, many of them when preys are not abundant. Floral nectaries can be at the same level as the flower surface or be deeply inside the organ that bears them. Exposed nectars might be collected by many animals and can evaporate fast, but if produced inside the corolla, it is sheltered against evaporation and fewer animals would be capable to feed on it (Pacini et al., 2003).

By examining the prospective of introducing flowering plants on agricultural landscapes, in this study we evaluated the potential of *C. abyssinica* as a floral resource provider to generalist predators. We tested the hypothesis that *C. abyssinica* flowers are beneficial to *C. externa* and *H. axyridis*, and that it can sustain these predaceous insects, improving conservation biological control of pest populations. We also tested if adults of the coffee leaf miner could benefit on *C. abyssinica* floral nectar or aphid-produced honeydew. Specifically, we analyzed the survival probability and oviposition rate of these two beneficial insects and one coffee pest in the presence and absence of *C. abyssinica* flowers.

2. MATERIAL AND METHODS

2.1 Green lacewing rearing

We used green lacewing larvae and adults of *C. externa* from the rearing of the Laboratory of Entomology at Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG) located in Viçosa, state of Minas Gerais (MG), Brazil . The predator was reared following the methodology of Venzon et al., (2006). Adults were kept in PVC tube cages (15x15 cm) lined with paper towels. The cages were supported on plastic trays lined with paper towels and closed with PVC film. They are fed with a diet of yeast and honey (1:1) offered on a parafilm stripe hanged inside the cage. The water was provided in a glass bottle (10 mL) with cotton soaked. Diet and water were replaced twice a week. Paper towels and PVC film where females lay their eggs were replaced weekly. Eggs and larvae were individualized in plastic container (10.0 cm high and 4.0 cm of diameter). The larvae were fed with eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), added every three days, until pupation. The rearing unit is kept at 25 ± 2°C, 70 ± 10% RH and 12h photophase.

2.2 Lady bugrearing

We used larvae and adults of *H. axyridis* collected on maize crop at the Agronomy field of the Federal University of Viçosa (UFV), Viçosa, MG, Brazil. The rearing was kept at the same place and conditions as described above for Chrysopidae rearing. Adults were kept in plastic containers (15x10 cm), fed with a honey solution soaked cotton, aphids and eggs of *E. kuehniella,* replaced twice a week. A cabbage leaf with its petiole wrapped in wet cotton to keep it turgid was replaced weekly as an oviposition site. A crumpled piece of paper was also weekly replaced, serving as shelter to them. The eggs were then separated in masses and the larvae were individualized in J10 (10 mL) containers, fed with *E. kuehniella* eggs every three days until turning into pupae.

2.3 Survival and reproduction of *C. externa* on *C. abyssinica* flowers

Here we analyzed the potential of *C. abyssinica* in increasing the survival probability and reproduction of *C. externa* due to the provision of floral resources. In the first experiment, newly-emerged larvae were placed in plastic containers (8x10 cm) under three treatments: i) presence of *E. kuehniella* eggs plus water-soaked cotton; ii) presence of “crambe” flowers (a bouquet of 3-5 flowers) plus water-soaked cotton; and iii) just water. Their survival was observed daily, eggs and water were renewed every three days and the flowers were replaced every two days before the pollen starts to turn gray. In total, 25 repetitions of each treatment were done. Similar experiment was conducted with the adults. The larvae were fed with *E. kuehniella* eggs twice a week until pupation. After adults emerged, they were sexed under microscope regarding their difference on the end of their abdomen (Morse, 1931). Couples were caged in plastic containers (8x10 cm) under three treatments: i) presence of yeast plus honey (1:1) diet and water-soaked cotton); ii) presence of “crambe” flowers (5-7) plus water-soaked cotton; and iii) just water-soaked cotton. In total, 20 repetitions of the first treatment were done and 30 repetitions of the second and third treatments were done. In this second experiment, male and female survival probability, mean number of eggs laid by female, total oviposition, and egg viability were determined. Eggs from couples fed with yeast, honey and water, were collected every three days and transferred individually to Eppendorf tubes (1.5 mL), before larvae emergence. In total, eggs were collected 10 times.

2.4 Survival and reproduction of *H. axyridis* on *C. abyssinica* flowers

Here we analyzed the potential of the *C. abyssinica* in increasing the survival probability and reproduction of *H. axyridis* through the provision of floral resources. In the first experiment, newly-emerged larvae were placed in plastic containers (8x10 cm) under three treatments: i) presence of *E. kuehniella* eggs plus water-soaked cotton); ii) presence of “crambe” flowers (3-5) plus water; and iii) just water. Their survival was observed daily, eggs and water were renewed every three days and the flowers were replaced every two days before the pollen starts to turn gray. In total, 25 repetitions of each treatment were done. Similar experiment was conducted with the adults. The larvae were fed with *E. kuehniella* eggs twice a week until pupation. After adults emerged, couples were caged in plastic containers (8x10 cm) under three treatments: i) presence of *E. kuehniella* eggs plus water-soaked cotton); ii) presence of “crambe” flowers (5-7) plus water; and iii) just water. Adults were sexed under microscope regarding their difference on prosternum pigmentation (McCornak et al., 2007). In this second experiment, male and female survival probability was determined. In total, 21 repetitions (couples) of each treatment were analyzed.

2.5 Survival and reproduction of *L. coffeella* on *C. abyssinica* floral nectar and aphid-secreted honeydew

Due to the possibility of feeding on floral nectar and honeydew, we tested if *L. coffeella* adults are capable of benefiting from *C. abyssinica* flowers and honeydew produced by aphids. Pupae of the coffee leaf miner were collected on a green house at Agriculture and

Livestock Research Enterprise of Minas Gerais (EPAMIG) located in Viçosa, MG. They were cut with a little piece of the leaf and put on Eppendorf tubes (1.5 mL) until adult emergence. New-born adults were sexed under microscope regarding their difference in the end of abdomen (Nantes and Parra, 1977). Couples were caged in plastic containers (8x10 cm), under three treatments: i) presence of "crambe" flowers (a bouquet of 5-7 flowers) plus water-soaked cotton; ii) just water; and iii) crambe flowers (5-7) infested with 7-10 green aphids *M. persicae* plus water. Each container had also one sanitized coffee leaf with its petiole submerged in water for oviposition. Every day, after couting oviposition, eggs were removed from the leaves. *L. coffeella* survival was observed daily, water and flowers were renewed every two days. Male and female survival probability, mean pre-oviposition period and mean oviposition were determined. In total, 32 repetitions of the first and second treatments were done and 25 repetitions from the third treatment were analyzed.

3. DATA ANALYSES

Survival data were tested by survival analysis with the Kaplan-Meier estimator (Kaplan, Meier, 1958). The general similarity among the curves was tested with log-rank tests (Mantel, 1966). Preoviposition and oviposition data were determined due to none statistic differences between treatments (overlap of the 95% confidence intervals). All data analyses were performed using R (version 4.4.2) statistical software package (R Development Core Team, 2024).

4. RESULTS

4.1.Survival and reproduction of *C. externa* on *C. abyssinica* flowers

The presence of *C. abyssinica* flowers increased the survival of *C. externa* larvae in comparison with the negative control (larvae fed with water). The median time to achieve 0.5 of survival probability increased from 2 to 3 days (χ^2 = 93.6; df = 2; p < 0.0001; Fig. 1). More than 90% of the larvae fed with water plus *E. kuehniella* eggs (positive control) turned into pupae. Females that had access to *C. abyssinica* flowers, increased significantly their survival. The median time to achieve 0.5 of survival probability increased from 1 day (negative control: individuals fed with water) to 4 days (χ^2 = 115.94; df = 2; p < 0.0001) and more than 80% of females fed with water-soaked cotton plus a diet of yeast and honey (positive control) survived from more than 30 days (Fig. 2 A). Males that had access to *C. abyssinica* flowers also increased their survival. The median time to achieve 0.5 of survival probability increased from 2 days (negative control) to 4 days (χ^2 = 110.17; df = 2; p < 0.0001) and more than 55% of males from positive control survived more than 30 days (Fig. 2 B). There was no difference between the sexes when adults were treated with only water (χ^2 = 0.59; df = 1; p = 0.44), water plus yeast and honey (χ^2 = 3.81; df = 1; p = 0.052) or water and *C. abyssinica* flowers (χ^2 = 0.23; df = 1; p = 0.63) (Figures 2 C, 2 D and 2 E, respectively). Just adults from positive control had the chance to reproduce. In total, 3.317 eggs were laid and analyzed. The mean number of eggs laid by female was 165.85, with a viability of 69.2%. Oviposition pattern is shown on figure 3.

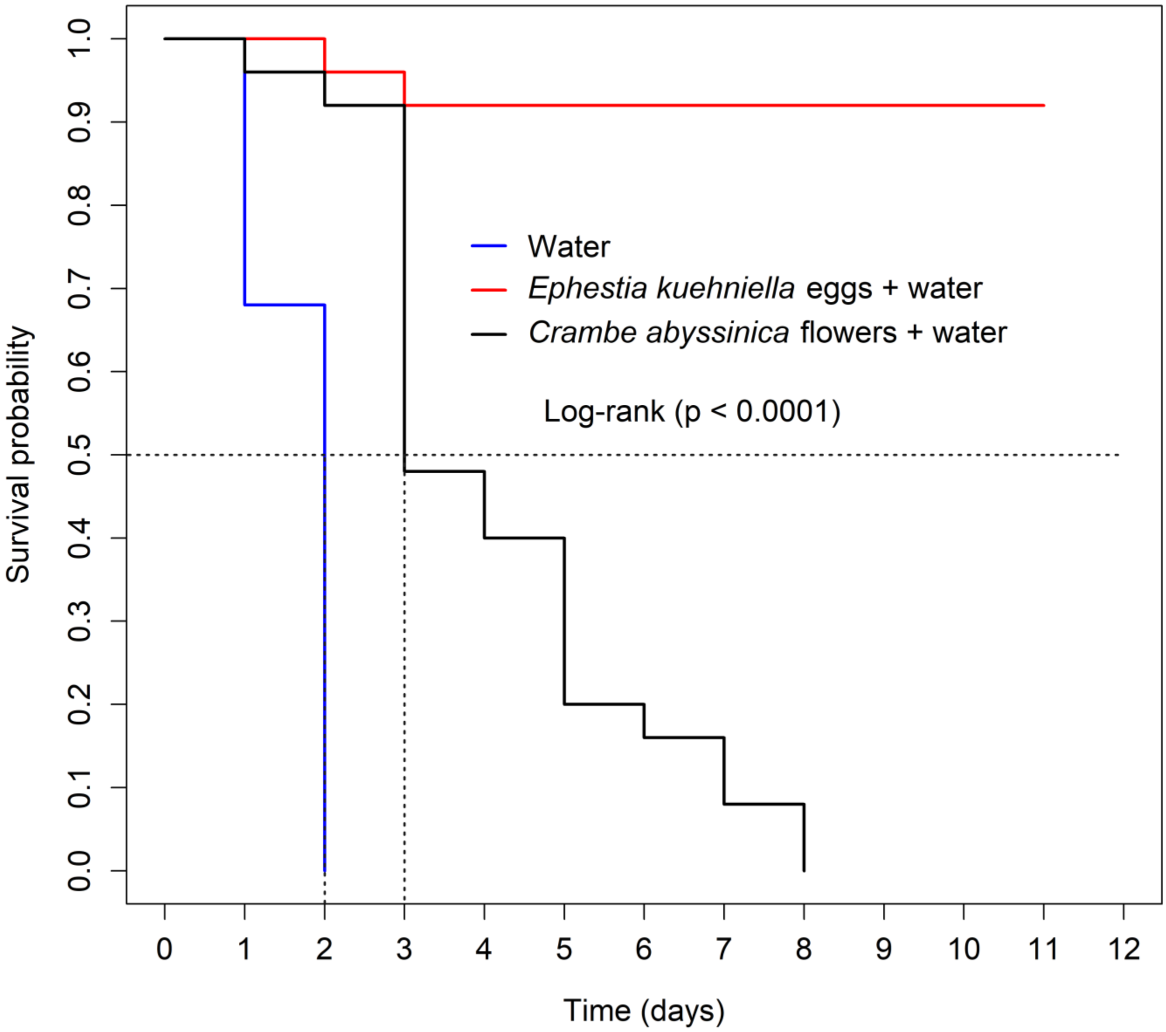

Figure 1. Survival probability of *C. externa* larvae in the presence of: *C. abyssinica* flowers plus water, just water or water plus *E. kuehniella* eggs (χ^2 = 93.6; df = 2; p < 0.0001).

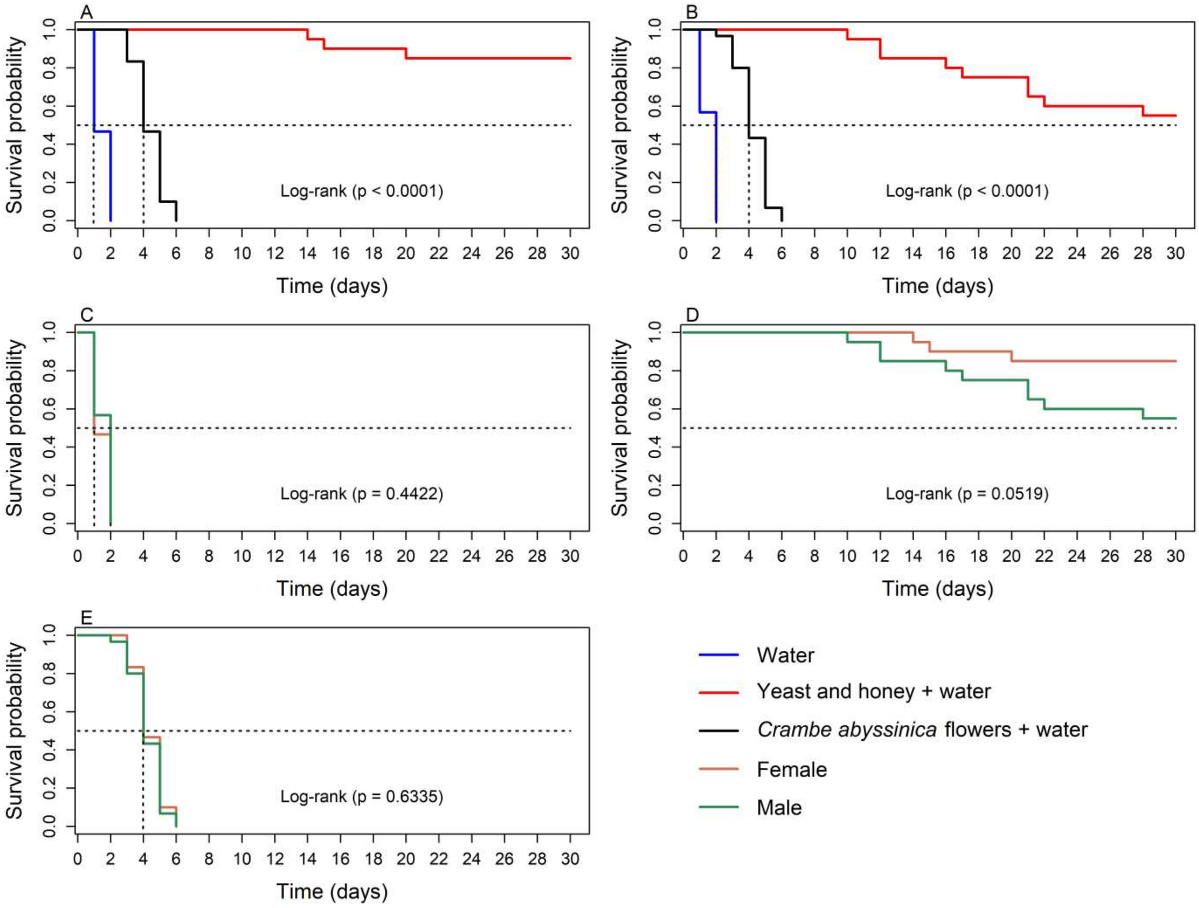

Figure 2. Survival probability of *C. externa* adults. **A** – female survival probability in the presence of: *C. abyssinica* flowers plus water, just water or water plus yeast and honey (χ^2 = 115.94; df = 2; p < 0.0001). **B** – male survival probability in the presence of: *C. abyssinica* flowers plus water, just water or water plus yeast and honey (χ^2 = 110.17; df = 2; p < 0.0001). **C** – female and male fed with only water (negative control) (χ^2 = 0.59; df = 1; p = 0.44). **D** – female and male in the presence of water plus yeast and honey (positive control) (χ^2 = 3.81; df = 1; p = 0.052). **E** – female and male in the presence of *C. abyssinica* flowers plus water (χ^2 = 0.23; df = 1; p = 0.63).

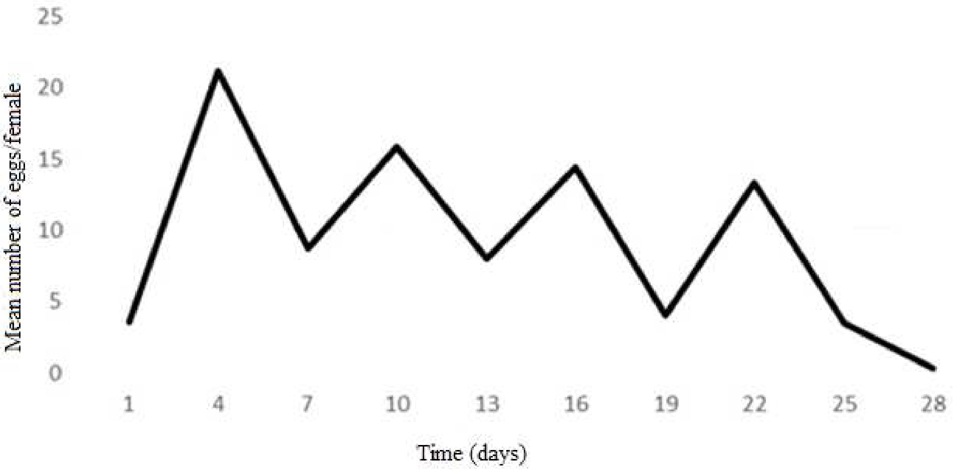

Figure 3. Pattern of *C. externa* oviposition rate, fed with a diet of yeast and honey (1:1) plus water-soaked cotton. Mean number of eggs laid by females in every three days.

4.2.Survival and reproduction of *H. axyridis* on *C. abyssinica* flowers

The presence of *C. abyssinica* flowers increased the survival of *H. axyridis* larvae in comparison with the negative control (larvae fed with water) (χ^2 = 67.30; df = 2; p < 0.0001; Fig. 4). The median time to achieve 0.5 of survival probability increased from 2 to 5 days. More than 80% of the larvae fed with water plus *E. kuehniella* eggs (positive control) turned into pupae. Females that had access to *C. abyssinica* flowers did not differ their survival in relation to the ones fed with only water, with a median time to achieve 0.5 of survival probability of 4 days (χ^2 = 2; df = 1; p = 0.2) but differed from females fed with water plus *E. kuehniella* eggs (χ^2 = 94.50; df = 1; p < 0.0001) and more than 95% of these females (positive control) lived longer than 30days (Fig. 5 A). Also, females fed with only water differed from the ones that fed on *E. kuehniella* eggs as well (χ^2 = 93.4; df = 1; p < 0.0001). Males that had access to *C. abyssinica* flowers did not differ their survival in relation to the ones fed with only water, with a median time to achieve 0.5 of survival probability of 4 days (χ^2 = 2; df = 1; p = 0.2) but differed from males fed with water plus *E. kuehniella* eggs (χ^2 = 94.50; df = 1; p < 0.0001) and more than 90% of these males (positive control) lived longer than 30days (Fig. 5 B). Also, males fed with only water differed from the ones that fed on *E. kuehniella* eggs (χ^2 = 93.4; df = 1; p < 0.0001). There was no difference between the sexes when adults were treated with only water (χ^2 = 0.28; df = 1; p = 0.59), water plus *E. kuehniella* eggs (χ^2 = 0.24; df = 1; p = 0.62) or water and

C. abyssinica flowers (χ^2 = 0.09; df = 1; p = 0.76) (Figures 5 C, 5 D and 5 E, respectively). Only 4 females from positive control could oviposit a few eggs.

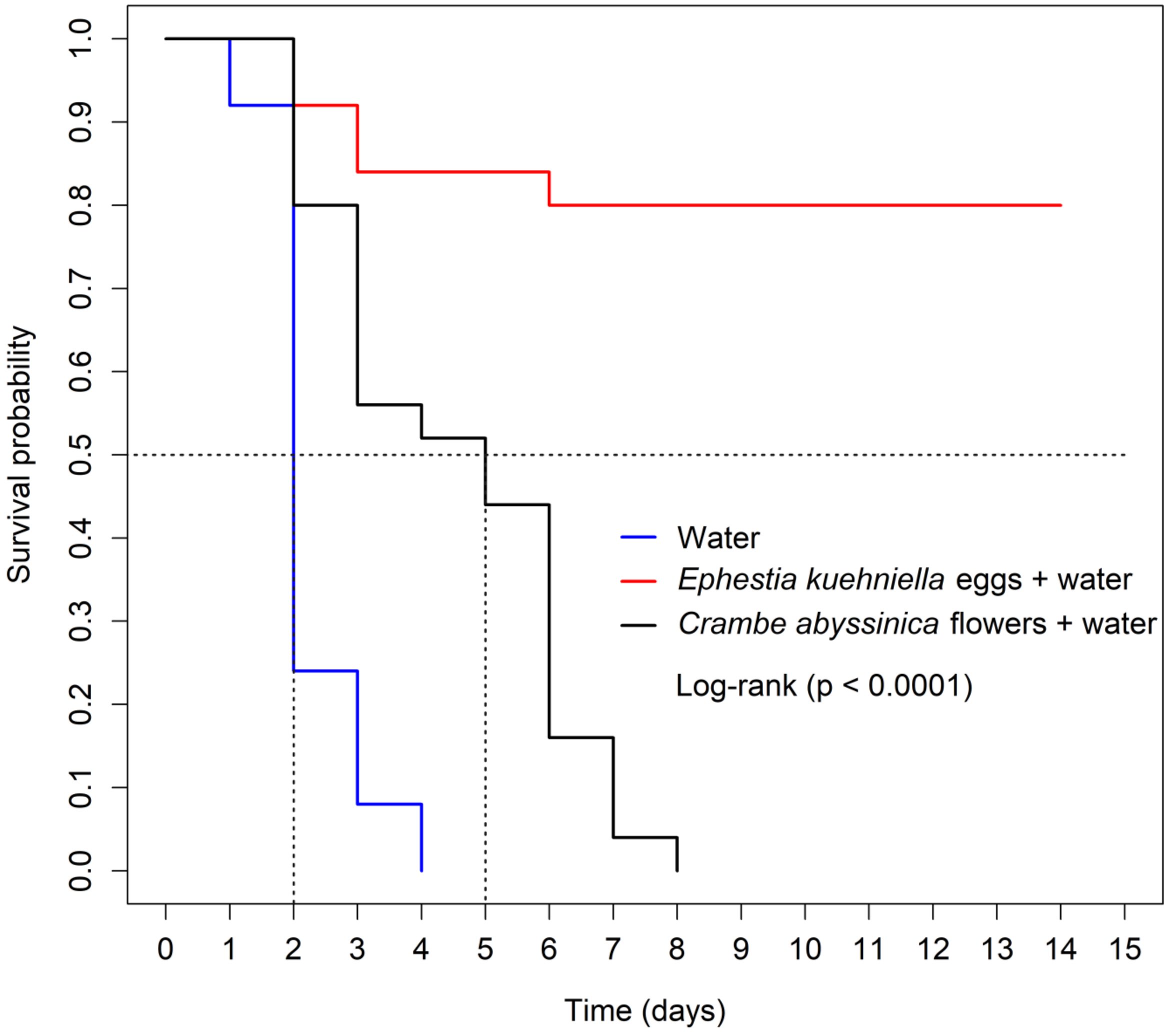

Figure 4. Survival probability of *H. axyridis* larvae in the presence of: *C. abyssinica* flowers plus water, just water or water plus *E. kuehniella* eggs (χ^2 = 67.30; df = 2; p < 0.0001).

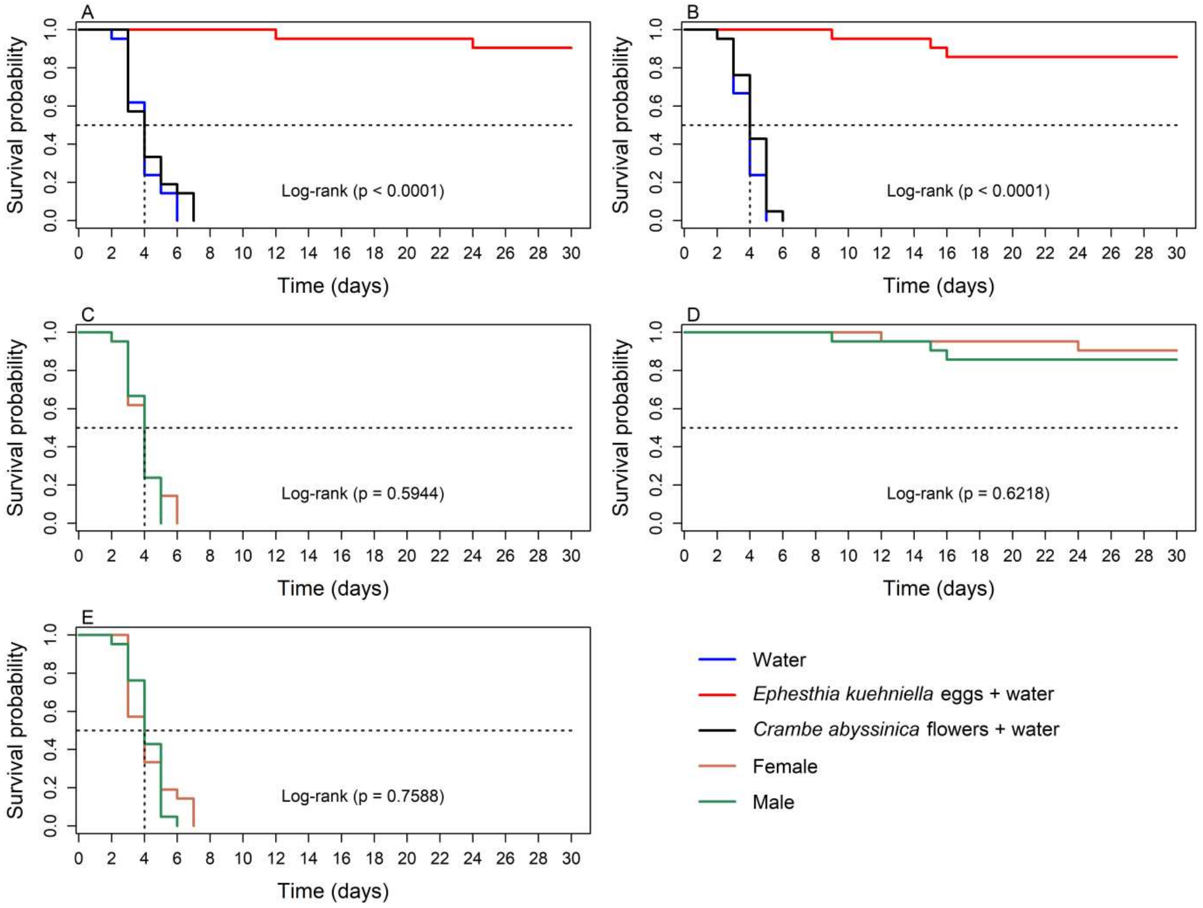

Figure 5. Survival probability of *H. axyridis* adults. **A** – female survival probability in the presence of: *C. abyssinica* flowers plus water, just water or water plus *E. kuehniella* eggs. **B** – male survival probability in the presence of: *C. abyssinica* flowers plus water, just water or water plus *E. kuehniella* eggs. **C** – female and male fed with only water (negative control) (χ^2 = 0.28; df = 1; p = 0.59). **D** – female and male in the presence of water plus *E. kuehniella* eggs (positive control) (χ^2 = 0.24; df = 1; p = 0.62). **E** – female and male in the presence of *C. abyssinica* flowers plus water (χ^2 = 0.09; df = 1; p = 0.76).

4.3. Survival and reproduction of *L. coffeella* on floral nectar of *C. abyssinica* and *M. persicae* honeydew

Neither the presence of *C. abyssinica* floral nectar nor the presence of honeydew made some difference on the survival of *L. coffeella* females in comparison with the ones from negative control (water) (χ^2 = 3.03; df = 2; p = 0.22; Fig. 6 A). The same occurred with the males (χ^2 = 2.89; df = 2; p = 0.24; Fig. 6 B). There was no survival difference between the sexes when adults were treated with only water ($\chi^2 = 0.20$; df = 1; p = 0.66), water and *C. abyssinica* flowers ($\chi^2 = 1.83$; df = 1; p = 0.18) or water plus *C. abyssinica* flowers and aphids ($\chi^2 = 0.14$; df = 1; p = 0.71) (Figures 6 C, 6 D and 6 E, respectively). There was no statistic difference in the pre-oviposition period of females (Fig. 7) and in the mean oviposition (Fig. 8) regarding these three treatments, due to the overlap of confidence intervals.

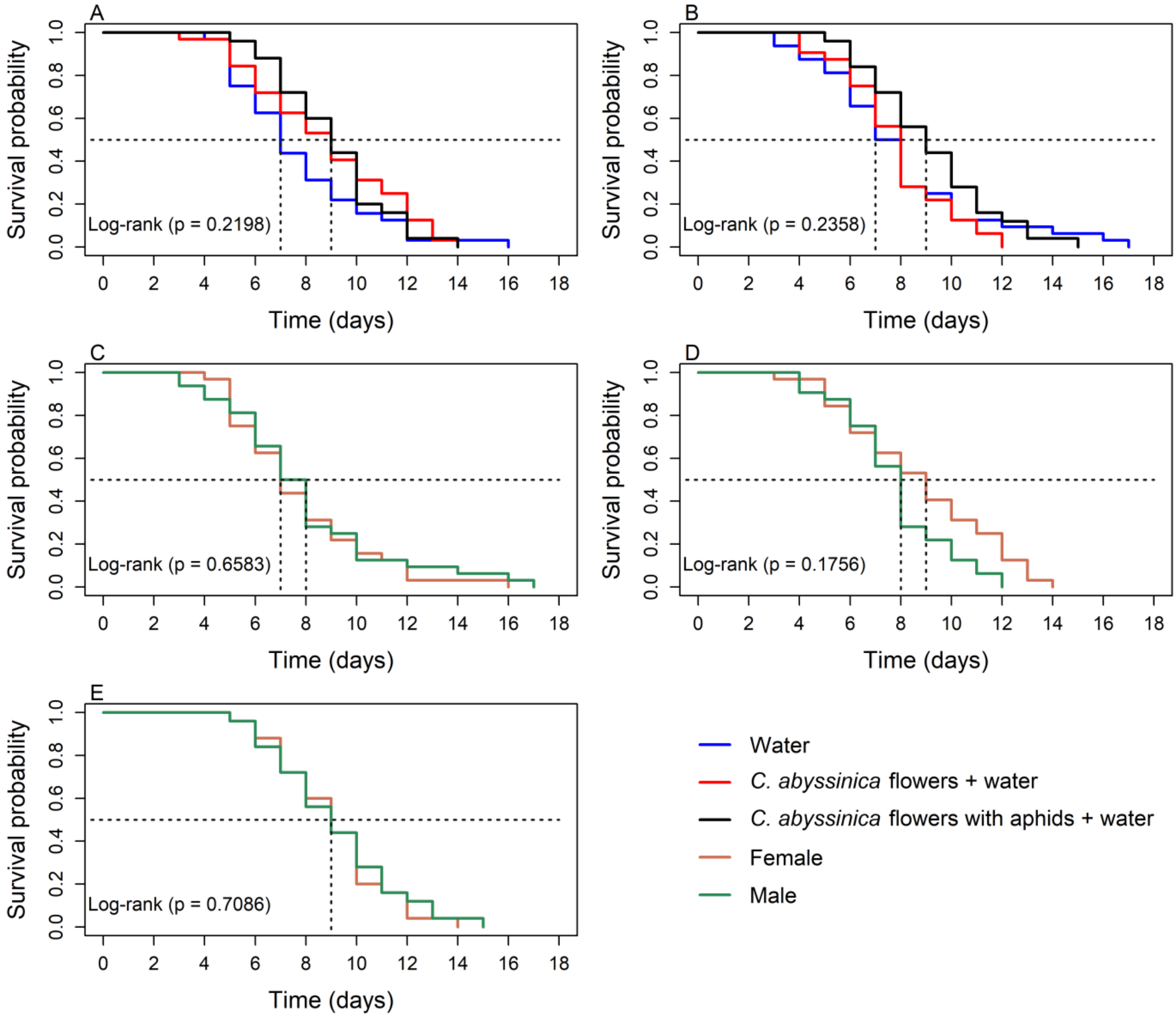

Figure 6. Survival probability of *L. coffeella* adults. **A** – female survival probability in the presence of: *C. abyssinica* flowers plus water, *C. abyssinica* flowers aphid-infested plus water or just water ($\chi^2 = 3.03$; df = 2; p = 0.22). **B** – male survival probability in the presence of: *C. abyssinica* flowers plus water, *C. abyssinica* flowers aphid-infested plus water or just water ($\chi^2 = 2.89$; df = 2; p = 0.24). **C** – female and male fed with only water (negative control) ($\chi^2 = 0.20$; df = 1; p = 0.66). **D** – female and male in the presence of water plus *C. abyssinica* flowers ($\chi^2 = 1.83$; df = 1; p = 0.18). **E** – female and male in the presence of *C. abyssinica* flowers aphid-infested plus water ($\chi^2 = 0.14$; df = 1; p = 0.71).

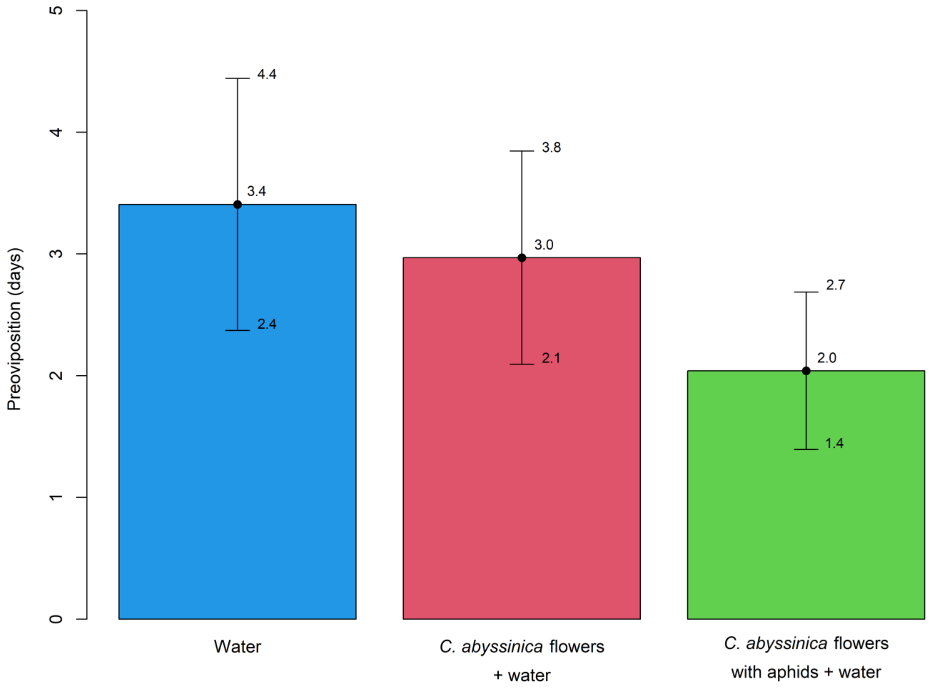

Figure 7. Mean pre-oviposition period (days) of *L. coffeella* females treated with only water, *C. abyssinica* flowers plus water, or *C. abyssinica* flowers with aphids plus water. Treatments with 95% of confidence interval.

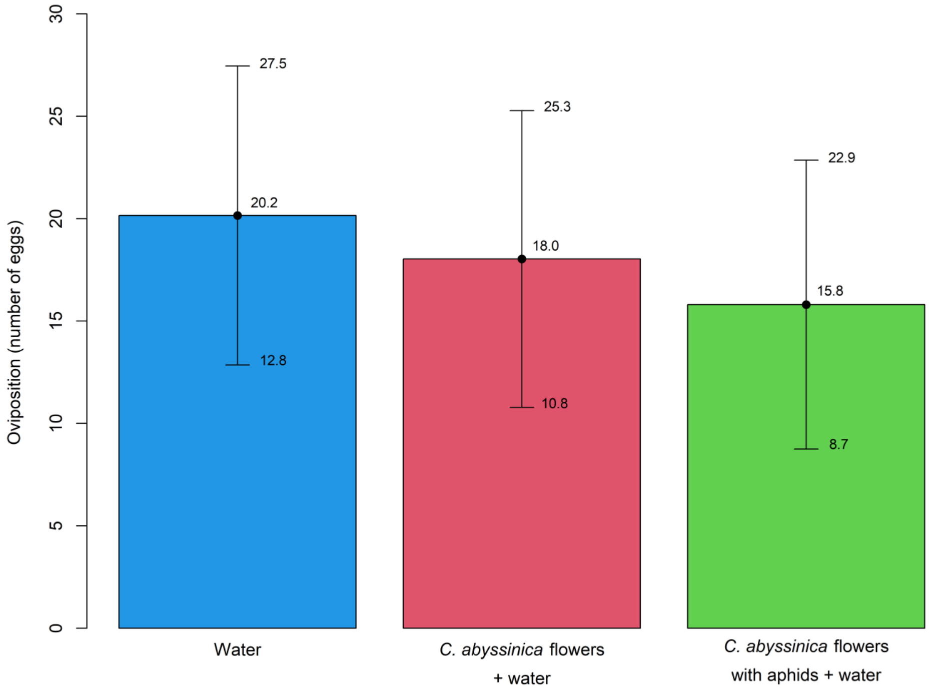

Figure 8. Mean oviposition (number of eggs) of *L. coffeella* females under three treatments: just water, water and *C. abyssinica* flowers and the flowers infested with aphids plus water. Treatments with 95% of confidence interval.

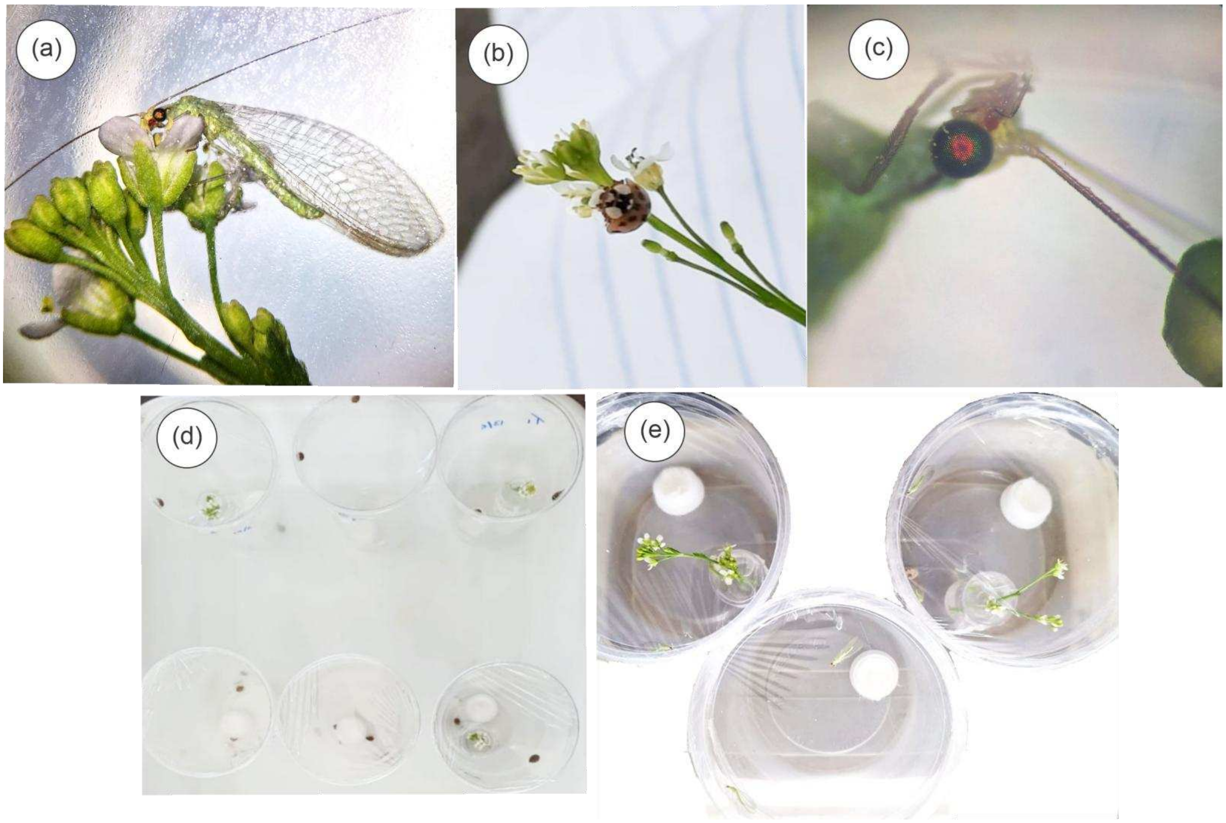

Figure 9. *C. externa* (a) and *H. axyridis* (b) adults on *C. abyssinica* flowers; *C. externa* adult with *C. abyssinica* pollen grains in its eyes (c); *H. axyridis* (d) and *C. externa* (e) adult's experiment with crambe flowers and other treatments. All photos were taken by Pedro Inocêncio Silveira at Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), Viçosa - MG, Brasil.

5. DISCUSSION

Flowers of *C. abyssinica* notably increased the survival of both *C. externa* and *H. axyridis*, two important natural enemies but did not bring any benefit to *L. coffeella*, neither did the honeydew. These results help to understand the mechanisms involved in conservation biological control to better apply its techniques in order to suppress pest populations in a more sustainable way, making use of ecosystem services. Plant-derived resources, such as pollen and nectar, contribute to attract and maintain beneficial insects for extended periods of time, for the purpose of controlling a diversity of pests more effectively in the field (Lu et al., 2012; Hassan et al., 2016), especially concerning generalist predators, such as green lacewings and ladybeetles. That being so, integrate *C. abyssinica* with other agricultural crops seems propitious.

Floral resources provided by *C. abyssinica* successfully increased the survival probability of *C. externa* larvae in comparison with the ones with only water available, with some individuals even reaching 7 or 8 days of survival. Just two individuals fed with *E. kuehniella* eggs plus water died before turning into pupae, the rest of them pupated in approximately 10 days. *C. abyssinica* flowers also increased the adult green lacewing's survival probability in comparison with individuals fed with just water. From 40 individuals fed with yeast, honey and water, 27 survived from more than the 30 days of analyses. Only in this treatment they could oviposit and the mean number of eggs laid by females was 165. Brassicaceae pollen was found on the gut of *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) and *Chrysoperla lucasina* Lacroix (Neuroptera: Chrysopidae) individuals (Villenave et al., 2006; María Villa et al., 2019). *C. carnea* adults fed with *Raphanus raphanistrum* (Brassicaceae) flowering plants survived an approximate interval of time from 3 to 6 days, but females did not oviposit (Gonzalez et al., 2016).

C. abyssinica floral resources increased the survival probability of *H. axyridis* larvae in comparison with individuals just in the presence of water. Only five individuals fed with *E. kuehniella* eggs plus water died before turning into pupae, the rest of them pupated in approximately 12 days. Sun et al., (2014), found that when *H. axyridis* larvae conventional diet was complemented with *Brassica napus* (Brassicaceae) pollen, the individuals increased their survival and turned into heavier adults. When fed with a diet of bee moist pollen (75-80% of Brassicaceae pollen), *H axyridis* larvae increased its survival and almost half of them could even reach adulthood, but in relation to the adults, the pollen just shortened female pre-oviposition period (Berkvens et al., 2008). Our results showed no difference in the survival probability between adults in presence or absence of *C. abyssinica* flowers. From 42 individuals fed with *E. kuehniella* eggs plus water, 37 survived more than 30 days of analyses. Just 4 females from this treatment could oviposit, probably because there were no aphids on their diets. It suggests that the ladybug, also predators when adults, requires nutritionally protein-richer diets to boost its reproduction. Chen et al., (2012), observed that *H. axyridis* females presented higher fecundity when fed with *Chaitophorus populeti* Panzer (Hemiptera: Aphididae) in relation with the ones offered with *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) eggs or artificial diets.

As floral nectar is an essential food source for many Lepidoptera (Kinoshita et al.,

2017) and since *L. coffeella* individuals were able to feed on artificial glucose and sucrose diets (Nantes and Parra, 1978), we offered the coffee leaf miner, *C. abyssinica* flowers and then flowers infested with *M. persicae* individuals. The presence of *C. abssynica* flowers did not affect the survival and reproduction of *L. coffeella* adults in relation to the control, which suggests that this microlepidoptera is not able to feed on floral nectar of this plant, maybe because it was inaccessible due to the flowers architecture and incompatibility with its proboscis. Many plant species pollinated by Lepidoptera and bees have their petals (corolla) fused like a tube (not the case of *C. abssynica*) where these insects fit their mouthparts to acquire the nectar from the base, and the ones with mouthparts shorter than the corolla tube cannot feed on them (Orr and Pleasant, 1996), which could be the case of the coffee leaf miner. Crambe corolla length is around 10mm, flower depht might be around 5mm and the overall length of these tiny moths is around 2mm (Almeida et al., 2020; Vergun et al., 2021; personal observations).

Floral nectar is a plant nutricious meal to attract visitors and increase pollen transfer, that being so, nectar traits are possibly established by pollinator-mediated selection (Parachnowitsch et al, 2019). Laboratory evaluations combined with field observations of foraging behavior of *Coleomegilla maculata* DeGeer (Coleoptera: Coccinelidae) and *C. carnea,* indicates that two plant species from the Apiaceae family, with white and yellow umbrella-shaped inflorescences, have floral architecture complementary with the head morphology of these two predators, allowing them to feed on floral nectar and increasing pest suppression when these plants were intercropped with eggplant (Patt et al., 1997). *C. abyssinica* flowers provided resources to larvae and adults of *C. externa*, and larvae of *H. axyridis*, but not to *L. coffeella*. The bigger size of the lacewing and ladybug adults may have prevented them to reach the nectar glands. On the other hand, new-born predator's larvae are very small and probably could have had access both to nectar and to the freely exposed pollen. The immature stages of *C. externa* are omnivorous predators and the adults have glycopalynophagous diets, feeding on pollen and nectar essentially (Resende et al., 2017, Souza et al., 2019; Fernandes et al., 2023). *H. axyridis* are generalist predators on immature stages as well as when they are adults, using non-prey resources as a complementary source of food, and this can be a reason that *C. abyssinica* flowers did not increase its adult survival. A total of 11,335 pollen grains, from 21 families, were extracted from the guts of 53 *C. externa* adults in contrast with 46 pollen grains, from 10 families, found on 46 adults of

Hippodamia convergens Guérin-Menéville (Coleoptera: Coccinelidae) (Medeiros et al., 2010).

The presence of *M. persicae* did not increased *L. coffeella* survival, which means it could not feed on honeydew. This result contradicts a field personal observation related by Crowe (1964) in which *Leucoptera meyrick* Ghesquiere (Lepidoptera: Lyonetiidae) adults were feeding on substances produced by aphids. On the other hand, the honeydew secreted by aphids from the genus *Melanaphis* was found to increase *C. externa* adult's survival and females could oviposit viable eggs (Barroso, 2022). To establish *C. abyssinica* as an adequate plant to be used in biological conservation control programs in coffee systems we might as well determine its effects on *L. coffeella* and other coffee pests besides on natural enemies. *C. abyssinica* do not host the black aphids from the genus *Toxoptera*, also present in coffee crops, but when hosting aphids from genus *Myzus* (which occurs occasionally) could possibly provide honeydew to the green lacewing, so intercropping it within coffee trees seems even more promising (Harelimana et al., 2024).

In this experiment, presence of aphids reduced *L. coffeella* oviposition and pre-oviposition period (not statistically different though). Kessler and Baldwin, (2001) found that herbivore-induced plant volatiles (HIPV) released by tobacco plants attacked by herbivores decreased oviposition of *Manduca quinquemaculata* Haworth (Lepidoptera: Sphingidae). *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) also reduced their oviposition on undamaged cotton plants adjacent to plants attacked by other caterpillars (Zakir et al., 2013). Our results showed similar pattern, probably because the coffee leaf miner tried to avoid inadequate oviposition sites such as weakened plants and accelerate its lifespan, granting more offspring generations in time.

Our approaches demonstrate the importance of floral resources availability to generalist predators, such as lacewings and ladybeetles. The increase on the survival of *C. externa* and *H. axyridis* suggests that *C. abyssinica* flowers produces beneficial non-prey resources to these natural enemies, which can be used as an alternative meal to complement their diets. Crop diversification with *C. abyssinica* seems optimistic because beside its potential to increase biological control of herbivore insects, it can also attract pollinators to the area and promote other ecosystem services. With the use of ecologically-friend techniques in agro ecosystems, we can perform a sustainable agriculture and find stability between the pressure of food production and the urgent need to protect the environment.

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CHAPTER III

Predation of main coffee pests by ladybugs

ABSTRACT

The coffee berry borer (CBB), *Hypothenemus hampei*, and the coffee leaf miner (CLM), *Leucoptera coffeella*, are key pests of coffee crops and both species are difficult to control due to their cryptic habit. Consequently, discovering insects that can prey upon any life stage of these pests is a matter of great importance and contribution to improve biological control practices and inversely, reduce chemical control. For the first time, we reported predation of CBB by the ladybugs *Eriopis connexa* and *Coleomegilla maculata*. In laboratory experiments, we investigated the predator's capability to access CBB galleries and prey on immature stages of this pest. First instars *E. connexa* and *C. maculata* larvae were able to enter the galleries and prey on eggs and larvae of CBB. We observed that the new-born predator's larvae can also feed on CBB pupae. In another experiment, we showed that from the second instars onwards, *Harmonia axyridis* larvae and adult could prey and feed on CLM pre-pupae. These ladybugs are generalist predators often found in coffee systems in Brazil, yet little is known about their potential in contributing to control pests in this specific crop. Here, we describe three new predators capable to help on biological control of CBB and CLM.

Keywords: Ladybeetles, beneficial insects, *Hypothenemus hampei*, *Leucoptera coffeella.*

1. INTRODUCTION

The coffee leaf miner (CLM) *Leucoptera coffeella* Guérin-Méneville (Lepidoptera: Lyonetiidae) and the coffee berry borer (CBB) *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae) are both key coffee pests in Brazil that causes substantial yield losses and are difficult to control (Venzon, 2021). While CLM control is hampered due to the fact that immature life stages feeds in the mesophyll, staying inside the leaves (Pereira et al., 2007; Dantas et al., 2021), CBB control is complicated because adult female dig galleries into the endosperm of coffee seeds, oviposit, and complete its life cycle inside the berries (Damon, 2000; Infante, 2018). Chemical control itself is hazardous to humans, wildlife and environment and the extensive use of synthetic pesticides to control these pests have selected resistant populations (Johnson et al., 2020; Dantas et al., 2021; Lemma and Abewoy, 2021), which emphasize the need to adopt integrative approaches to deal with these insects. Thus, discovering insects that can prey upon any life stage of these harmful pests, which are physically protected either inside mined-leaves or bored-berries are of great importance for coffee farms.

Some natural enemies were reported to prey or parasitize on CLM, such as ants (De la Mora et al., 2008; Lomelí-Flores et al., 2009), predatory wasps, hymenopteran parasitoids (Pereira et al., 2007; Fernandes., 2012; Rezende et al., 2014; David-Rueda et al., 2016; Androcioli et al., 2018) and green lacewings (Ecole et al., 2002; Martins et al., 2021). Likewise, parasitoids have been described to parasitize on CBB (Vega et al, 1999; Damon, 2000; Aristizábal et al., 2016) and few predators, mostly ants (Pardee and Philpott, 2011; Botti, 2021; Newson et al., 2021), but also species of Hemiptera (Bustillo et al., 2002), Neuroptera (Botti et al., 2022), Thysanoptera (Jaramillo et al., 2010; Rezende et al., 2014); and Coleoptera (Follet et al., 2016; Sim et al., 2016). Botti et al., (2021) found an adult green lacewing inside one bored coffee berry collected from a diversified coffee system in the Cerrado of Brazil. Later, the authors confirmed that Chrysopidae larvae (*Chrysoperla externa*) (Hagen) can enter the berry through CBB gallery and prey on the pest.

Ladybugs (Coccinellidae) are generalist predators that feed on aphids and a variety of soft-body insects such as mites, scales, Lepidoptera eggs and caterpillars (Koch, 2003; Koch et al., 2003; Hoogendoorn and Heimpel, 2003) and are often found on coffee crops (Venzon personal information), however their role on coffee pest control is unclear. Due to the morphological similarities (both in size and shape) of their immature bodies in comparison with the lacewings, especially newly-emerged larvae, we tested the hypothesis that first instar of *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), *Cycloneda sanguinea* Linnaeus (Coleoptera: Coccinellidae), *Eriopis connexxa* Germar (Coleoptera: Coccinellidae) and *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae) would be able to access the galleries and prey on some CBB life stage. *E. conexxa* is native from South America (Santos et al., 2016), *C. sanguinea* is native from South, Central and south of North America (Michaud, 2002), *C. maculata* is native from North America (Hesler and Brust, 2024) while *H. axyridis* is native from Asia (Paula et al., 2021). Thus, we carried on laboratory experiments to test whether native and exotic ladybugs prey upon this key coffee pest. Moreover, due to their diversified feeding habits and high prey searching ability, we also hypothesize that *H. axyridis* (the first species we reared) can prey upon vulnerable CLM life stage.

2. MATERIAL AND METHODS

2.1. Ladybug rearing

Adults of *H. axyridis, C. sanguinea, E. conexxa* and *C. maculata* were collected on maize crop at the Agronomy field of the Federal University of Viçosa (UFV), Viçosa, state of Minas Gerais, Brazil. Adults were kept in plastic containers (15x10 cm), fed with a honey-soaked cotton, *Myzus persicae* Sulzer (Hemiptera: Aphididae) and eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Food was replaced twice a week. A cabbage leaf with its petiole wrapped in wet cotton to keep it turgid was replaced weekly as an oviposition site. A crumpled piece of paper was also weekly replaced, serving as shelter to the predators. The egg clutches were then cut from the cabbage leaves and separated until larvae emergence. Then, larvae were individualized in J10 (10 mL) containers, fed with *E. kuehniella* eggs every three days until turning into pupae. The rearing unit was kept at 25 ± 2°C, 70 ± 10% RH and 12h photophase in the Laboratory of Entomology at Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG) in Viçosa, state of Minas Gerais, Brazil.

2.2. Predation of coffee leaf miner

We tested whether *H. axyridis* were able to prey on any life stage of the coffee leaf miner. Coffee leaf miner rearing is kept on seedlings inside a green house at EPAMIG, located in Viçosa. The rearing is similar with natural conditions, besides they are protected from rain, where adults can freely reproduce and generate offspring. Pupae were then cut with a little piece of the leaf and put on Eppendorf tubes (1.5 mL) until adult emergence. New-born adults were sexed under microscope regarding their difference in the end of abdomen (Nantes and Parra, 1977). Couples were caged in plastic containers (8x10 cm) with one sanitized coffee leaf with its petiole submerged in water for oviposition. In Petri-dishes, CLM new-laid eggs, larvae (inside the mines) and pupae (attached to the coffee leaf), both collected at the green house, were offered to first and fourth-instar *H. axyridis* larvae and also adults (5 individuals of each life stage). After 24 hours, none predation occurred in these pre-tests. Then, pre-pupae of *L. coffeella* were collected at the moment they were leaving the coffee leaf before pupation (at the green house) and offered to second-instar *H. axyridis* larvae (first-instar were fed with *E. kuehniella* eggs). Due to the lack of pre-pupae individuals in the green house, larvae were removed from their mines to complement the diet. In total, an average of 5 individuals (pre-pupae and larvae from different instars), in every two days, was offered to 30 *H. axyridis* larvae and their survival probability was recorded and compared with the control (30 second-instar *H. axyridis* larvae fed with water-soaked cotton). *L. coffeella* pre-pupae were also offered to a few adults from this ladybug species, predation occurred but no survival data was recorded.

2.3. Predation of coffee berry borer

We tested if the ladybug species can access CBB gallery and prey on this pest. Bored and healthy coffee berries were collected at the Infectarium from Plant Pathology Department, at UFV, Viçosa. In a pre-test, 5 new-born larvae of *H. axyridis*, *C. sanguinea* and *E. connexxa* were confined in Petri-dishes, each individual with one bored-coffee berry. After 30 minutes observations, we recorded whether they were able to access the CBB gallery. Just one individual of *E. connexxa* accessed the gallery. Then, we simulated the entrance of CBB galleries. We used green healthy coffee berries and pinned a hole of 1 mm in diameter and 1 cm depth in the berry crown. Thus, we collected CBB smaller larvae and eggs of the bored-berries from Infectarium and introduced each one of them, individually, with a brush, inside the pinned holes. Finally, we trapped the larvae in the berry surface as we seized the grain against a helmet-type cage (1 cm in diameter and 1 cm height) for 24 hours. In total, 30 CBB larvae and 30 eggs were caged with 60 *E. connexxa* first-instar larvae to analyze the ladybug capability to access the galleries and prey on this pest.

Walking once more through the maize crop in the Agronomy field we found adults of *C. maculata* and due to the morphological similarities in their adult's bodies with *E. connexxa* (smaller narrow-shaped body) in contrast with *H. axyridis* and *C. sanguinea* (bigger rounded bodies), we investigated the capability of this species larvae in entering CBB gallery and preying on them. For this evaluation, we repeated the exact same experiments cited above, but now with 20 CBB eggs, 20 larvae and 40 *C. maculata* first-instar larvae. We also observed whether these two ladybug species are able to prey on CBB pupae and adults in J10 containers.

3 DATA ANALYSES

Survival data were tested by survival analysis with the Kaplan-Meier estimator (Kaplan and Meier, 1958). The general similarity among the curves was tested with log-rank tests (Mantel, 1966). Descriptive statistic was used for predation data (yes or no, percentage). All data analyses were performed using R (version 4.4.2) statistical software package (R Development Core Team, 2024).

4 RESULTS

4.1 Predation of coffee leaf miner

As mentioned above, in the pre-tests, first and fourth-instar *H. axyridis* larvae and adults did not prey on CLM eggs, pupae, adults or larvae (inside the mines). But outside the leaves, from second instar onwards, the ladybug could feed on CLM pre-pupae. The diet of CLM pre-pupae and larvae increased the survival of *H. axyridis* second-instar larvae in comparison with the control. The median time to achieve 0.5 of survival probability increased from 4 to 17 days ($\chi^2 = 68.80$; df = 1; $p < 0.0001$; Fig. 1). Two individuals even pupated and one of them turned into adult. Adults of *H. axyridis* were also observed to feed on CLM pre-pupae and larvae but their survival was not analyzed.

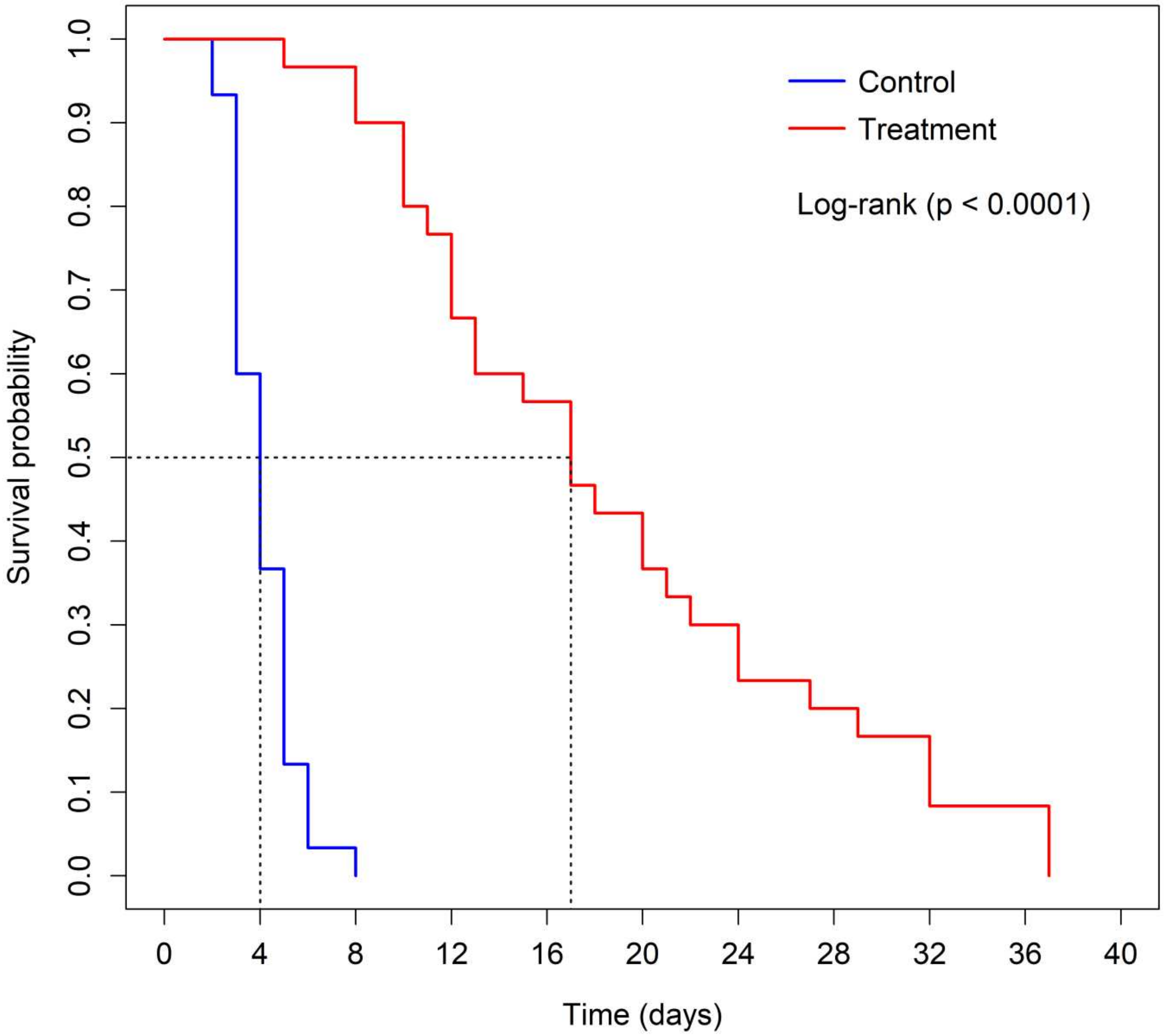

Figure 1. Survival probability of second-instar *H. axyridis* larvae fed with CLM pre-pupae and larvae or fed with just water ($\chi^2 = 68.80$; df = 1; $p < 0.0001$).

4.2 Predation of coffee berry borer

Both ladybug species could successfully enter CBB galleries and prey upon eggs and larvae. For *E. connexa*, 80% of new-born larvae accessed the galleries and preyed on the eggs, and 30% preyed on larvae. For *C. maculata,* 25% of first-instar larvae accessed the galleries and preyed on CBB eggs and 15% preyed on larvae. The ladybugs did not prey on CBB adults but both species preyed on pupae. CBB pupae size is bigger than the gallery entrance diameter, so we could not introduce the pupae inside the coffee berry and conduct the experiment, that being so, we observed predation only in J10 plastic containers. In field conditions though, once inside the bored-berries, first instar larvae of these ladybug species could also prey on CBB pupae.

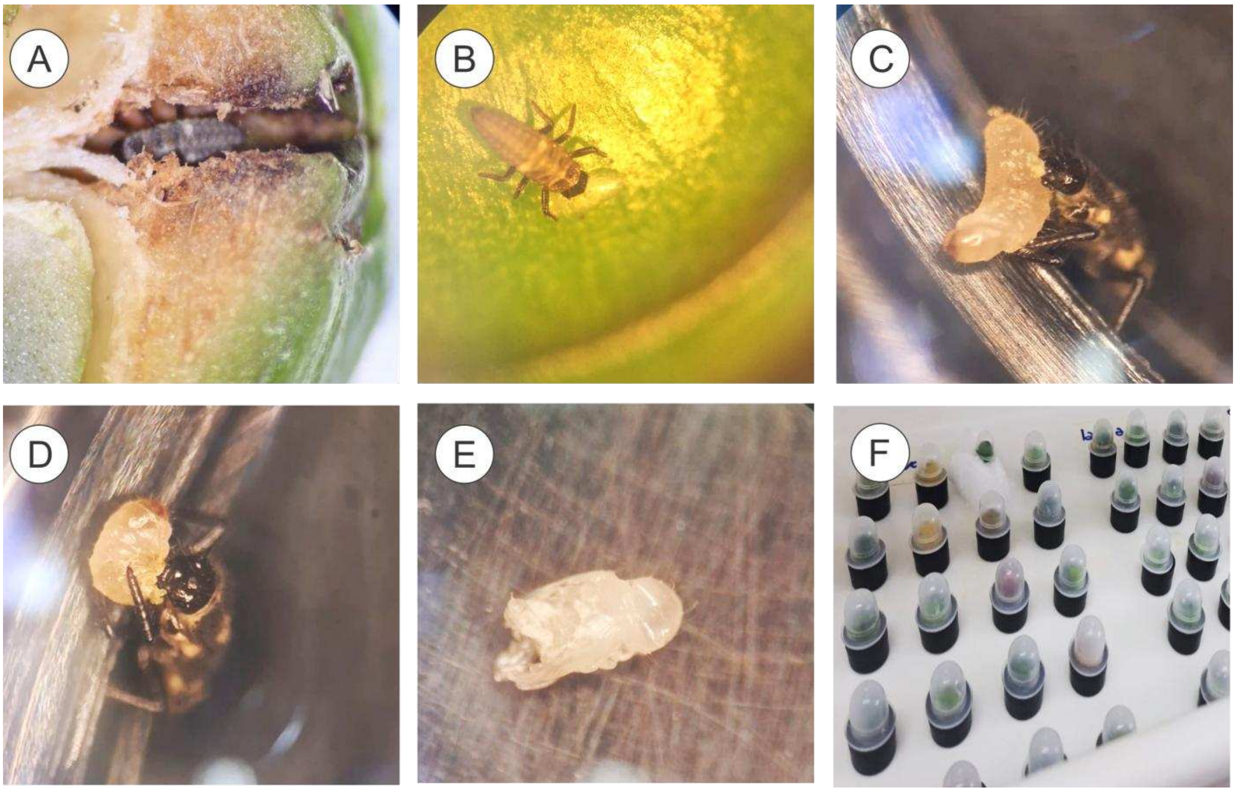

Figure 2: First instar *E. connexxa* larva inside the gallery (A); first instar *C. maculata* preying on CBB egg (B); first instar *C. maculata* starting (C) and finishing (D) CBB larva predation; CBB pupa predated by *E. connexxa* first instar larvae (E); arenas used for predation experiments (F). All photos were taken by Pedro Inocêncio Silveira at Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), Viçosa - MG, Brasil.

5 DISCUSSION

A clear understanding about plants, herbivores and natural enemies is essential to control important pests in a more sustainable and efficient way (Venzon et al., 2011). Ladybugs are important generalist predators in all life stages (Kim et al., 2022; Hsu et al., 2025) preying on a variety of soft-body insects and our objective here were to discover the potential of new species that can prey upon key coffee pests. In regarding to CLM and CBB, which are difficult to control, conserving Coccinellidae predators in coffee crops over longer periods of time (besides the blooming season) is an important strategy that must be adopted through landscape heterogenization, selecting plants that strategically provides plant derived alternative foods, such as nectar and pollen and also shelter and oviposition sites (Berkvens et al.,2008; Venzon and Sujii, 2009; Santos et al., 2016; D'Ávila et al., 2017).

Some predators were found to prey on the coffee leaf miner. Ants can prey upon immovable life stages such as eggs and pupae (Lomeli-Flores et al., 2009). Vespidae species, with their strong mandibles, torn CLM mines and ripe the larvae out (Fernandes et al., 2009; Rosado et al., 2021). Second and third *Ceraeochrysa cubana* Hagen (Neuroptera: Chrysopidae) preyed on CLM eggs and pupae, piercing the pest with their mandibles and sucking it (Martins et al., 2021) and third instar *Chrysoperla externa* Hagen (Neuroptera: Chrysopidae) could feed on CLM pupae (Ecole et al., 2002). This is the first record of a Coccinellid preying on the coffee leaf miner. Larvae of *H. axyridis* and adults could not feed on CLM eggs, probably because of their tiny size (0.3 mm) and translucent color. The ladybug could not prey on CLM pupae either, their chewing mouthparts are possibly not strong enough to tear the pupae silk apart or long enough to get to the insect. Also, the predators could not torn CLM mines and remove the larvae inside. On the other hand, from second instar onwards, *H. axyridis* successfully fed on CLM pre-pupae, a brief period where larvae are vulnerable to predation.

One native ladybug species and one exotic accessed CBB galleries and preyed on the pest (*E. connexxa* and *C. maculata*, respectively). Similarly, one native species and one exotic did not enter the galleries (*C. sanguinea* and *H. axyridis*, respectively). The asian ladybeetle, is a bigger species in comparison with the other ones (Mirande et al., 2015). First instar larvae pronotum reaches almost 1 mm width (Dmitriew and Rowe, 2007) and its body lengths reaches approximately 2mm (Sasaji, 1977; Koch, 2003). Likewise, *C. sanguinea* did not differ its average weight in first and second larvae instar in comparison with *H. axyridis* (Santos et al., 2013). *E. connexxa* and *C. maculata* larvae are overall very similar, and adults, besides coloration, are similar in size, and shape (smaller narrow-shaped body) in contrast with *H. axyridis* and *C. sanguinea*, which are bigger and have rounded bodies. That being so, size and similarities among these species could have prevented or not their access inside CBB galleries.

Botti et al., (2022) found that first instar larvae of *Chrysoperla externa* Hagen (Neuroptera: Chrysopidae) could enter CBB gallery, remove eggs and larvae and prey on them in 30 min observations. In my half-hour evaluations, just one *E. conexxa* individual accessed the gallery, so I left them for 24 hours. CBB egg and larvae predation occurred by first instar of *E. connexxa* and *C. maculata*, but the coccinellids could not remove the immature from the berries probably because of their mouthparts. The ladybugs larvae have chewing mouthparts, with smaller mandibles, in contrast with the long mandibles of the green lacewing larvae, which possibly pierced and dragged the pest to the outside, finally sucking it.

The higher predation of CBB eggs is probably because eggs are immobile and defenseless. In contrast, larvae could dig dipper inside the gallery. I observed in Petri dishes, that the predators eat the whole larvae body, except the head, which is thicker. So, if the encounter of them (inside the coffee berry) occurs face-to-face, CBB larvae would possibly avoid predation. I also observed that first instar larvae can prey upon CBB pupae, which are immobile as well. Neither larvae nor adults from ladybeetles could feed on CBB adults due to hardness of elytra.

For the first time, predation of such dangerous coffee pests, like CBB and CLM, is reported by coccinellid species. Control of these pests is difficult due to physical protection inside the galleries or mines, so integrative measures are essentially important. Conservation of these natural enemies in field conditions can be achieved by diversifying coffee crops, either using cover crops, maintaining spontaneous flowering plants or introducing extrafloral nectar bearing plants to provide alternative meal when preys are scarce (Venzon et al., 2011; Rezende et al., 2014; Venzon, 2021). Our aim is to bring new allies capable to help in the biological control of important coffee pests, such as *L. coffeella* and *H. hampei*, reducing chemical inputs and contributing to a regenerative and sustainable agriculture.

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GENERAL CONCLUSIONS

Extrafloral nectar of *Senna cernua* successfully increased *Chrysoperla externa* fitness, once it increased larvae survival and adult's survival and reproduction. The nectar also increased *Harmonia axyridis* larvae and adult survival.

Crambe abyssinica floral resources successfully increased *C. externa* larvae and adult survival and also increased *H. axyridis* larvae survival. *Leucoptera coffeella* did not feed on *C. abyssinica* floral nectar and *Myzus persicae* honewdey.

For those reasons cited above, *S. cernua* and *C. abyssinica* should be introduced in coffee agro ecosystems, providing ecosystem services and contributing to a sustainable and regenerative agriculture.

H. axyridis preyed on *L. coffeella* pre-pupae, a brief life stage susceptible to predation. Thus, we add a new predator to the list of *L. coffeella* natural enemies.

Eriopis connexxa and *Coleomegilla maculata* first instar larvae successfully accessed *Hypothenemus hampei* galleries and fed on immature stages of this pest. Eggs, larvae and pupae were preyed by these ladybug species. Therefore, we add two new predators to the list of *H. hampei* natural enemies.