ANDRÉ MAZOCHI BARROSO

APHID HONEYDEW AFFECTS POSITIVELY *Chrysoperla externa* FITNESS

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

Adviser: Madelaine Venzon

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BIOGRAPHY

André Mazochi Barroso is son of Guilherme de Castro Barroso and Sylvia de Moraes Mazochi. He was born in Belo Horizonte-MG, on April 3rd, 1995.

In 2013, he started the Agronomy course at the Universidade Federal de Lavras (MG) and completed it in December of 2018.

During his graduation, he was a research scholarship holder for one year under the advice of Professor Dr. Brígida Souza from the Department of Entomology, where he worked with methods of releasing Chrysoperla externa. Still in graduation, he was monitor of the discipline of Plant Morphology and Systematics and worked on his graduation thesis with morphological identification of forage grasses and legumes, under the advice of Professor Dr. Mariana Esteves Mansanares.

In March 2020, he started the Master's course in Entomology at the Universidade Federal de Viçosa under the advice of Professor Dr. Madelaine Venzon. He presented this thesis defense on the 24th of February of 2022.

ABSTRACT

BARROSO, André Mazochi, M.Sc., Universidade Federal de Viçosa, February, 2022. **Aphid honeydew affects positively *Chrysoperla externa* fitness**. Adviser: Madelaine Venzon.

Dealing with arthropod pests is one of the main problems of modern agriculture. The use of agrochemicals has often become inefficient due to the acquisition of resistance by pests, in addition to being harmful to human health and to the environment. For these reasons, eco-friendly methods of combating pests are currently gaining ground. This is the case of Conservation Biological Control, which involves practices such as the use of companion plants to provide shelter and/or food resources and to attract natural enemies of pests, allowing their population establishment in the agroecosystem. The objective of this dissertation was to study the suitability of food resources directly or indirectly provided by sorghum (*Sorghum bicolor*) as a companion plant to the lacewing *Chrysoperla externa*. First, I assessed the suitability of honeydew produced by aphids (*Melanaphis* sp.) on *S*. *bicolor* as food resource for *C*. *externa* adults. The survival of *C. externa* adults was higher and females did oviposit viable eggs when they had access to *Melanaphis* sp. honeydew. Females without access to honeydew did not oviposit. I followed by evaluating whether pollen of sorghum is suitable to *C. externa* adults when offered alone or in combination with *Melanaphis* sp. honeydew. The lacewings survived longer and females oviposited viable eggs when they had access to honeydew but the pollen presence did not influence oviposition or adult survival. These results show that sorghum can act as a companion plant to provide honeydew for *C. externa* adults when infested by aphids of the genus *Melanaphis*, aiming for the conservation of these biocontrol agents in agroecossystems due to longer survival and oviposition of viable eggs. This strategy should only work when the aphids are not pests of the main crops.

Keywords: Green lacewing. *Sorghum bicolor*. *Melanaphis*. Pollen. Conservation Biological Control.

RESUMO

BARROSO, André Mazochi, M.Sc., Universidade Federal de Viçosa, fevereiro de 2022. **Honeydew de afídeos afeta positivamente a performance de *Chrysoperla externa***. Orientadora: Madelaine Venzon.

Lidar com artrópodes praga é um dos principais problemas da agricultura moderna. O uso de agroquímicos com frequência tem se mostrado ineficiente devido à aquisição de resistência por pragas, além de ser prejudicial à saúde humana e ao meio ambiente. Por essas razões, métodos ecologicamente sustentáveis de combate a pragas estão ganhando espaço. É o caso do Controle Biológico Conservativo, que envolve práticas como o uso de plantas companheiras para fornecer abrigo e/ou recursos alimentares e atrair inimigos naturais de pragas, promovendo o estabelecimento de sua população no agroecossistema. O objetivo desta dissertação foi estudar recursos alimentares fornecidos direta ou indiretamente pelo sorgo (*Sorghum bicolor*) para o predador *Chrysoperla externa*. Primeiramente, foi avaliada a sobrevivência, taxa de oviposição (ovos por fêmea) e viabilidade de ovos de *C. externa* com acesso ao honeydew produzido por afídeos (*Melanaphis* sp.). A sobrevivência de adultos de *C. externa* foi maior e as fêmeas ovipositaram ovos viáveis quando tiveram acesso ao honeydew. Fêmeas sem acesso ao honeydew não ovipositaram. Posteriormente, foi avaliada a sobrevivência, taxa de oviposição e viabilidade de ovos de adultos de *C. externa* com acesso ao pólen de sorgo oferecido sozinho ou em combinação com honeydew de *Melanaphis* sp. Os crisopídeos sobreviveram por mais tempo e as fêmeas ovipositaram ovos viáveis quando tiveram acesso ao honeydew, mas a presença de pólen não influenciou a oviposição ou a sobrevivência dos adultos. Esses resultados mostram que o sorgo pode atuar como planta companheira para fornecer honeydew para adultos de *C. externa* quando infestados por afídeos do gênero *Melanaphis*, visando a conservação desses agentes de controle biológico em agroecossistemas, devido à maior sobrevivência e oviposição de ovos viáveis. Esta estratégia só poderá funcionar quando os afídeos não são pragas da cultura principal.

Palavras-chave: Crisopídeo. *Sorghum bicolor*. *Melanaphis*. Pólen. Controle biológico conservativo.

SUMMARY

1. INTRODUCTION

Agriculture is a human activity that needs to deal with the attacks of arthropod pests, which can cause damage and even devastate crops if they are poorly managed. An extensive monoculture field is a large area of abundant resources for crop pests, because they can easily find the resources they need to survive and reproduce. On the other hand, their natural enemies commonly have difficulties in establishing populations due to the lack of alternative food, microclimatic conditions and refuge areas. (VENZON et al., 2019b). However, plant diversification in the agroecosystem brings many benefits for production, such as cycling of nutrients in the soil, promoting pollination and promoting biological and cultural control of pests and diseases (SUJII et al., 2010). Greater plant diversification in the agroecosystem generally means more ecological functions being performed in the field. This is to say that monocultures and simplified ecosystems that have lost several ecological functions and are more vulnerable to pest outbreaks (PEÑALVER-CRUZ et al., 2019; BLASSIOLI-MORAES et al., 2022). Therefore, it is important to study plants that can bring services and benefits to agricultural crops, such as natural regulation of insect populations, serving as companion plants to promote conservation biological control (BLASSIOLI-MORAES et al., 2022).

Conservation Biological Control (CBC) is an alternative that has been gaining space in agricultural pest control, with information and related research increasing since the 1990s (PEÑALVER-CRUZ et al., 2019). Conservation Biological Control seeks to make the agroecosystem more propitious to the establishment of populations of natural enemies of insect pests (VAN DRIESCHE; HODDLE; CENTER, 2008). It happens through diversification of plants in the field which includes companion plants that provide shelter and food resources such as nectar, pollen, honeydew and alternative prey to natural enemies. These plant-provided food resources are important to complement the diet of entomophagous arthropods or as essential food resource in non-carnivorous life stages and the presence of these plants can increase the efficiency of these biocontrol agents on controlling agricultural pests (VENZON et al., 2019a; VENZON, 2021)(VENZON et al., 2019a). The companion plants in the agroecossystem, however, must not benefit pest insects, just beneficial organisms, and it also must not reduce crop productivity (POVEDA et al., 2008; VENZON et al., 2018, 2019a).

The increase in CBC research in the last decades has been due to the search for sustainable alternative strategies to chemical control, which has been promoting pest resistance to pesticides (OLIVEIRA et al., 2014). These chemicals also cause environmental impacts and reach other non-target species that play important roles in human activities, such as pollination, cycling of soil nutrients and biological control (COUX et al., 2016). In addition to these consequences, the large amount of pesticides used has affected human health (MENDES et al., 2020). In 42 years (1975-2017), an increase of more than 400 times in cases of pesticide poisoning was recorded in Brazil (Ministério da Saúde/SVS, 2020).

Among the potential biological control agents in Neotropical America, the insects of the Chrysopidae family stand out, mainly the species *Chrysoperla externa* (Hagen) and *Ceraeochrysa cubana* (Hagen) (Neuroptera: Chrysopidae), which are efficient biological control agents of various pests (ALBUQUERQUE et al., 1994; SOUZA et al., 2008; SOUZA et al., 2019). They are generalist predators in the larval stage, which is the stage where biological control happens. The adult stage feeds on pollen, nectar and honeydew, thus it is important that these food resources are available in the agroecosystem for the establishment of this predator populations. For instance, Venzon et al. (2006) demonstrated that fertility and longevity of *C. externa* adults varied between individuals that fed on pollen (protein source) from different species, that the addition of honey (carbohydrate source) increased their reproductive success and that individuals without a protein source did not reproduce.

There are several studies on the importance of providing plant resources, such as floral and extrafloral nectar and pollen and how these resources should not benefit populations of the pests themselves (SOUZA et al., 2010; VENZON et al., 2018, 2019a, 2021). Another strategy, less studied, to provide suitable food complement for predators and non-carnivorous life stages of natural enemies, is the presence of honeydew excreted by hemipterans (e.g. aphids, scales, white flies and mealybugs) (LANDIS et al., 2000). Honeydew is a sugary substance rich in carbohydrates that also contains amino acids and proteins (SABRI et al., 2013; SHAABAN et al., 2020). The excretion of honeydew by hemipterans is due to a peculiarity in their digestive system that eliminates, mainly, excess carbohydrates and water, but also amino acids, sucked from the sap (GULLAN; CRANSTON, 2010). Lee et al. (2004) demonstrated that honeydew-fed adult parasitoid wasps of the species *Diadegma insulare* Cresson

(Hymenoptera: Ichneumonidae) survived longer than when there were no food resources. Leroy et al. (2011), using the hoverfly *Episyrphus balteatus* De Geer (Diptera: Syrphidae) as a model, suggested greater attractiveness and effectiveness of natural enemies in the presence of volatiles produced by microorganisms found in honeydew. The presence of honeydew is one more nutritional alternative in the field for beneficial insects, decreasing the chances of food resources to deplete. Furthermore, unlike pollen and nectar, it does not depend on the flowering season nor the time of flower opening to be accessed (LEE et al., 2004). Although frequently considered to be pests, aphids can be allies when the focus is to provide resources for natural enemies. However, it is extremely important that the honeydew-producing hemipteran do not threaten the main crop and that they will feed only on a companion plant chosen for that, otherwise it could jeopardize crop yield.

Sorghum (*Sorghum bicolor* (L.) Moench) is an easily accessible grass in Brazil. It has several uses, like in human food (QUEIROZ et al., 2014) and animal feeding (FERNANDES et al., 2014; BARBERO et al., 2014), ethanol production (MAY et al., 2014) and intercropping in crop-livestock-forest systems (ALBUQUERQUE et al., 2014). Among its main leaf pests there are the aphids of the genus *Melanaphis* (SINGH et al., 2004, BOWLING et al., 2016, NIBOUCHE et al., 2018; NIBOUCHE et al., 2021). These aphids also attack sugarcane, corn crops and other grasses and are present worldwide (EMBRAPA, 2015; SINGH et al., 2004; LOPES-DA-SILVA; ROCHA; DA SILVA, 2014; RAZMJOU; GOLIZADEH, 2010). Palomares-Pérez et al. (2020) demonstrated that *C. externa* are able to complete its life cycle when larvae are fed exclusively on *M. sacchari* (Zehntner) (Homoptera: Aphididae), what shows the value of these grass aphids as an alternative prey. Therefore, this study tested the nutritional value of honeydew from *Melanaphis* sp. on sorghum for the green lacewing *C. externa*.

Sorghum can produce more than 20 million grains per panicle (EMBRAPA, 2015) and thus it can also serve as a pollen source for *C. externa* adults. In different agroecosystems, Medeiros et al. (2010) and Andrade et al. (2017) found that more than 90% of the pollen grains in the digestive tract of *C. externa* adults were from grasses. In addition of being a plant of economic interest, sorghum also has the potential to be an ally in the establishment of natural enemies in the field. Due to the potential to sustain aphid populations harmless to non-grass crops and to the abundant pollen production, sorghum was chosen to be included in this work.

To assess whether honeydew of *Melanaphis* sp. favors the establishment of *C. externa* populations, the following two hypothesis were tested: (1) the survival of *C. externa* adults in the presence of aphids excreting honeydew is greater than in the absence of aphids; (2) *C. externa* are able to lay viable eggs when in the presence of aphids excreting honeydew.

To assess the value of sorghum pollen as a food resource, the following hypotheses were proposed: (3) the survival of *C. externa* is greater when sorghum pollen is offered than when pollen is absent; (4) *C. externa* oviposits viable eggs when sorghum pollen is offered. To test the value of pollen and honeydew together, two new hypotheses were made: (5) the survival of *C. externa* is greater when sorghum and honeydew pollen are offered together than when only one of these resources is offered; (6) *C. externa* oviposits in greater numbers when sorghum and honeydew pollen are offered together than when only one of these resources is offered.

The objectives of this work were to study the nutritional value of sorghum pollen and of honeydew from *Melanaphis*. sp. on sorghum for *C. externa* by assessing biological parameters of its adults.

2. MATERIALS AND METHODS

The experiments were carried out in a greenhouse at the Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), in Viçosa, MG, Brazil, between July, 2021 and February, 2022. Two experiments were made, with similar methodolodies.

2.1. Plants and insects

Three seeds of Grain sorghum (*S. bicolor*) were sown in pine bark substrate of the brand Mec Plant (Mec Plant Agrícola LTDA., Telêmaco Borba, PR, Brazil) in 800 mL pots around 14 days before the introduction of the aphids and lacewings in the repetitions cages. The cages for the experiments units were made with 2L transparent PET bottles, which had its bases and tops removed to obtain cylindrical shapes with opened ends. Each repetition arena consisted of fitting the adapted PET bottle onto the vase, surrounding the plants, and covered with a thin porous fabric tied with elastics (Figure 1). For the maitenance of each repetition arena, when sorghum plants were growing close to the top of the cage, three new seeds were sown and when germinated, the old plants were removed.

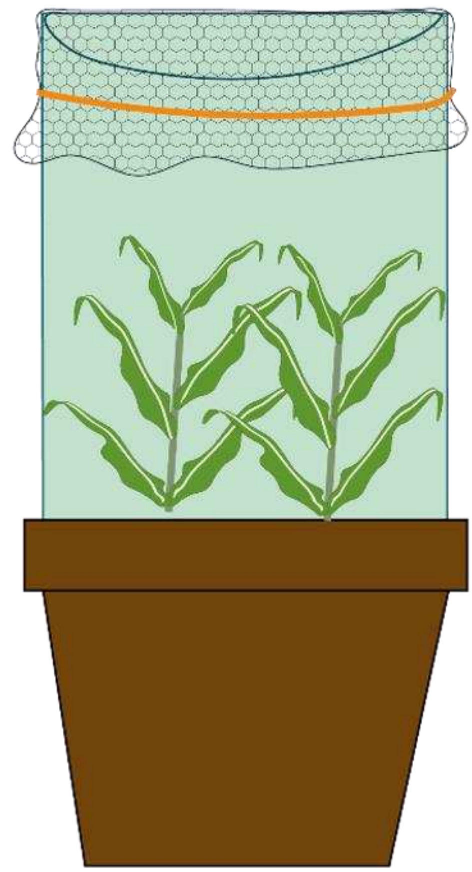

Figure 1. Illustration representing the arenas of the experimental units, with the porous fabric covering the adapted PET bottle fitted onto the pot with the sorghum plants.

To obtain the aphid *Melanaphis* sp., individuals were collected from sorghum plants at Vale da Agronomia at Universidade Federal de Viçosa (Viçosa, MG, Brazil). A rearing was established at the EPAMIG greenhouse in potted sorghums and kept in an acrylic cage of 45 cm width x 65 cm length x 60 cm height, for maintenance of the experiment, when needed. Sorghum pollen grains were also obtained from Vale da Agronomia, where anthers were collected and kept in an ultrafreezer at -80ºC until the beginning of the experiment.

The individuals of *C. externa* used were obtained from the rearing of the EPAMIG entomology laboratory (Viçosa, MG, Brazil), where adults are kept in a room at 25 ± 2ºC, 70 ± 10% RH and 12h photophase in PVC tube cages (Ø 15 cm x 15 cm height), with a diet based on brewer's yeast and honey in a 1:1 ratio (CARVALHO; SOUZA, 2009). The adults used in the experiments were obtained from eggs collected from the rearing, individualized in small cylindrical pots (Ø 2cm x 4cm high) and kept in the same room. After hatch, these individualized larvae were fed twice a week with abundant supply of eggs of *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) until their pupation. Newly emerged adults had their sex identified, were grouped into couples and then were used in the experiments.

2.2. Experiment with Honeydew

The first experiment consisted in two treatments. Each experimental unit was represented by one pot with a PET cage. In the first treatment, each repetition was consisted of a cage with three sorghum plants, with around 10 cm high, infested by 50 *Melanaphis* aphids, while control-group units were composed of sorghum plants of same height without aphids. One newly emerged couple of *C. externa* adults was placed inside each repetition cage. The cages were arranged on benches at the greenhouse in a completely randomized design. Due to problems with predation of lacewings by ants, the pots were placed inside trays filled with water mixed with detergent. In each repetition, the aphids and lacewings couple were introduced into the cages on the same day. In total, there were 30 repetitions for each treatment. All repetitions had a piece of wet cotton inside the cage to make sure that water was available to the insects. The cotton pieces were replaced every week.

To assess survival of *C. externa* adults, cages were observed daily until the death of the last individual, recording the date of death of males and females separately. To assess oviposition rate (number of eggs per female) and egg viability, the methodology was based on the work of Venzon et al. (2006). Every four days, all eggs present in each repetition were counted, recorded and removed. Before this procedure, the lacewings were removed from the cage by sucking them with a mouth sucker made of plastic tubes and, after the process, returned. From the total number of eggs found, a sample of five eggs per repetition was randomly chosen and taken to the breeding room in the laboratory to be incubated and to have the viability evaluated.

Eggs from which larvae hatched were considered viable, while eggs from which no larvae hatched were considered non-viable.

2.3. Experiment with Pollen and Honeydew

As in the first experiment, the cages were placed on benches in the greenhouse in a completely randomized design and each experimental unit was represented by one cage with one couple of *C. externa* adults. Trays with water and detergent were used to isolate the pots of each repetition to protect them from ant predation.

The experiment consisted of four treatments. The control-group consisted of three sorghum plants in the PET cage arena, with no aphids nor sorghum pollen. Treatment 1 consisted of three sorghum plants infested with *Melanaphis* aphids and no sorghum pollen. Treatment 2 consisted of three sorghum plants without aphids but with sorghum pollen supplied apart. Treatment 3 consisted of three sorghum plants infested with *Melanaphis* aphids and also sorghum pollen supplied apart. The sorghum plants were about 10 cm tall and the sorghum anthers with pollen grains were available *ad libitum* in a 2 cm diameter plastic cap at the bottom of the cage and replaced twice a week. A total of 10 repetitions per treatment were performed. All of the repetitions had also a piece of wet cotton to provide water to the insects, which was replaced once a week. The aphids and pollen were introduced into the cages on the same day the *C. externa* individuals were introduced.

Survival, oviposition rate and viability of eggs were evaluated like in the first experiment. According to the studies of Palomares-Pérez et al. (2020) and Costa et al. (2012), oviposition per female (number of eggs/day) starts to decline consistently about 20 to 30 days after female emergence from the pupa. Therefore, due to the limited time in the schedule, the experiment was evaluated until the 41st day after the adult emergence, which was enough time to compare the treatments.

2.4. Statistical Analysis

A survival analysis with exponential distribution was used for the data, with the proportion of survivors being a variable response as a function of the explanatory variable time (days) and co-variable treatments (presence and absence of honeydew and/or pollen). An ANOVA was performed to assess whether there is a difference in survival between treatments. The Akaike Information Criterion (AIC) was used to assess whether the model as a function of treatments and interaction of treatments was better explanatory than the null model. The Tukey test of the “emmeans” package was used for pairwise comparison (LENGTH et al., 2021). For the analysis of number of eggs per female, a Generalized Linear Model (GLM) with Poisson distribution was performed. Then, an ANOVA followed by a Tukey test in the “emmeans” package tested the difference between treatments (LENGTH et al., 2021). Viability of eggs was compared by an ANOVA. The analysis were performed in the R statistical program (R Core Team, 2019) with $p < 0.05$.

3. RESULTS

3.1. Adult fitness of C. externa as affected by Melanaphis sp. honeydew

Females of *C. externa* survived longer in the presence of honeydew than in the absence ($z = 6.8$, $df = 1$, $p < 0.05$) (Figure 2) and males also survived longer in the presence of honeydew than in the absence ($z = 9$, $df = 1$, $p < 0.05$) (Figure 3). Females and males that did not have access to honeydew survived for an average of 10.4 ± 8.3 days and 5.9 ± 4.3 days (Mean ± SD), respectively, while females and males that did have access to honeydew survived for an average of 68.4 ± 13.5 days and 71.4 ± 18.7 days, respectively. The AIC demonstrated that the survival model as function of the treatment is better than the null model.

The oviposition rates differed significantly between the treatments ($t = 7.6$, $df = 1$, $p < 0.05$). *Chrysoperla externa* females did oviposit when aphids were present, while the females that did not have access to a honeydew source did not oviposit (Figure 4). The mean number of eggs oviposited by females with access to honeydew was 38.3 ± 22.6 and the viability of eggs was 77.3 ± 10.5%.

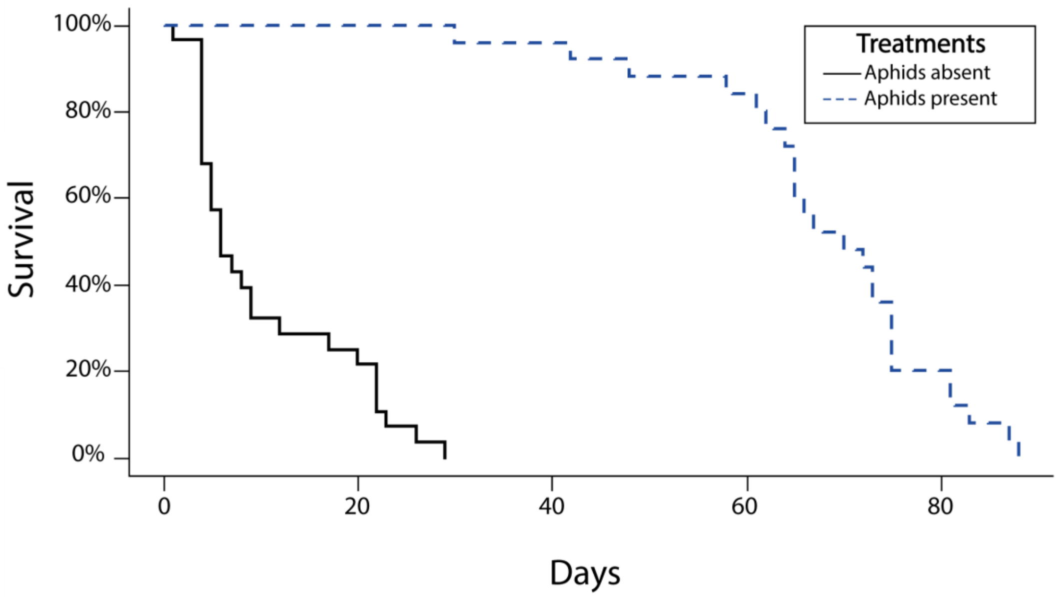

Figure 2. Survival curves of females of *Chrysoperla externa* in the presence and absence of the aphids *Melanaphis* sp. on *Sorghum bicolor*. Survival was different between treatments.

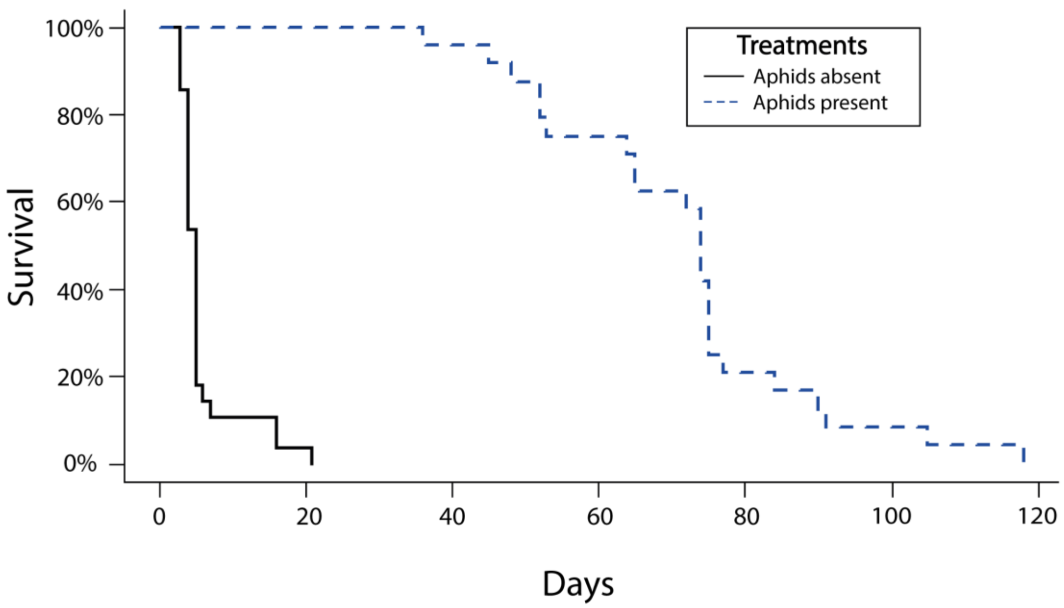

Figure 3. Survival curves of males of *Chrysoperla externa* in the presence and absence of the aphids *Melanaphis* sp. on *Sorghum bicolor*. Survival was different between treatments.

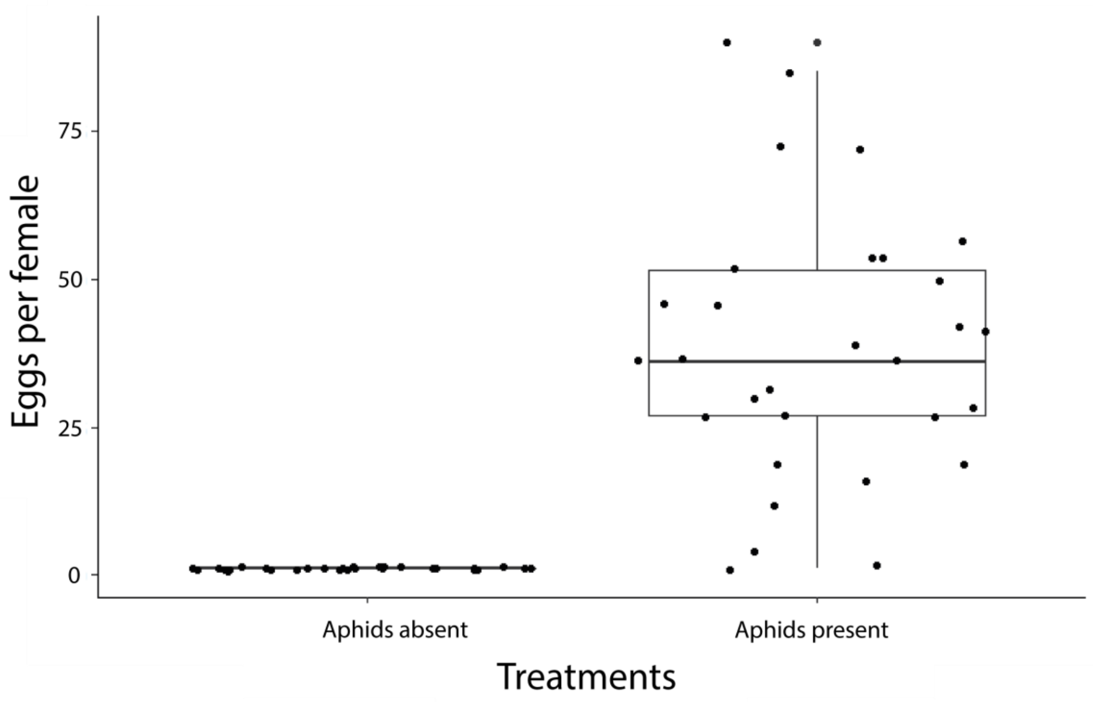

Figure 4. Number of eggs per female of *Chrysoperla externa* in the presence and absence of the aphids *Melanaphis* sp. on *Sorghum bicolor*. The number of eggs differed between treatments.

3.2. Adult fitness of *C. externa* as affected by *Melanaphis* sp. honeydew plus sorghum pollen

The different diets available influenced the survival of *C. externa* females ($\chi^2 = 42.9$, df = 3, $p < 0.05$) and males ($\chi^2 = 27.1$, df = 3, $p < 0.05$) (Figures 5 and 6). The survival of females with no access to the two food resources did not differ from the females with access to only pollen (t = -0.4, df = 3, p = 0.98) but did differ from females with access to only honeydew (t = -4.8, df = 3, $p < 0.05$) and from females with access to honeydew and pollen (t = -4.9, df = 3, $p < 0.05$). Females with access to only honeydew survived longer than females with access to only pollen (t = 4.4, df = 3, $p < 0.05$) but it did not differ from females with access to honeydew and pollen (t = -0.5, df = 3, p = 0.96). Females with access to honeydew and pollen survived longer than females with access to only pollen (t = -4.5, df = 3, $p < 0.05$). The survival of males with no access to the two food resources did not differ from the males with access to only pollen (t = -0.6, df = 3, p = 0.94) but did differ from males with access to only honeydew (t = -3.3, df = 3, p = 0.01) and from males with access to honeydew and pollen ($t = -4.5$, df = 3, $p < 0.05$). Males with access to only honeydew survived longer than males with access to only pollen ($t = 2.8$, df = 3, $p = 0.04$) but it did not differ from males with access to honeydew and pollen ($t = -1.3$, df = 3, $p = 0.57$). Males with access to honeydew and pollen survived longer than males with access to only pollen ($t = -4$, df = 3, $p < 0.05$).

The survival of female *C. externa* was 3.9 ± 1.0 (Mean ± SD) days when there was no food and 4.7 ± 1.3 days when there was pollen but no aphids. Three out of the 10 females that had access to only honeydew were still alive by the 41st day after emergence, when the experiment ended. Five out of the 10 females that had access to both pollen and honeydew were still alive by the 41st day after emergence. The survival of male *C. externa* was 3.2 ± 0.6 days when there was no food, 4.1 ± 0.9 days when there was pollen but no aphids, 14.1 ± 14.8 days when there were aphids but no pollen and 23.1 ± 11.9 days when there were both pollen and aphids. The AIC demonstrated that the survival model as function of the presence of honeydew is the best explanatory model.

The oviposition rates differed significantly between treatments ($\chi^2 = 236.3$, df = 3, $p < 0.05$). Females of *C. externa* exposed to sorghum with aphids oviposited, while those exposed to plants without aphids and plants only with pollen did not. The total number of eggs per female did not differ from females exposed to only aphids to females exposed to aphids and sorghum pollen ($z = 1.7$, df = 3, $p = 0.31$) (Figure 7). The viability of eggs from females that had acess to only honeydew was 74.7 ± 22.9% and did not differ from those that had access to honeydew and pollen, which was 87.7 ± 9.7% ($t = -1.3$, df = 1, $p = 0.24$).

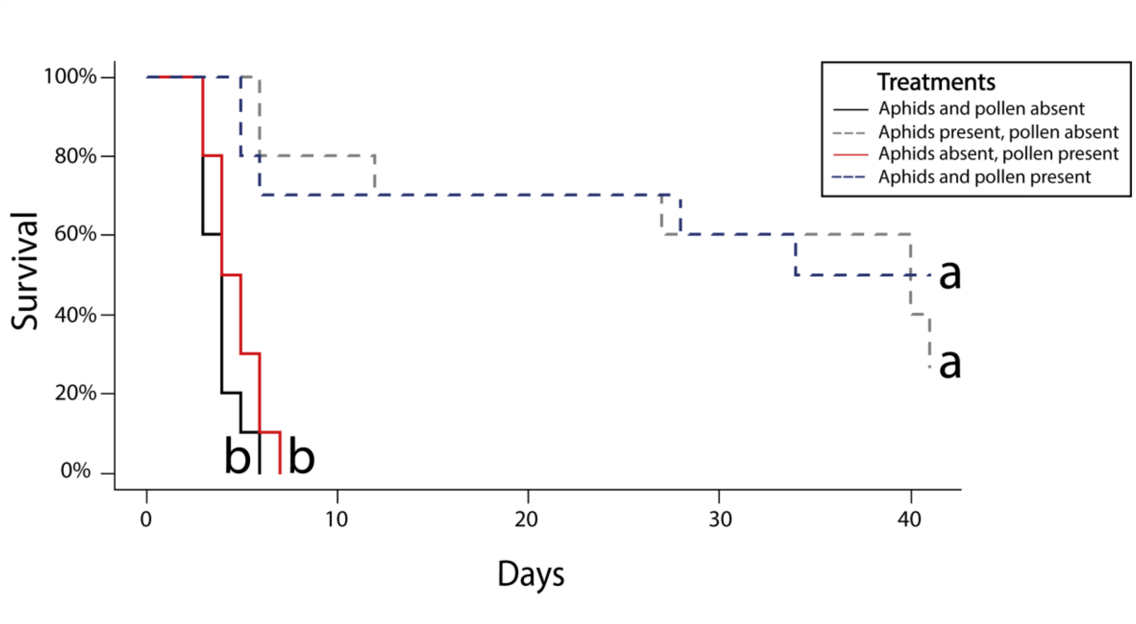

Figure 5. Survival curves of females of *Chrysoperla externa* in the presence and absence of pollen and the aphids *Melanaphis* sp. on *Sorghum bicolor*. Treatments with the same letter at the end of the curve did not differ.

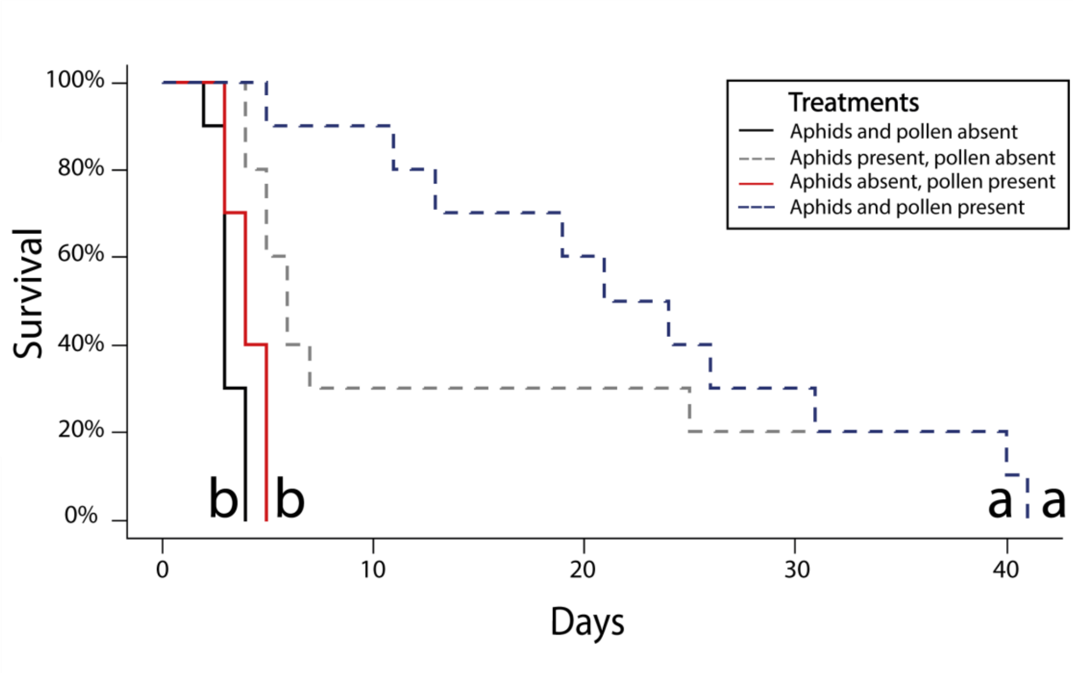

Figure 6. Survival curves of males of *Chrysoperla externa* in the presence and absence of pollen and the aphids *Melanaphis* sp. on *Sorghum bicolor*. Treatments with the same letter at the end of the curve did not differ by the test of Tukey.

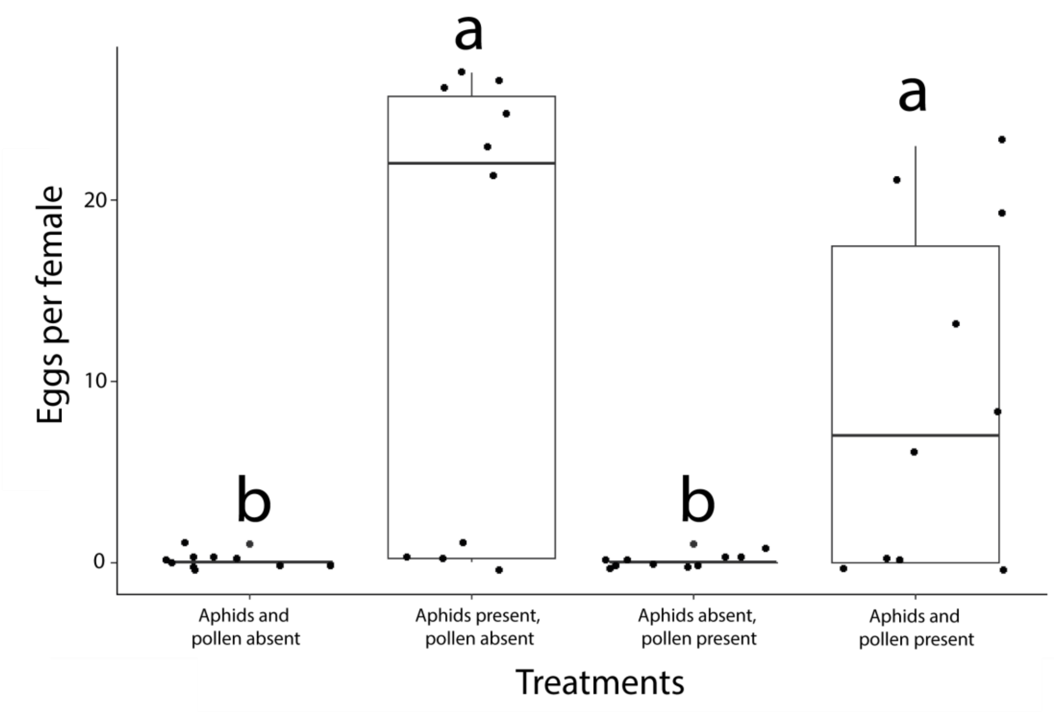

Figure 7. Number of eggs per female of *Chrysoperla externa* in the presence and absence of pollen and the aphids *Melanaphis* sp. on *Sorghum bicolor*. Treatments with the same letter did not differ.

4. DISCUSSION

Access to honeydew from *Melanaphis* sp. on sorghum allows the oviposition of viable eggs by *C. externa* and increases the longevity of its adults compared to individuals without access to this substance. Individuals deprived of honeydew survived less time and did not lay eggs. Pollen of sorghum did not influence survival or oviposition of *C. externa*.

The presence of sugars and amino acids is a general characteristic of honeydew but the composition of this substance is influenced by the species and nutrition of the host plant, species of the substance-producing hemipteran, species of symbiotic bacteria in the hemipteran cells and even the occurrence of mutualism with ants (SABRI et al., 2013). Venzon et al. (2006) demonstrated that females of *C. externa* fed only on honey did not oviposit, but when the diet was supplemented with pollens from different plant species, oviposition occurred. These results showed that it is essential that a protein source takes part in the diet of *C. externa* for reproduction and development to be possible. The oviposition and survival were not constant in the two experiments, these attributes were lower in the second one and we observed a vast number of deaths within the first week after emergence of every treatment of the second experiment but we could not detect any factor responsible for that. The two experiments allow us to conclude that the honeydew from *Melanaphis* sp. feeding on sorghum is a balanced food. This honeydew allows *C. externa* to reproduce and complete its life cycle and probably to establish a population in the field. Although, it is interesting to have more food resources available because when we compare with other works like the one by Venzon et al. (2006), we can see that oviposition might be much higher when multiple food resources compose a diet for *C. externa*.

Sabri et al. (2013) studied the aphid *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) in fava beans (*Vicia faba* L.) and found that honeydew, in addition to sugars and amino acids, also has a high diversity and abundance of proteins. According to these authors, these proteins come mainly from the renewal of tissues in the aphids digestive system and elimination of degraded tissue (43.8%), and from the gut symbiotic bacterial flora (22.7%). We believe that not only *M. sacchari* honeydew is a source of nutrients, but honeydew in general.

In addition to the nutritional benefits, honeydew can also work as an attractant for natural enemies, what is a very important attribute in CBC. Studies have registered honeydew volatiles attracting different natural enemies, such as lady beetles (LEROY et al., 2012) and parasitoids (MEHRNEJAD; COPLAND, 2006). The bacteria present in honeydew also have a role on producing volatiles that attract biological control agents (LEROY et al., 2011; FAND et al., 2020). Honeydew can also work as an ovipositional stimulant (LEROY et al., 2011; BUDENBERG; POWELL, 1992).

The excretion of honeydew is not the only benefit on having aphids in the agroecossystem. Aphids are prey of many generalist and specialist predators, including *C. externa* larvae (CARVALHO; SOUZA, 2009), lady beetles (MICHAUD, 2012), syrphids (TENHUMBERG; POEHLING, 1995; HOPPER et al., 2011) and many species of parasitoids (BOIVIN et al., 2012). This mean that when other preys are scarce, the presence of aphids can serve as alternative prey to maintain populations of biological control agents established in the field. Companion plants as aphid hosts make the agroecosystem more prone and favorable for the establishment of biological control agents (e.g., *C. externa*), increasing food availability (honeydew and alternative preys) and even working as attractants for predators. Also, the presence of aphids in seasons that the main crop is not in bloom means a source of food in times that floral resources are scarce.

However, we need to be aware that the presence of aphids in the field can mean a threat to the main crop in many cases, causing direct damage and acting as vectors of viral diseases. Although, the presence of these phytophagous, when previously planned, can be positive for maintaining the availability of nutritional resources for beneficial insects. For that, it is very important that aphids feed specifically on the companion plants, not attacking the main crop, otherwise they will become the pests themselves. This is an initial criterion, as well as adding only plants that will not benefit the crop pests with the resources provided (VENZON et al., 2019b).

Many cultures may benefit from the use of sorghum as companion plant for CBC, once that sorghum herbivores are hosted mostly by grasses (YOUNG; TEETES, 1977). For instance, *C. externa*, as we demonstrated, are organisms that can benefit on aphids on sorghum and they are oftenly present in coffee crops, being ecologically important for controlling the culture main pests (BOTTI et al., 2021; VENZON, 2021). Because of that, we suggest that coffee crops are strong candidates to benefit from intercropping with sorghum and field tests would be propitious.

Despite the results that did not sugest sorghum pollen as a suitable food source, we believe that the results of the works by Andrade et al. (2017) and Medeiros et al. (2010), which demonstrate the predominance of grass pollens in the digestive tract of *C. externa*, are strong premises to demonstrate the importance of these pollens in the diet of these insects. Andrade (2013) showed that when pollen of another sorghum species (*S. halepense* L. (Pers.)) was offered in addition to honey, *C. externa* oviposited viable eggs and had prolonged survival, but when only pollen was offered, there was no oviposition and survival was lower. There are no records about antinutritional factors of *S. bicolor* pollen and there are some records about other beneficial insects feeding on it, such as honey bees (SIEDE et al., 2021) and a syrphid species (NUNES-SILVA et al., 2010). Possibly, the individuals of our experiment were not able to feed on pollen due to the way it was offered, in the anthers of the flowers. Another possibility is that this pollen should pass through a drying process before being stored in the ultrafreezer, like in the methodology of Rodríguez-Cruz et al. (2013).

During pollen collections, we noticed several arthropods present in sorghum inflorescences such as spiders, coleopterans and hymenopterans. We believe that other species of beneficial organisms may feed on this resource and we suggest further studies with this plant and pollen.

Conservation Biological Control is gaining ground in the last decades (PEÑALVER-CRUZ et al., 2019), once that agriculture is looking for alternative sustainable pathways. To discover new companion plants and the ways it can contribute to beneficial insects and crop production is the essence of these science. We discovered an interesting potential use of *Sorghum bicolor* for agriculture as companion plant by hosting aphids. Other plants also have the potential of hosting aphids and should be studied. The presence of honeydew and aphids benefit not only *Chrysoperla externa* but also other natural enemies, such as parasitoids (LEE et al., 2004; BOIVIN et al., 2012), syrphids (LEROY et al., 2011; TENHUMBERG; POEHLING, 1995; HOPPER et al., 2011) and lady beetles (MICHAUD, 2012; LEROY et al., 2012). Honeydew can be seen as more than just waste and aphids can be seen as beneficial organisms instead of pests but it will depend on CBC strategies.

CONCLUSIONS

Honeydew, the excreta of aphids, allowed the reproduction and increased survival of adult *C. externa*, proving to be a resource that meets the nutritional requirements of this species. The presence of the aphid *Melanaphis* sp. is sufficient for *C. externa* to complete the entire life cycle, as larvae can feed on them. These results allow us to conclude that strategies including the use of aphid-hosting companion plants can be useful for crop protection.

Pollen of *S. bicolor* did not influence the survival or reproduction of adult *C. externa* when offered alone or with honeydew. These results show that either sorghum pollen does not meet the nutritional requirements of adult *C. externa* or that the pollen grains should be supplied in a different way than we did. Despite these results, during the collection of pollen, several arthropods were observed in the inflorescences of sorghum plants, which may indicate that other organisms of interest may benefit from this resource. We suggest further studies on the organisms that frequent sorghum panicles.

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