University of São Paulo
"Luiz de Queiroz" College of Agriculture

Environmental management strategies for pest control in strawberry crop

Fernanda de Cássia Neves Esteca

Thesis presented to obtain the degree of Doctor in Science. Area: Entomology

Piracicaba
2021

Fernanda de Cássia Neves Esteca
Agronomic Engineer

Environmental management strategies for pest control in strawberry crop

Advisor:
Prof. Dr. **GILBERTO JOSÉ DE MORAES**

Thesis presented to obtain the degree of Doctor in Science. Area: Entomology

Piracicaba
2021

Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP

Esteca, Fernanda de Cássia Neves

Environmental management strategies for pest control in strawberry crop / Fernanda de Cássia Neves Esteca - - Piracicaba, 2021.

179 p.

Tese (Doutorado) - - USP / Escola Superior de Agricultura “Luiz de Queiroz”.

1. Drosófila-da-asa-manchada 2. Ácaro-rajado 3. Ácaros predadores edáficos 4. Fungo entomopatogênico 5. Controle biológico 6. Mulching I. Título

This work is especially dedicated to

God

My parents

João Luiz Esteca and Angela Tereza de Oliveira Neves Esteca

My brother

Antonio Marcos Neves Esteca

My nephews

Yago Esteca Albuquerque Saraiva and Sophia Esteca Carvalho

My goddaughters

Mariana Baccan Esteca and Letícia Baccan Esteca

My fiancé

Andre Ricardo Machi

My dog

Belinha

To Prof. Dr. Gilberto José de Moraes

For his valuable guidance, friendship and recognition of my work.

Special thanks

ACKNOWLEDGEMENTS

To God for allowing all this to happen, always granting me health, peace, protection and helping me in the most difficult hours, not only in University life, but in all moments of my existence.

To the "Luiz de Queiroz" College of Agriculture, University of São Paulo (USP), and to all teachers who helped me during the undergraduate and graduated courses, for the necessary support to carry out all works.

To my parents, João Luiz Esteca and Angela Tereza de Oliveira Neves Esteca, for always encouraging me to study, aiming at a quality education and above all always respecting others; without their encouragement, support and help I would never have made it this far.

To my brother, Antonio Marcos Neves Esteca, for all his teachings, collaboration, dedicated time and patience when teaching me various subjects during my undergraduate and graduate time. Thank you so much, my brother, for always wanting my best. To my sister, Ariane Neves Esteca.

To my nephews, Yago Esteca de Albuquerque Saraiva and Sophia Esteca de Carvalho, to my goddaughters, Mariana Baccan Esteca and Letícia Baccan Esteca, for making me happy and always trying to understand that the moments of my absence (dedicated to higher education) are for the future and that this is only done from the constant dedication in the present.

To my fiancé, Andre Ricardo Machi, for the attention, support, collaboration in various moments of this work and mainly always encouraging me to move forward in the troubled moments during my graduate program.

To my puppy, Belinha, for being my angel and my support, for calming me down, bringing me happiness, confidence and security to my life.

To my advisor, Prof. Dr. Gilberto José de Moraes, for always being available to solve doubts and problems that arose during the development of the thesis. There were many years of living together, and he helped me a lot to overcome my fears and try new challenges. I am very grateful.

To Dr. Ingeborg Klingen and Dr. Nina Trandem for having welcomed me so well and having guided me during my marvelous time in Norway. I can say that I have developed many skills under their guidance, both for academic life and for personal life. To Dr. Marit Helgheim at NIBIO for practical assistance in conducting the expriments in Norway.

To Prof. Dr. Italo Delalibera Júnior for the contact with the SMARTCROP project and for all the help and incentive provided for my work in Norway. I am very thankful.

To Prof. Dr. Carlos H. W. Flechtmann (ESALQ/USP) for his assistance in resolving doubts and providing material for the preparation of this work. Thank you very much professor.

To colleagues at the Laboratory of Acarology, ESALQ/ USP, who were with me during all or part of the period I was conducting my doctoral research and who always provided me full support, MSc. Elias Soares de Figueiredo, MSc. Mariana Yamada, MSc. Camila Nascimento Dainese, MSc. Camila Ferreria Amorim, Dr. Jandir C. Santos, Dr. Marcela Massaro da Silva, Marielle M. Berto, Dr. Márcia Daniela Santos, MSc. Eliamara Marques, Dr. Letícia H. de Azevedo, Dr. Marina F. C. Barbosa, Prof. Dr. Raphael de C. Castilho, MSc. Lina Marcella Gonzalez Cano, Dr. Sofia Jimenez Jorge, MSc. Vinicius Borges. To colleagues at NIBIO, who were with me in Norway, Marta Kanoo, Clara Romeu, Zarah Hallalae and Stéphanie Saussure.

Especially to Elias Soares de Figueiredo and his wife, Nancy Monteiro, who helped me kindly with practically all the field work, lots of advice and sincere friendship.

Especially too, to Mariana Yamada for her companionship and for making the environment happier in the difficult times that graduate education provides, in addition to helping with field work, data tabulation carried out in the south of Minas Gerais and revision of references.

To Prof. Dr. Sônia Maria De Stefano Piedade and Dr. José Bruno Malaquias who helped me with parts of the statistical analysis.

To the growers Adilson Domingues, Caio Coluci, Cleone dos Santos, Cláudio Santos, Daniel Franco, Ademir Amâncio for providing me part of their crops to conduct field experiments in southern Minas Gerais and in Atibaia.

To my great collaborators Ana Clara Arantes Villas Bôas de Barros, Murilo Prudente Ferreira, Matheus Fernandes Pereira Moreira, Jessica de Campos, Ivan Valerio, Tiago Casaroto and Gabriela Silva de Almeida who assisted me during part of several experiments. Especially to Ana Clara, Murilo, Matheus and Tiago who at many times were at my side supporting in various field and laboratory works.

To Dr. Jandir C. Santos for helping me identify several mesostigmatid mites; to Prof. Carlos Ribeiro Vilela at Instituto de Biociências USP for help in identifying *Drosophila suzukii* and *Zaprionus indianus*. To Prof. Sinval Silveira Neto and Dr. Élison Fabrício Bezerra Lima for confirming the identification of the insects.

To the laboratory technician Solange Aparecida Vieira Barros for the support in conducting all the works related to fungi. To the field technician, Josenilton Mandro, who contributed by supporting the logistics and development of this study. To the laboratory technician, Lásaro Silva (*in memoriam*), for his support in logistics during the conduction of the field work in southern Minas Gerais.

EPIGRAPH

“Joy lies in the fight, in the attempt, in the suffering involved, not in the victory itself”.

Mahatma Gandhi

“It is the mark of an educated mind to be able to entertain a thought without accepting it.”

Aristóteles

SUMMARY

RESUMO

Estratégias de manejo ambiental para o controle de pragas na cultura do morangueiro

O morango (*Fragaria* x *ananassa* Duch.) é produzido principalmente no sul de Minas Gerais e áreas adjacentes de São Paulo, sendo suscetível a muitas pragas, como por exemplo, a *Drosophila suzukii* (Matsumura, 1931). Estudos recentes com o fungo *Metarhizium humberi* Luz et al. (Hypocreales: Clavicipitaceae) (ESALQ 1638) têm indicado esta espécie como fungo promissor para o controle de pragas. A cobertura de solo é uma prática importante no cultivo do morango. Muitos produtores utilizam polietileno como mulching, porém este é caro e não biodegradável, sendo necessário buscar materiais orgânicos, como feito em outras partes do mundo. Os objetivos desse trabalho foram avaliar: a) a dinâmica populacional de drosofilídeos em morangueiro; b) o potencial de predação, oviposição e sobrevivência de ácaros Gamasina quando oferecidos diferentes estágios de *D. suzukii* como presa*;* c) o efeito da inoculação, via sistema de irrigação, do *M. humberi* na cultura do morangueiro; d) a composição da fauna de ácaros e a variação da densidade destes na palhada de café e em fragmentos florestais no sul de Minas Gerias; e) o efeito do uso de palhada de café no microclima do morangueiro, na qualidade dos pseudofrutos e a habilidade de Gamasina edáficos, que sobe nas plantas de morango à noite, em predar *Tetranychus urticae* (Koch, Tetranychidae); f) diversidade e prevalência de ácaros em palha de cereal submetida a diferentes períodos de exposição no cultivo morangueiro, na Noruega, avaliando a movimentação durante a noite do habitat edáfico para os folíolos. Os resultados mostraram que *D. suzukii* e *Zaprionus indianus* Gupta foram encontrados no sul de Minas Gerais em morango, mas não parecem causar perdas significativas aos produtores locais. Alguns dos predadores apresentam capacidade de predar e ovipositar em *D. suzukii*. *Metarhizium humberi* foi recuperado em amostras de substrato de plantio, raiz, pecíolo e folíolo de plantas inoculadas. Em alguns dos meses, as plantas inoculadas apresentaram número médio estatisticamente inferior: de tripes/ flor, do ácaro-rajado/ folíolos e flor-preta/ planta, enquanto houve maior número médio de Phytoseiidae/ folíolos Ácaros predadores foram encontrados em números maiores na palhada de café adquirida no início do ano A manutenção da palhada de café no solo do fragmento florestal antes do uso como mulching promove a incorporação dos ácaros predadores edáficos na lavoura de morangueiro, mantém um microclima próximo às plantas favorável ao desenvolvimento de ácaros predadores e prejudicial ao ácaro-rajado, além de produzir pseudofrutos mais pesados e mais doces. Ácaros predadores edáficos podem mover-se para plantas de morango à noite no Brasil e na Noruega. Pelo menos três dos predadores edáficos avaliados foram capazes de predar e ovipositar quando oferecidos ovos de *T. urticae* como presa. O estudo mostrou que o uso da palha de café como mulching e a aplicação de *M. humberi* em plantas de morango podem melhorar a produção de morango.

Palavras-chave: Drosófila-da-asa-manchada, Ácaro-rajado, Ácaros predadores edáficos, Fungo entomopatogênico, Controle biológico, Mulching

ABSTRACT

Environmental management strategies for pest control in strawberry crop

Strawberry (*Fragaria* x *ananassa* Duch.) is produced mainly in the south of Minas Gerais and adjacent areas of São Paulo state, being susceptible to many pests, such as *Tetranychus urticae* (Koch, Tetranychidae) and *Drosophila suzukii* (Matsumura, 1931). Recent studies with *Metarhizium humberi* Luz et al. (Hypocreales: Claviccipitaceae) (ESALQ 1638) have indicated this species as a promising fungus for pest control. Mulching is an important practice in strawberry cultivation. Many growers use polyethylene as mulching, but this is expensive and non-biodegradable, making it necessary to look for organic materials, as done in other parts of the world. The objectives of this work were to evaluate: a) the population dynamics of drosophilids in strawberry; b) the predation, oviposition and survival potential of Gamasina mites when offered different stages of *D. suzukii* as prey; c) the effect of inoculation, via irrigation system, of *M. humberi* in strawberry crop; d) the composition of the mite fauna and the variation in their density in coffee husk and pulp and in forest fragments in southern Minas Gerias; e) the effect of using coffee husk and pulp on strawberry microclimate, on the quality of the pseudofruits and on the ability of edaphic Gamasina, which climb the strawberry plants at night, to prey on *T. urticae;* f) the diversity and prevalence of mites in cereal straw subjected to different periods of exposure in strawberry crops in Norway, evaluating overnight movement from edaphic habitat to leaflets. The results showed that *D. suzukii* and *Zaprionus indianus* Gupta are presently found in southern Minas Gerais on strawberry, but that they do not seem to cause significant losses to local growers. Some of the predators were able to attack and oviposit when fed with *D. suzukii*. *Metarhizium humberi* was recovered from samples of planting substrate, roots, petioles and leaflets of inoculated plants. In some months, the inoculated plants had a statistically lower mean number of the thrips/flower, of the two-spotted spider mite/leaflet and black flower/plant, while there was a higher mean number of Phytoseiidae/leaflet. Predatory mites are found in higher numbers in coffee husk and pulp acquired at the beginning of the year. Maintenance of coffee husk and pulp on the forest fragment floor before using as mulching promoted the incorporation of the edaphic predatory mites in the strawberry field, maintained a microclimate close to the strawberry plants favorable to the development of predatory mites and harmful to the two-spotted spider mites and result in the production of heavier and sweeter strawberries. Edaphic predatory mites may move to strawberry plants at nighttime in Brazil and in Norway. At least three of the evaluated edaphic predator were able to attack and oviposit when offered *T. urticae* eggs as prey. The study showed that the use of coffee husk and pulp as mulching and the application of *M. humberi* on strawberry plants can improve strawberry production.

Keywords: Spotted-wing drosophila, Two-spotted spider mite, Edaphic predatory mites, Entomopathogenic fungi, Biological control, Mulching

1. GENERAL INTRODUCTION

In Brazil, the strawberry crop (*Fragaria* x *ananassa* Duch.) is localize in temperate and subtropical climate regions. It is estimated that Brazilian production is around 165,000 tons in an area of 4,500 hectares (FAOSTAT 2021). About 50% of the strawberry consumed in Brazil is produced in the south of Minas Gerais, where approximately 82 thousand tons are produced annually (Antunes et al. 2020). In contrast to this promising scenario, pests and diseases are considered serious problems for the crop.

The two-spotted spider mite (*Tetranychus urticae* Koch, Acari: Tetranychidae) has traditionally been considered one of its main pests in Brazil and elsewhere (Solomon et al. 2001). Additionally, several other pests are considered important to the crop worldwide, such as western flower thrips (*Frankliniella occidentalis* (Pergande), Thysanoptera: Thripidae); aphids (*Chaetosiphon fragaefolli* (Cockerell), Hemiptera: Aphididae), whiteflies (*Trialeurodes vaporariorum* (Westwood), Hemiptera: Aleyrodidae), bugs (*Neopamera bilobata* (Say), Hemiptera: Rhyparochromidae), flies (*Drosophila suzukii* (Matsumura), Diptera: Drosophilidae), and beetles (*Maecolaspis* sp., reported as *Colaspis* sp., Coleoptera, Chrysomelidae-Eumolpinae) (Kuhn et al. 2014, Bernardi et al. 2015, Andreazza et al. 2016, Dara 2016, Montagnana et al. 2017). The African-fig-fly, *Zaprionus indianus* Gupta is a primary pest of figs and a secondary pest of more than 70 other fruit species, always attacking preferably rotting fruits (Joshi et al. 2014), for the difficulty of females to oviposit in undamaged fruits (Fartyal et al. 2014).

Gray mold, *Botrytis cinerea* (Persoon: Fries), is the main fungus associated with fruits under field and postharvest conditions (Barakat and Al-Masri 2017). Micosferela leaf spot (*Mycosphaerella fragariae* (Tul.) Lindau) and dendrophoma (*Dendrophoma obscurans* (Ell & Ev.) HW Anderson) are the main foliar diseases and can be found in all regions where the crop is grown (Ueno et al. 2014). Anthracnose (*Colletotrichum acutatum* Simmonds) is one of the most important and destructive strawberry diseases in all production regions in Brazil (Dias et al. 2007; Ueno et al. 2014). Angular spot, also known as "bacterial spot", (*Xanthomonas fragariae* Kennedy and King), can cause high losses, especially where sprinkler irrigation is used (Tanaka et al. 2005; Ueno et al. 2014).

Due to the high demand of the market for pesticide-free fruits, as well as the enormous problems that the indiscriminate use of chemical products has generated, the need to integrate other management methods, especially biological control, increasingly arises. In Brazil, predatory mites of the Phytoseiidae and Laelapidae family have been marketed to control several pests, such as phytophagous mites and edaphic pests, respectively. In Europe, mites of the Macrochelidae family are already commercialized for the control of flies and thrips (Azevedo et al. 2015). Furthermore, literature data suggest that predatory mites are potentially useful for the control of drosophilids (Houck 1994).

Entomopathogenic fungi have a global distribution, wide host range, and often the ability to regulate insect populations. *Metarhizium anisopliae* (Metschn.) Sorokin and *Beauveria bassiana* (Bals.) Vuill are currently being used as commercial biocontrol agents. Moreover, these entomopathogens can be plant growth promoters, which increase the productivity of many crops (Machado et al. 2012). *Metarhizium humberi* Luz et al. (ESALQ 1638) was collected from soil for the first time in 2001 in Goiás state (Luz et al. 2019). Evaluations of this fungus since then have indicated it as a promising species for pest control (Siqueira et al. 2020).

In strawberry crop management, another relevant aspect refers to the use of soil cover (mulching), made among other objectives, to reduce the development of spontaneous plants. Nowadays, the mulching in the south of Minas Gerais is made exclusively with the use of polyethylene films. The use of organic cover can bring recognized benefits, such as improved humidity retention inside the tunnels and in the soil (Resende et al. 2005) and conservation of soil microbiota (increasing beneficial soil organisms such as fungi and predatory mites) (Brust 1994). Some authors have already reported that the use of organic mulching can increase the abundance and diversity of predatory mites and/or promote a reduction in the pest population in different crops (Hoddle et al. 2002; Jamieson and Stevens 2006).

In Norway, about half of the strawberry production area uses cereal straw mulching, while in the rest of the area straw is combined with a black plastic film below it (Haslestad 2020). Strawberry is an important horticultural crop in Norway, accounting for 70% of the country's soft-skinned fruit production yield (Vi Spiser 2020). In 2019, around 9,000 tonnes of strawberries were produced by Norwegian farmers, with revenue of around NOK 455 million (US$45.5 million) (Statistics Norway, 2020). Most strawberry production is done in open fields, where the plants are harvested for 2-3 years. The most common pests in strawberry cultivation in Norway are: two-spotted spider mite, the tarsonemid mite, *Phytonemus pallidus* (Banks) (Acari: Tarsonemidae); two weevils, *Anthonomus rubi* Herbst. (Coleoptera: Curculionidae) and *Otiorhynchus sulcatus* F. (Coleoptera: Curculionidae); and a capsid, *Lygus rugulipennis* Poppins (Hemiptera: Miridae) (Trandem 2003; Klingen et al. 2015; Aasen and Trandem 2006).

Assessing this situation and the need generated from it, the hypotheses of this work were: i) that in strawberry cultivation in southern of Minas Gerais, *D. suzukii* and *Z. indianus* would only be found at low levels; ii) some Gamasina mites found in southern Minas Gerais are able to attack and reproduce on immature stages of the spotted-wing drosophila; iii) the application of the ESALQ 1638 isolate of *M. humberi* in strawberry cultivation leads to its colonization of the growing substrate and plants (endophytically), reducing the occurrence of pests and pathogens and increasing productivity; iv) the mites are present in the coffee husk and pulp in density and specific composition that vary along the year, including no detrimental mite species; v) that representative forest fragments in southern Minas Gerais have variable diversity and density of Gamasina, some of which may contribute to pest control; vi) the maintenance of the organic material (coffee husk and pulp) destined for later use as mulching on the floor of a forest fragment for a given period allows local predatory mites to move into it, what could be considered "an enrichment" of that material; vii) organic mulching causes changes in the microclimate close to the plants, favoring the presence of predatory mites and/ or disfavoring the presence of the two-spotted spider mite; viii) mulching with coffee husk and pulp influences the quality of strawberry pseudofruits; ix) predators that climb the plants at night can act as biological control agents of the two-spotted spider mite, which is considered important as management of mulching may directly impact the edaphic mites; x) species richness and abundance of predatory mites (especially Gamasina) in organic material increases with the time the matter has been exposed on the soil surface in the strawberry field in Norway; and mites present in the organic mulching at daytime climb to strawberry plants at nighttime in potting experiments in climatic chambers.

The objectives of this thesis were:

- To evaluate of *D. suzukii* and *Z. indianus* population dynamics in strawberry grown under two cultivation systems commonly adopted in the region, suspended and in low tunnels.
- To study the ability of Gamasina found in southern Minas gerais to attack and reproduce on immature stages of the spotted-wing drosophila.
- To determine whether the inoculation of ESALQ 1638 isolate of *M. humberi* in strawberry cultivation leads to its colonization of the growing substrate and plants (endophytically), reducing the occurrence of pests and pathogens and increasing productivity.

- To evaluate the composition of the mite fauna and variation in their density in coffee husk and pulp recently acquired along the year in southern Minas Gerais and the composition of the mite fauna in four forest fragments located in the municipality of Senador Amaral and Bom Repouso, within the strawberry growing areas of southern Minas Gerais.
- To analyze: a) whether the use of coffee husk and pulp as mulching could lead to the introduction of harmful mites for strawberry crop; b) if the maintenance of this material in a forest fragment would allow the movement of predatory mites to this material. Furthermore, to evaluate the effect of coffee husk and pulp on: c) populations of strawberry pest arthropods; d) temperature, relative humidity and dew point; e) the weight and Brix° of the pseudofruits and the macro and micronutrient levels of the strawberry leaflets. Finally, evaluate the ability of edaphic Gamasina, which climbs on plants at night, to prey on *T. urticae*.
- To compare the diversity and prevalence of mites in cereal straw subjected to different periods of exposure in the strawberry field, in Norway, evaluating their possible temporary movement from the edaphic habitat to strawberry plant leaves at nighttime.

1.1. Literature review

1.1.1. The problem generated by *Drosophila suzukii*

The Diptera of the Drosophilidae family include more than 4,000 species (Yassin 2013). These develop into rotten fruit and other decaying organic matter, but most of them are of no economic importance. Only the African-fig-fly, *Zaprionus indianus* Gupta (Diptera: Drosophilidae) and the spotted-wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), are considered pests, as they can attack fruits for human consumption before harvest (Van Timmeren and Isaacs 2013).

Drosophila suzukii is native to Japan, endemic to southwest Asia, but has been reported in America and Europe (Lee et al. 2011; Calabria et al. 2012; Deprá et al. 2014; Kinjo et al. 2014). Of special concern is the rate of global spread of this pest, as its first record outside Asia took place in Hawaii, in 1980, and is now spread across Europe and the continental region of the United States, where its occurrence was recorded in 2008, almost simultaneously in both regions (Cini et al. 2012). In 2013, spotted-wing drosophila was recorded for the first time in

South America (southern Brazil) (Deprá et al. 2014) and, in 2016, in the Viçosa region, in southern Minas Gerais, Brazil (Andreazza et al. 2016).

Considered a polyphagous pest, spotted-wing drosophila became a pest recently, causing severe damage to thin skin fruits (plum, blackberry, persimmon, cherry, apricot, raspberry, blueberry, strawberry and peach) (Cini et al. 2012), generating significant economic losses, since females are able to lay eggs on ripening fruits before harvesting. The larvae destroy the pulp, making the fruit unmarketable (Bolda et al. 2010). This species has a high reproduction rate and a short development period, and can theoretically have up to 13 generations per year, which may contribute to its rapid spread, given the high range of suitable hosts available (Cabi 2017). Although this pest is able to develop on multiple hosts, many studies report that strawberry is the preferred fruit (Arnó et al. 2012).

When no control method is adopted, economic losses can range from 60 to 100% (Grassi et al. 2011). In 2008, in California, Oregon and Washington, losses were estimated to be 20% for strawberries, 33% for cherries, 40% for blueberries and 50% for raspberries. Only in the California alone, the estimated reduction in gross income due to *D. suzukii* infestation, in the absence of management, was estimated to be 37% for raspberries and 20% for processed strawberries (Goodhue et al. 2011).

Producers in small fruit producing regions in the North American currently apply pesticides an average of 5-7 times per season, but with the invasion of *D. suzukii* there was an increase of 5 to 8 additional sprays of insecticides (Van Timmeren and Isaacs 2013). When taking into account current harvest levels, input costs, and losses generated by *D. suzukii* in Oregon range between US$12 and US$16 million (Julian et al. 2011). Chemical control of *D. suzukii* can also lead to the rejection of exported fruits due to residual pesticide levels that exceed maximum residue limits (Haviland and Beers 2012).

1.1.2. Morphological and biological aspects of *Drosophila suzukii*

The eggs are whitish and measure approximately 0.62 x 0.18 mm in length, have two filaments in the terminal portion, which are used for respiration. Newly hatched larvae are transparent. In general, they have 3 larval instars, and when they reach the last instar, they measure, on average, 3.9 x 0.9 mm. Initially, the pupa coloration is grayish-yellow with a soft consistency, later hardening and acquiring brown coloration. They also have two anterior extensions linked to breathing. (Walsh et al. 2011).

Morphologically, drosophilids cannot be identified to species in the immature stages, as they are all very similar; thus, specific identification depends on adult emergence or molecular biology techniques (Walsh et al. 2011). The spotted-wing drosophila presents some peculiarities that allow its easy identification. Adult males have a small dark spot at the apex of the wings, which refers to the popular name of this species. In addition, they have a row of bristles on the first and another on the second tarsal segment of the first pair of legs, known as the tarsal comb.

Females do not have wing spots, but have a narrow and double serrated ovipositor, with a series of sclerotized teeth, which allows them to be inserted into the fruit. This is six to seven times longer than the spermatheca, while in most *Drosophila* females this is only two to four times longer than the spermatheca, while the ovipositor is more fragile and without sclerotized teeth (Cini et al. 2012). The adult is small, between 2 and 3 mm in length, with red eyes, yellow to pale brown chest, and longitudinal black bands along the abdomen.

Like other drosophilids, spotted-wing drosophila has a high biotic potential. With favorable conditions of temperature and humidity, females reach sexual maturity in 1 or 2 days, with 13 days being the maximum recorded time. Oviposition begins on the second day after emergence of the adults, and can lay up to three eggs per fruit. Each female lays an average of 7 to 16 eggs per day, being able to lay up to 600 eggs in total (400 eggs on average). Eggs hatch 2 to 72 h after oviposition and larvae develop in 3 to 13 days. The pupa remains within the fruit or fallen to the ground, lasting 3 to 15 days (Kanzawa 1939; Walsh et al. 2011). Thus, the full development of the immature phase requires 8 to 18 days. Adult longevity ranges from 21 to 66 days. The most suitable temperature range is 13.4 to 28.1°C, with a lower upper limit of 7.2°C and an upper upper limit of 30°C (Tochen et al. 2014).

1.1.3. The problem generated by *Zaprionus indianus*

Zaprionus indianus is native to Tropical Africa (Chassagnard and Kraaijeveld 1991; Bächli 2020) and was first reported in Brazil in 1999, in the municipality of Santa Isabel (SP), infesting persimmon fruits, *Diospyros kaki* L. (Ebenaceae) (Vilela 1999), and in March of that same year, it was reported in the fig crop, *Ficus carica* L. (Moraceae), in the municipality of Valinhos, SP (Stein et al. 2003).

Factors related to high adaptability, short period of development and high biotic potential, made the species provide a high population distribution throughout the Brazilian territory, being detected in several states, such as Rio Grande do Sul (Castro and Valente 2001), Santa Catarina (De Toni et al. 2001), Paraíba, Pernambuco, Bahia (Santos et al. 2003), Distrito Federal, Maranhão, Pará and Rondônia (Tidon et al. 2003), Minas Gerais (Kato et al. 2004), followed by Mato Grosso and Rio de Janeiro (David et al. 2006).

Zaprionus indianus is considered the main pest of the fig crop, inserting its eggs in the fruit's ostiole. The larvae penetrate the interior, making it unsuitable for consumption (Stein et al. 2003), it is usually associated with decomposing fruits, but it has already been found in unripe fruits. This drosophilid is considered a secondary pest to more than 70 other fruit species due to its preference for attacking and feeding on decaying fruit (Joshi et al. 2014). This is related to the difficulty for females to lay eggs on ripe fruit without injury or with the presence of mechanical damage caused by other insect pests (Fartyal et al. 2014).

The major problem with this pest is its association with other pest species, for example, with *D. suzukii*, as it is known that lesions caused by spotted-wing drosophila cause the release of volatiles (Abraham et al. 2015) that attract other drosophilid species such as *Z. indianus* (Van Timmeren and Isaacs 2013; Joshi et al. 2014; Lasa and Tadeo 2015).

When no method is used to control *Z. indianus*, economic losses reach 50% for production and 80% for export, and estimated monetary losses with this pest in São Paulo were already US$ 767 thousand per year (Vilela et al. 2001; Stein et al. 2003).

1.1.4. Morphological and biological aspects of *Zaprionus indianus*

Zaprionus indianus is yellowish, approximately 2 mm long, has silvery-white longitudinal bands on the dorsal region of the head and chest, usually bordered by black bands, which contrast with the adjacent velvety-brown areas, in addition, they have a series of 4 to 6 distinct compound spines on the anterior femur. These spines are not located in small tubercles, and each has a short second branch at its base, acting as a rest for the bent-leg tibia. The narrow black bands (compared to those of other species) surrounding the silver thoracic bands do not widen into the scutellum, and the scutellum does not have a white tip. This combination of characters is sufficient to distinguish *Z. indianus* from all other known species of the genus (Vilela et al. 2001).

The environmental factors that favor the occurrence of this pest have already been studied by some authors. A study related to the thermal range of development of *Z. indianus* showed that this species is able to develop when raised at 14 to 32ºC (Karan et al. 1999). When raised at 13ºC, *Z. indianus* has a very low survival rate (Karan et al. 1999). When created at 12ºC, there is total lethality in the pupal stage. At the other extreme of the temperature range, complete lethality also occurs when development occurs at 33°C (Karan et al. 1999). Araripe et al. (2004) observed that the species *Z. indianus* is more sensitive to cold, which would probably explain the fact that this fly is restricted to tropical and subtropical climates. According to Silva et al. (2005), carried out a survey of drosophilids in the south of the country, as higher frequencies of *Z. indianus* were adapted during seasons with high temperatures, especially during the summer.

1.1.4. Integrated Pest Management

The history of Integrated Pest Management (IPM) in Brazil is linked to the change in the concept of pest control that took place in the 1960s, a period in which the world was alerted to the dangers of the abusive use of phytosanitary products. Since then, production standards based on high energy expenditure on pesticides and fertilizers are being reassessed regarding their sustainability over time and their consequences for man and the environment. The IPM is compatible with other integrated pest management practices and, when well planned, adds value to the agricultural product in more demanding consumer markets (Maniania et al. 2008).

As emphasized by Norris et al. (2003), an IPM system must go far beyond the pure and simple control of chemical applications. According to Moraes and Flechtmann (2008), one of the most relevant aspects of an IPM system refers to what has been designated as “system management”, which refers to a group of procedures related to changes in the environment. Ultimately, these procedures seek to make the environment more favorable to the development of its natural enemies and less suitable for the development of pests and diseases.

The IPM consists of reconciling various management tactics, such as the use of resistant varieties, control chemical, cultural, biological, genetic manipulation, pheromones and environmental manipulation. The IPM usually requires correct identification of the pest and/or disease, periodic sampling of populations, adoption of control levels, knowledge of natural mortality, etc.

There is in Brazil a famous program entitles as “Produção Integrada do Morango (PIMo)” in which specific technical standards and accompanying documents were published in Normative Instruction number 14 of April 1, 2008. This program allows the fruits produced to compete both in the domestic and foreign market and offer differentiated products, capable of granting farmers better remuneration and guaranteeing the sustainability of the crop.

PIMo is involved in the management of this culture, from implementation to post-harvest, following up-to-date standards and techniques appropriate to the national reality, aiming to solve the different problems through a multidisciplinary view (Brasil 2008).

1.1.5. Use of biological control

Biological control consists of using natural enemies to control organisms considered pests. This can be done through three strategies: classic biological control, increment and conservation. Classic biological control involves the introduction of natural enemies, usually from the place of origin of the pest. The increment consists of the mass multiplication of the natural enemy under controlled conditions for later release in the field. Conservation involves the maintenance of natural enemies in agroecosystems through actions that favor their survival and multiplication and, consequently, increase their effectiveness (Gallo et al. 2002).

The biological control of phytosanitary agents in strawberry plants is carried out using increment and conservation strategies, which are usually made compatible with other control methods control in integrated pest management processes. Within the IPM, if the use of pesticides is necessary, the decision is always to use selective products, so as not to harm natural enemies.

However, there are still few farmers who use biological control in the cultivation of strawberries. For the control of phytophagous mites in Brazil, two species of predatory mites of the Phytoseiidae family have been used: *Phytoseiulus macropilis* (Banks) and *Neoseiulus californicus* (McGregor). These are already produced and sold in Brazil to control the spotted spider mite. In addition to these, *Stratiolaelaps scimitus* (Womersley) (Laelapidae) is commercially produced for the control of thrips and flies of the Sciaridae family.

1.1.6. Predatory mites in drosophilid control

Predatory mites are commonly found in the soil and on cultivated plants and plants of the natural vegetation. Most of these belong to the cohort Gamasina, of the order Mesostigmata. Although many of them have been demonstrated to have diverse feeding habits, most are known to prey on small invertebrates, including mites, nematodes and insects. For this reason, members of this group have been extensively studied as candidates for the control of aerial and edaphic pests (McMurtry et al. 2013; Carrillo et al. 2015).

Species of some families of mites of the order Mesostigmata are often mentioned as effective predators of fly larvae, thrips, pest mites, whitefly and nematodes. Mites of the Phytoseiidae and Laelapidae family are already commercialized for the control of phytophagous mites and flies, respectively. Laelapids deserve considerable attention as possible edaphic pest control agents, as they are found mainly on the soil surface and are reported to be satisfactory for the control of some species of Thripidae, Sciaridae and Diptera (Moreira and Moraes 2015).

Gamasina have been reported in association with different groups of Diptera. *Stratiolaelaps scimitus* (Womersley), of the family Laelapidae, has been used commercially in Brazil and other countries for the control of fungus gnats (Sciaridae) in strawberry and several other cultures (Knapp et al. 2018). Also, many studies have been conducted on the role of Gamasina of the family Macrochelidae as predators of Diptera of economic importance, especially of the family Muscidae (Azevedo et al. 2015; 2018).

Some authors reported the predation of *Drosophila melanogaster* Meigen by *Arctoseius cetratus* (Sellnick) (Ascidae) (O'Farrell and Butler 1948; Binns 1972), *Gaeolaelaps aculeifer* (Canestrini) (Laelapidae) (Ignatowicz 1974) and *Proctolaelaps regalis* De Leon (Melicharidae) (Houck et al. 1991; Houck 1994). A crash in the population of *Drosophila* species was related with the increase in the population of *Proctolaelaps drosophilae* Karg et al., although actual feeding on the flies was not reported by the authors (Karg et al. 1995).

1.1.7. Entomopathogenic Fungi

Belonging to the phylum Ascomycota, order Hypocreales, the entomopathogenic fungi of the genre *Beauveria* and *Metarhizium* were the first pathogens to be used in the biological control of arthropods (Davidson 2012). These fungi have a global distribution (Zimmerman 1993) and a wide host range. Their ability to regulate insect populations is well established in tropical, temperate and Nordic environments (Zimmermann 2007) and are currently being used commercially as biocontrol agents. The use of microbial control is a potentially valuable alternative compared to chemical insecticides due to its selectivity benefits, producer and consumer health, non-target organisms and environmental preservation (Sosa-Gomez and Moscardi 1994).

Entomopathogenic fungi are good candidates for implementation in integrated pest management, a practice increasingly adopted in different agroecosystems, which includes the maintenance of natural enemies within crops. They are commonly found in soil, can also be found in insects and mites and act on different stages of development of hosts (insects and mites), such as eggs, larvae, pupae and adults. Its action usually occurs through penetration via the insect's integument, where the fungus, with full colonization, leads the insect to its death.

Metarhizium spp. can infect more than 200 species of insects and other arthropods. According to Zimmermann (1993), beetles, grasshoppers, leafhoppers and termites represent the groups most susceptible to these fungi. For these reasons, *Metarhizium* is one of the most studied groups of insect pathogens for use in biological pest control.

Metarhizium anisopliae (Metschn.) Sorokin and *Beauveria bassiana* (Bals.) Vuill have been recognized as the most important entomopathogens of insects of the order Diptera (Watson et al. 1995). Regarding *D. suzukii*, *M. anisopliae* isolates were evaluated as potential biological control agents (Naranjo-Lázaro et al. 2014), just as *B. bassiana* isolates caused 50 to 80% mortality, respectively, in the spotted-wing drosophila (Gargani et al. 2013).

These entomopathogens can additionally participate in other processes. They can colonize the rhizosphere, as plant exudates serve as nutrients for the fungi; solubilize inorganic nutrients to plants; when they kill an insect, they can transfer nitrogen from the insect to the plant, generating a tritophic relationship: host insect, rhizosphere and plant; perform endophytic colonization generating production of phytohormones that result in an increase in the growth of roots and aerial parts of the plants; because they colonized endophytically, they can compete with phytopathogens, induce systemic resistance and increase plant size through the induction or synthesis of growth-regulating substances, that is, they act as plant growth promoters, which increase the productivity of many crops (Bixby-Brosi and Potter 2012; Canassa et al. 2020).

1.1.8. Predatory mites and organic soil cover (mulching)

The Gamasina cohort which comprises a large group of mites found in different habitats, but especially in the soil. Although many of them feed on fungi, they are known to prey on small invertebrates, including mites, nematodes and insects. Mites, like Collembola and some other Hexapoda, are part of the soil mesofauna (length 200 µm – 2 mm), many of which break down the organic matter available on the soil surface (Walter and Proctor 2013).

Macrofauna (> 2 mm), especially earthworms, also participate in the process of formation of organic matter and fragmentation (Bottinelli et al. 2015). Soil microbiota, which includes microfauna and other small organisms (<100 µm), including bacteria, fungi and nematodes, is also a key element in the matter cycle (Raven et al. 2001). Thus, it is expected that throughout the process of decomposition of organic matter, such as straw cover, soil characteristics will vary, influencing the edaphic predatory mite fauna.

Therefore, soil with better coverage, naturally represented by structures of dead plants on its surface or resulting from deliberate introduction by producers, has a higher density of predatory mites, as widely reported in the literature (Esteca et al. 2018; Karg 1967; Van de Bund 1970; Walter and Behan-Pelletier 1999). Consequently, in agricultural lands, the use of organic coverings should benefit pest control, acting as a reservoir for edaphic predatory mites.

For this reason, members of Gamasina have been studied as predators of edaphic pests in plants (Carrilo et al. 2015). These beneficial mites can also benefit from organic mulch. An increase in the abundance and diversity of Gamasina mites and/or a decrease in pest populations in different crops has been reported by several authors (Hoddle et al. 2002; Jensen et al. 2002; Jamieson and Stevens 2006; Sánchez-Moreno and Ferris 2007).

An aspect little cited in the literature on predatory mites is the movement of soil mites to exposed environments, such as plants, or from one part of the plant to another, at night (Onzo et al. 2003; Parecis-Silva et al. 2016; Fagam et al. 2006). In Brazil, Esteca et al. (2018) found *Proctolaelaps pygmaeus* (Müller) (Melicharidae) and *Blattisocius dentriticus* (Berlese) (Blattisociidae) present in strawberry leaflets mainly at night, indicating their possible daily migration from the edaphic environment to the plants. The reason for this behavior has not been properly studied. In another study, unidentified species of *Proctolaelaps* and of the blattisociid *Lasioseius* were also reported to migrate from the soil to strawberry leaflets, mainly at night (Esteca et al. 2020).

REFERENCES

Aasen, S.S.; Trandem, N. (2006) Strawberry blossom weevil *Anthonomus rubi* Herbst (Col.: Curculionidae): Relationships between bud damage, weevil density, insecticide use, and yield. Journal of Pest Science, 79: 169-174.

Abraham, J.; Zhang, A.; Angeli, S.; Abubeker, S.; Michel, C.; Feng, Y.; Rodriguez-Saona, C. (2015) Behavioral and antennal responses of *Drosophila suzukii* (Diptera: Drosophilidae) to volatiles from fruit extracts. Environmental Entomology, 44(2): 356-367.

Andreazza, F.; Haddi, K.; Oliveira, E.E.; Ferreira, J.A.M. (2016) *Drosophila suzukii* (Diptera: Drosophilidae) arrives at Minas Gerais State, a main strawberry production region in Brazil. Florida Entomologist, 99(4): 796-798.

Antunes, L.E.C.; Bonow, S.; Reisser Jr., C. (2020) Morango: Crescimento constante em área e produção. Campo e Negócios, 1: 88-92.

Araripe, L.O.; Klaczko, L.B.; Moreteau, B.; David, J.R. (2004) Male sterility thresohlds in a tropical cosmopolitan drosophilid, *Zaprionus indianus*. Journal of Thermal Biology, 29: 73-80.

Arnó, J.; Riudavets, J.; Gabarra, R. (2012) Survey of host plants and natural enemies of *Drosophila suzukii* in an area of strawberry production in Catalonia (northeast Spain). IOBC/WPRS Bull, 80: 29–34.

Azevedo, L.H.; Emberson, R.M.; Esteca, F.C.N.; Moraes, G.J. (2015) Macrochelid mites (Mesostigmata: Macrochelidae) as biological control agents. In: Prospects for biological control of plant feeding mites and other harmful organisms; Carillo, D.; Moraes, G.J.; Peña, J.E. (Eds.). Springer International Publishing: Cham, Switzerland, chap. 4, pp 103–132.

Azevedo, L.H.; Ferreira, M.P.; Castilho, R.C.; Cançado, P.H.D.; Moraes, G.J. (2018) Potential of *Macrocheles* species (Acari: Mesostigmata: Macrochelidae) as control agents of harmful flies (Diptera) and biology of *Macrocheles embersoni* Azevedo, Castilho and Berto on *Stomoxys calcitrans* (L.) and *Musca domestica* L. (Diptera: Muscidae). Biological Control, 123: 1-8.

Bächli, G. (2020) TaxoDros: The database on Taxonomy of Drosophilidae, v1.04, Database 2020/9. Available from: https://www.taxodros.uzh.ch/ (Accessed January 7, 2020).

Barakat, R.M.; Al-Masri, M.I. (2017) Effect of *Trichoderma harzianum* in combination with fungicides in controlling gray mould disease (*Botrytis cinerea*) of strawberry. American Journal of Plant Sciences, 8(4): 65-665.

Bernardi, D.; Botton, M.; Nava, D.E.; Zawadneak, M.A.C. (2015) Guia para identificação e monitoramento de pragas e seus inimigos naturais em morangueiro. Embrapa, Brasília.

Binns, E.S. (1972) *Arctoseius cetratus* (Sellnick) (Acarina: Ascidae) phoretic on mushroom sciarid flies. Acarologia, 14: 350-356.

Bixby-Brosi, A.J.; Potter, D.A. (2012) Endophyte-mediated tritrophic interactions between a grass feeding caterpillar and two parasitoid species with different life histories. Arthropod-Plant Interact, 6: 27-34.

Bolda, M.P.; Goodhue, R.E.; Zalom, F.G. (2010) Spotted Wing Drosophila: Potential Economic Impact of Newly Established Pest. Agricultural and Resource Economics Update, California, 13(3): 5-8.

Bottinelli, N.; Jouquet, P.; Capowiez, Y.; Podwojewski, P.; Grimaldi, M.; Peng, X. (2015) Why is the influence of soil macrofauna on soil structure only considered by soil ecologists? Soil and Tillage Research, 146: 118–124.

Brasil. Instrução Normativa n.14, de 01 de abril de 2008. Aprova as Normas Técnicas Específicas para a Produção Integrada de Morango – NTEPI-Morango, na forma do Anexo à presente Instrução Normativa. Available online: http://www.agricultura.gov.br/ (accessed on 17 December, 2020).

Brust, G.E. (1994) Natural enemies in straw mulch reduce Colorado potato beetle populations and damage in potato. Biological Control, Amsterdam, 4: 163-69.

Cabi (2017) Invasive Species Compendium. Available online: http://www.cabi.org/isc/datasheet/109283 (accessed on June 29, 2017).

Calabria, G.; Máca, J.; Bachli, G.; Serra, L.; Pascual, M. (2012) First records of the potential pest species *Drosophila suzukii* (Diptera: Drosophilidae) in Europe. Journal of Applied Entomology, 136: 139-147.

Canassa, F.; Esteca, F.C.N; Moral, R.A.; Meyling, N.V.; Klingen, I.; Delalibera Jr, I. (2020). Root inoculation of strawberry with the entomopathogenic fungi *Metarhizium robertsii* and *Beauveria bassiana* reduces incidence of the twospotted spider mite and selected insect pests and plant diseases in the field. Journal of Pest Science, 93(1): 261-274.

Carrillo, D.; Moraes, G.J.; Peña, J.E. (2015) Prospects for Biological Control. of Plant. Feeding Mites and Other Harmful Organisms; Springer International Publishing: Cham, Switzerland, p. 337.

Castro, F.L.; Valente, V.L.S. (2001) *Zaprionus indianus* is invading Drosophilid communities in the southern Brazilian city of Porto Alegre. Drosophila Information Service, 84: 15-16.

Chassagnard, M.T.; Kraaijeveld, A.R. (1991) The occurrence of *Zaprionus* sensu strict in the Paleartic region (Diptera: Drosophilidae). Annales de Société Entomologique de France, 27: 495-496.

Cini, A.; Ioriatti, C.; Anfora, G. (2012) A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. Bulletin of Insectology, 65(1): 149-160.

Dara, S.K. (2016) IMP solutions of insect pests in California strawberries: efficacy of botanical, chemical, mechanical, and microbial options. CAPCA Adviser, 19(2): 40-46.

David, J.R.; Araripe, L.O.; Bitner-Mathé, B.C.; Capy, P.; Goñi, B.; Klaczko, L.B.; Legout, H.; Martins, M.; Vouidibio, J.; Yassin, A.; Moreteau, B. (2006) Quantitative trait analysis and geographic variability of natural populations of *Zaprionus indianus*, a recent invader in Brazil. Heredity, London, 96(1): 53-62.

Davidson, E.W. (2012) History of insect pathology. In: Vega, F.E.; Kaya, H.K. (Ed.). Insect pathology. 2nd ed. San Diego: Academic Press, chap. 2, pp 13-28.

Deprá. M.; Poppe, J.L.; Schmitz, H.J.; Toni, D.C.de; Valente, V.L.S. (2014) The first records of the invasive pest *Drosophila suzukii* in the South American continent. Journal of Pest Science, 87(3): 379-383.

De Toni, D.C.; Hofmann, P.R.P.; Valente, V.L.S. (2001) First register of *Zaprionus indianus* (Diptera: Drosophilidae) in the state of Santa Catarina. Biotemas, 14(1): 71-85.

Dias, M.S.C.; Silva, J.J.C.; Pacheco, D.D.; Rios, S.A.; Lanza, F.E. (2007) Produção de morangos em regiões não tradicionais. Informe Agropecuário, Belo Horizonte, 8(236): 24-33.

Esteca, F.C.N.; Rodrigues, L.R.; Moraes, G.J.; Delalibera Jr., I.; Klingen, I. (2018) Mulching with coffee husk and pulp in strawberry affects edaphic predatory mite and spider mite densities. Experimental and Applied Acarology, 76: 161-183.

Esteca, F.C.N.; Trandem, N., Klingen, I., Cruz, J. S.; Delalibera Jr., I.; Moraes, G.J. (2020) Cereal Straw Mulching in Strawberry-A Facilitator of Plant Visits by Edaphic Predatory Mites at Night? Diversity, 12(6), 242.

Fagan, L.L.; Didham, R.K.; Winchester, N.N.; Behan-Pelletier, V.; Clayton, M.; Lindquist, E.E.; Ring, R.A. (2006) An experimental assessment of biodiversity and species turnover in terrestrial versus canopy leaf litter. Oecologia, 147: 335-347.

Fartyal, R.S.; Sarswat, M.; Lhamo, N.; Sati, P.C.; Asha, L. (2014) Records of *Zaprionus indianus* and *Drosophila suzukii* indicus as invasive fruit pest from mid valley region of Garhwal Uttarakhand. India. *Drosophila* Information Service, 97: 119-123.

Food and Agriculture Organization of the United Nations (FAO). Available online: http://www.fao.org/faostat/en/#data/QC (Accessed on 04 January, 2021).

Gallo, D.; Nakano, O.; Silveira Neto, S.; Carvalho, R.P.L.; Batista, G.C.; Berti Filho, E.; Parra, J.R.P.; Zucchi, R.A.; Alves, S.B.; Vendramin, J.D.; Marchini, L.C.; Lopes, J.R.S.; Omoto, C. (2002) Entomologia agrícola. Piracicaba: FEALQ, pp. 20.

Gargani, E.; Tarchi, F.; Frosinini, R.; Mazza, G.; Simoni, S. (2013) Notes on *Drosophila suzukii* Matsumura (Diptera Drosophilidae): Field survey in Tuscany and laboratory evaluation of organic products. Redia-Giornale Di Zoologia, 96: 85-90.

Goodhue, R.E.; Bolda, M.; Farnsworth, D.; Williams, J.C.; Zalom, F.G. (2011) Spotted wing drosophila infestation of California strawberries and raspberries: economic analysis of potential revenue losses and control costs. Pest Management Science, Oxford, 67(11): 1396-1402.

Grassi, A.; Giongo, L.; Palmieri, L. (2011) *Drosophila* (*Sophophora*) *suzukii* (Matsumura), new pest of soft fruits in Trentino (North-Italy) and in Europe. Integrated Plant Protection in Soft Fruits. IOBC wprs Bulletin, Budapest, 70: 121-128.

Haviland, D.R.; Beers, E.H. (2012) Chemical control programs for *Drosophila suzukii* that comply with international limitations on pesticide residues for exported sweet cherries. Journal of Integrated Pest Management, 3(2): F1-F6.

Haslestad, J. (2020) (Norsk Landbruksrådgivning, Ribadu, Hedmark, Norway). Personal communication.

Hoddle, M.S.; Morse, J.G.; Phillips, P.A.; Faber, B.A.; Jetter, K.M. (2002) Avocado thrips: a new challenge for growers. California Agriculture, Richmond, 56: 103-107.

Houck, M.A. (1994) Mites as potential horizontal transfer vectors of eukaryotic mobile genes: *Proctolaelaps regalis* as a model. In: Houck MA (ed) Mites: ecological & evolutionary analyses of the life-history patterns. Chapman & Hall, London, pp 45-69.

Houck, M.A.; Clark, J.B.; Peterson, K.R.; Kidwell, M.G. (1991) Possible horizontal transfer of *Drosophila* genes by the mite *Proctolaelaps regalis*. Science, 253: 1125-1129

Ignatowicz, S. (1974) Observations on the biology and development of *Hypoaspis aculeifer* Canestrini, 1885 (Acarina: Gamsides). Zoologica Poloniae, 24: 41-59.

Jamieson, L.E.; Stevens, P.S. (2006) The effect of mulching on adult emergence of Kelly's citrus thrips (*Pezothrips kellyanus*). New Zealand Plant Protection, Auckland, 59: 42-46.

Jensen, L.; Simko, B.; Shock, C.; Saunders, L. (2002) Alternative Methods for Controlling Onion Thrips (*Thrips tabaci*) in Spanish Onions; Malheur Experiment Station Annual Report 2001; Oregon State University Agricultural Experiment Station: Ontario, OR, Canada, Volume 1038, pp. 104-111.

Joshi, N.K.; Biddinger, D.J.; Demchak, K.; Deppen, A. (2014) First report of *Zaprionus indianus* (Diptera: Drosophilidae) in commercial fruits and vegetables in Pennsylvania. Journal of Insect Science, 14(259): 1-4.

Julian, J.; Strik, B.; Yang, W. (2011) Blueberry Economics. The costs of establishing blueberries in the Willamette Valley. AEB 0022. Oregon, USA: Oregon State University Extension Service.

Karan, D.; Moreteau, B.; David, J. R. (1999) Growth temperature and reaction norms of morphometrical traits in a tropical drosophilid: *Zaprionus indianus*. Heredity, 83: 398-407.

Kanzawa, T. (1939) Studies on *Drosophila suzukii* Mats. Kofu, Yamanashi Agricultural Experimental Station. pp 49 (translation courtesy of Biosecurity Australia).

Karg, W.; Baker, R.A.; Jenkinson, L. (1995) Mite infestation in *Drosophila* cultures and description of a new mite species of the genus *Proctolaelaps* Berlese with notes on its control in the laboratory (Acarina, Parasitiformes). Deutsche Entomologische Zeitschrift, 42(1): 187-190.

Karg, W. Syno¨kologische (1967) Untersuchungen von Bodenmilben aus forstwirtschaftlich und landwirtschaftlich genutzten Böden. Pedobiologia, 7: 198-214.

Kato, C.M.; Foureaux, L.V.; César, R.A.; Torres, M.P. (2004) Ocorrência de *Zaprionus indianus* Grupta, 1970 (Diptera: Drosophilidae) no Estado de Minas Gerais. Ciência e Agrotecnologia, Lavras, 28(2): 457-458.

Kinjo, H.; Kunimi, Y.; Nakai, M. (2014) Effects of temperature on the reproduction and development of *Drosophila suzukii* (Diptera: Drosophilidae). Applied Entomology and Zoology, 49: 297–304.

Klingen, I.; Westrum, K.; Meyling, N.V. (2015) Effect of Norwegian entomopathogenic fungal isolates against *Otiorhynchus sulcatus* larvae at low temperatures and persistence in strawberry rhizospheres. Biological Control, 81: 1-7.

Knapp, M.; van Houten, Y.; van Baal, E.; Groot, T. (2018**)** Use of predatory mites in commercial biocontrol: Current status and future prospects. Acarologia, 58: 72-82.

Kuhn, T.N.A.; Loeck, A.E.; Zawadneack, M.A.C.; Garcia, M.S.; Botton, M. (2014) Biological parameters and fertility life table of *Neopamera bilobata* (Hemitera: Rhyparochromidae) on strawberry. Pesquisa Agropecuária Brasileira, 49(6): 422-427.

Lasa, R.; Tadeo, E. (2015) Invasive drosophilid pest *Drosophila suzukii* and *Zaprionus indianus* (Diptera: Drosophilidae) in Veracruz, Mexico. Florida Entomologist, 98: 987-988.

Lee, J.C.; Bruck, D.J.; Dreves, A.J.; Ioriatti, C.; Vogt, H.; Baufeld, P. (2011) In focus: Spotted wing drosophila, *Drosophila suzukii*, across perspectives. Pest Management Science, 67: 1349-1351.

Luz, C.; Rocha, L.F.; Montalva, C.; Souza, D.A.; Botelho, A.B.R.; Lopes, R.B.; Faria, M.; Delalibera Jr, I. (2019) *Metarhizium humberi* sp. nov. (Hypocreales: Clavicipitaceae), a new member of the PARB clade in the *Metarhizium anisopliae* complex from Latin America. Journal of Invertebrate Pathology, 166: 107216.

Machado, D.F.M.; Parzianello, F.R.; Silva, A.C.F. da; Antoniolli, Z.I. (2012) *Trichoderma* no Brasil: o fungo e o bioagente. Revista de Ciências Agrárias, 35(1): 274-288.

Maniania, N.K.; Bugeme, D.M.; Weksaj, V.W.; Delalibera, I.; Knapp, M. (2008) Role of entomopathogenic fungi in the control of *Tetranychus evansi* and *Tetranychus urticae* (Acari: Tetranychidae), pests of horticultural crops. Experimental and Applied Acarology, Amsterdam, 46: 259-274.

McMurtry, J.A.; Moraes, G.J.; Sourassou, N.F. (2013) Revision of the lifestyles of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. Systematic and Applied Acarology, 18(4): 297-320.

Montagnana, J.M.; Costa, A.L.; Pereira, J.M.; Tonelli, M.; Leite, L.G. (2017) Eficiência de Steinernema Brazilense no controle de *Colaspis* sp. em morangueiro. In: 9ª Jornada Científica e Tecnológica e 6º Simpósio da Pós-graduação do IFSULDEMINAS, Machado.

Moraes, G. J.; Flechtmann, C. H. W. (2008) Manual de acarologia: acarologia básica e Ácaros de plantas cultivadas no Brasil. Ribeirão Preto: Holos, Editora, 308.

Moreira, G.F.; Moraes, G.J. (2015) The potential of free-living laelapid mites (Mesostigmata: Laelapidae) as biological control agents. In: *Prospects for biological control of plant feeding mites and other harmful organisms*; Carillo, D.; Moraes, G.J.; Peña, J.E. (Eds.). Springer International Publishing: Cham, Switzerland, chap. 3, pp. 77-102.

Naranjo-Lazaro, J.M.; Mellin-Rosas, M.A.; Gonzalez-Padilla, V.D.; Sanchez-Gonzalez, J.A.; Moreno-Carrillo, G.; Arredondo-Bernal, H.C. (2014) Susceptibility of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) to entomopathogenic fungi. The Southwestern Entomologista, 39: 201-203.

Norris, R.F.; Caswell-Chen, E.P.; Kogan, M. (2003) Concepts in integrated pest management. Prentice Hall, New Jersey, 586.

O'Farrell, A.F.; Butler, P.M, (1948) Insects and mites associated with the storage and manufacture of foodstuffs in Northern Ireland. The Economic Proceedings of the Royal Dublin Society, 3: 343-407.

Onzo, A.; Hanna, R.; Zannou, I.; Sabelis, M.W.; Yaninek, J.S. (2003) Dynamics of refuge use: Diurnal, vertical migration by predatory and herbivorous mites within cassava plants. Oikos, 101: 59-69.

Parecis-Silva, P.V.; Nuvoloni, F.M.; Feres, R.J. (2016) Day vs. night: The importance of the circadian cycle over metacommunities and predator–prey densities. International Journal of Acarology, 42: 141-148.

Raven, P.H.; Evert, R.F.; Eichorn, S.E. (2001) Biologia Vegetal; Guanabara-Koogan: Rio de Janeiro, Brasil. p. 906.

Resende, F.V.; Souza, L.S.D.; Oliveira, P.S.R.D.; Gualberto, R. (2005) Uso de cobertura morta vegetal no controle da umidade e temperatura do solo, na incidência de plantas invasoras e na produção da cenoura em cultivo de verão. Ciência e Agrotecnologia, Lavras, 29(1): 100-105.

Sánchez-Moreno, S.; Ferris, H. (2007) Suppressive service of the soil food web: Effects of environmental management. International Journal of Acarology, 19: 75-87.

Santos, J.F.; Rieger, T.T.; Campos, S.R.C.; Nascimento, A.C.C.; Felix, P.T.; Silva, S.V.O.; Freitas, F.M.R. (2003) Colonization of Northeast region of Brazil by the drosopilid flies *Drosophila malerkotliana* and *Zaprionus indianus*, a new potential pest for Brazilian fruit culture. Drosophila Information Service, 86: 92-95.

Silva, N.M.; Fantinel, C.C.; Valente, V.L.S.; Valiati, V.H. (2005) Population Dynamics of the Invasive Species *Zaprionus indianus* (Gupta) (Diptera: Drosophilidae) in Communities of Drosophilids of Porto Alegre City, Southern of Brazil. Neotropical Entomology, 34(3): 363-374.

Siqueira, A.C.O.; Mascarin, G.M.; Gonçalves, C.R.N.C.B.; Marcon, J.; Quecine, M.C.; Figueira, A.; Delalibera Jr, I. (2020) Multi-trait biochemical features of *Metarhizium* species and their activities that simulate the growth of tomato plants. Frontiers in Sustainable Food Systems, 4(137): 1-15.

Solomon, M.G.; Jay, C.N.; Innocenzi, P.J.; Fitzgerald, J.D.; Crook, D.; Crook, A.M.; Easterbrook, M.A.; Cross, J. V. (2001) Natural enemies and biocontrol of pests of strawberry in northern and central Europe. Biocontrol Science and Technology, 11(2): 165-216.

Sosa-Gomez, D.R.; Moscardi, F. (1994) Effect of till and no-till soybean cultivation on dynamics of entomopathogenic fungi in the soil. Florida Entomologist, 77(2): 284.

Statistics Norway. Available online: https://www.ssb.no/en (accessed on 24 May 2020).

Stein, C.P.; Teixeira, E.P.; Novo, J.P.S. (2003) Aspectos biológicos da mosca do figo, *Zaprionus indianus* Grupta, 1970 (Diptera: Drosophilidae). Entomotropica, Maracay, 18(3): 219-221.

Tanaka, M.A.S; Betti, J.A.; Kimati, H. (2005) Doenças do morangueiro. In: Kimati, H.; Amorim, L.; Rezende, J.A.M; Bergamin Filho, A.; Camargo, L.E.A. (Ed). Manual de Fitopatologia, São Paulo: Agronômica Ceres, pp. 489-499.

Tidon, R.; Leite, D.F.; Leão, B.F.D. (2003) Impact of the colonization of *Zaprionus* (Diptera: Drosophilidae) in different ecosystems of the Neotropical region: 2 years after the invasion. Biological Conservation, 112: 299-305.

Tochen, S.; Dalton, D.T.; Wiman, N.; Hamm, C.; Shearer, P.W.; Walton, V.M. (2014) Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on cherry and blueberry. Environmental Entomology, 43: 501-510.

Trandem, N. (2003) Greenhouse production of strawberries and blackberries in Norway–arthropod pests and biological control. In Proceedings of the IOBC/ WPRS Working Group "Integrated Plant Protection in Orchards" Subgroup "Soft Fruits", Dundee, Scotland, 18–21 September 2001; Gordon, S.C., Cross, J.V., Eds.; Scottish Crop Research Institute: Dundee, Scotland; volume 26, pp. 45-50.

Ueno, B.; Costa, H.; Dias, M.S.C.; Jesus, A.M. (2014) Manejo integrado das principais doenças do morangueiro. Informe Agropecuário, Belo Horizonte, 35(279): 82-91.

Van de Bund, C.F. (1970) Gamasides as predators of phytophagous nematodes. In Problems of Acarology, Symposium; Bozek, J., Suski, Z.W., Jakubowska, J., Eds.; Publisher: Polska Akademia Nauk, Polska; p. 280.

Van Timmeren, S.; Isaacs, R. (2013) Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs. Crop Protection, 54: 126-133.

Vi Spiser Mer Utenlandske Enn Norske Jordbær. Available online: https://www.ssb.no/jord-skogjakt-og-fiskeri/artikler-og-publikasjoner/vi-spiser-mer-utenlandske-enn-norske-jordbaer (accessed on 22 January 2020).

Vilela, C.R. (1999) Is *Zaprionus indianus* Gupta, 1970 (Dipera, Drosophilidae) currently colonizing the Neotropical Region? Drosophila Information Service, Oklahoma, 82: 38-39.

Vilela, C.R.; Teixeira, E.P.; Stein, C.P. (2001) Mosca-africana-do-figo, *Zaprionus indianus* (Diptera: Drosophilidae). In: Vilela, E.F., Zucchi, R.A., Cantor, F. (Eds.) Histórico e impacto das pragas introduzidas. Ribeirão Preto: Holos Editora, p. 48-52.

Walsh, D.; Bolda, M.; Goodhue, R.; Dreves, A.; Lee, J.C.; Bruck, D.J.; Walton, V.M.; O'Neal, S.D.; Zalom, F.G. (2011) *Drosophila suzukii* (Diptera: Drosophilidae): Invasive pest of ripening soft fruit expanding its geographic range and damage potential. Integrated Pest Management, 106(2): 289-295.

Walter, D.E.; Behan-Pelletier, V. (1999) Mites in forest canopies: Filling the size distribution shortfall? Annual Review of Entomology, 44: 1-19.

Walter, D.E.; Proctor, H.C. (2013) Mites: Ecology, Evolution and Behaviour Life at a Microscale, 2nd ed.; Springer Science and Business Media: Dordrecht, The Netherlands; p. 509.

Watson, D.W.; Geden, C.J.; Long, S.J.; Rutz, D.A. (1995) Efficacy of *Beauveria bassiana* for controlling the house fly and stable fly (Diptera: Muscidae). Biological Control, 5: 405-411.

Yassin, A. (2013) Phylogenetic classification of the Drosophilidae Rondani (Diptera): the role of morphology in the postgenomic era. Systematic Entomology, 38: 349-364.

Zimmerman, G. (1993) The entomopathogenic fungus *Metarhizium anisopliae* and its potential as a biocontrol agent. Pesticide Science, Oxford, 37: 375-379.

Zimmermann, G. (2007) Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. Biocontrol Science and Technology, Oxford, 17(9): 879-920.

2. POPULATION DYNAMICS OF *Drosophila suzukii* (MATSUMURA), *Zaprionus indianus* GUPTA AND OTHER DROSOPHILIDS (DIPTERA: DROSOPHILIDAE) IN SOUTHERN MINAS GERAIS, BRAZIL

Abstract

Strawberry (*Fragaria* x *ananassa* Duch.) is one of the main small fruit crops grown in Brazil, being particularly susceptible to pests and diseases. The spotted-wing drosophila, *Drosophila suzukii* (Matsumura, 1931) and African-fig-fly, *Zaprionus indianus* Gupta, 1970 have been mentioned to damage strawberry in Brazil and other countries. The objective of this work was to evaluate the population dynamics of those insects in strawberry grown under two cultivation systems, suspended cultivation under plastic and in low tunnels in southern Minas Gerais state, Brazil, monitoring adults of the insect with traps made of plastic bottles. For most of the study, the levels of occurrence of *D. suzukii* and *Z. indianus* were relatively small. Overall average numbers of *D. suzukii* flies were significantly higher in field 1 and 2 (suspended cultivation) than in field 3 (low tunnels) (respectively 36.2 ± 13.7, 25.2 ± 6.2 and 7.1 ± 1.8 flies/ trap collected during about one month). Similar pattern of occurrence was observed for *Z. indianus* (respectively 17.9 ± 2.7, 31.0 ± 5.6 and 4.5 ± 0.9 flies per trap). Almost invariably, the numbers of other drosophilids as a group were higher than the numbers of *D. suzukii* or *Z. indianus*. The peak population of *D. suzukii* occurred from late spring to late summer (December through March), whereas the peak population of *Z. indianus* occurred slightly later, from mid-summer to mid-winter (February through July). Other drosophilids occurred at higher levels throughout the year. In summary, *D. suzukii* and *Z. indianus* are presently found in southern Minas Gerais on strawberry, but they do not seem to cause significant losses.

Keywords: 1. Spotted-wing drosophila 2. African-fig-fly 3. Monitoring 4. Ecology

2.1. Introduction

Strawberry (*Fragaria* x *ananassa* Duch.) is the main small fruit crop grown in Brazil (Fachinello et al. 2011), being particularly susceptible to attack by pests and diseases. The spotted-wing drosophila, *Drosophila suzukii* (Matsumura), is a pest of fruits of thin tegument, such as apricot, blueberry, cherry, cranberry, peach, persimmon, plum, raspberry and strawberry (Cini et al. 2012). Arnó et al. (2012) reported strawberry as the preferred host of this pest, which attacks mainly unripe fruits. The African-fig-fly, *Zaprionus indianus* Gupta, is a primary pest of figs and a secondary pest of more than 70 other fruit species, always attacking preferably rotting fruits (Joshi et al 2014), for the difficulty of females to oviposit in undamaged fruits (Fartyal et al. 2014).

Drosophila suzukii is considered native to Japan, and until recently endemic to southwest Asia. However, it has more recently been reported from America and Europe (Lee et al. 2011b; Calabria et al. 2012; Deprá et al. 2014). In Brazil, *D. suzukii* was reported for the first time in subtropical southern areas, in Rio Grande do Sul state, next to Uruguay (Deprá et al. 2014, Santos 2014). In the same year, it was reported from São Paulo state, in southeastern Brazil (Vilela and Mori 2014), and in Brasília, in the central part of the country (Paula et al. 2014). Shortly afterward, it was found in Minas Gerais (Andreazza et al. 2016), also in southeastern Brazil. These reports show that in a period of 1–2 years, *D. suzukii* was found in Brazil in places up to 1400 km apart, which could be explained by the transport in commercialized infested fruits.

The damage caused by *D. suzukii* is also expressive, for the large and serrated ovipositor, that allows it to oviposit in unripe fruits, followed by damage to the pulp by the larvae and subsequent entry of microorganisms (Van Timmeren and Isaacs 2013), turning fruits unsuitable for commercialization (Bolda et al. 2010). The appropriate temperature range for the spotted-wing drosophila development is 13.4 to 28.1°C, with lower and upper threshold of 7.2 and 30°C (Tochen et al. 2014). When not controlled, *D. suzukii* can cause up to 100% economic losses (Grassi et al. 2011). In California, the estimated reduction in gross revenue due its infestation in the absence of management was estimated at 20% for processed strawberries (Goodhue et al. 2011). *Zaprionus indianus* is native to Tropical Africa (Chassagnard and Kraaijeveld 1991). It was first reported in Brazil in 1999, in Sao Paulo state, infesting persimmon (*Diospyros kaki* L., Ebenaceae) (Vilela 1999) and figs (*Ficus carica* L., Moraceae) (Stein et al. 2003). High adaptability, short developmental period and high biotic potential allowed this species to become widely distributed in Brazil, as in the states of Rio Grande do Sul (Castro and Valente, 2001), Santa Catarina (De Toni et al. 2001), Bahia, Paraíba and Pernambuco (Santos et al. 2003), Federal District, Maranhão, Pará and Rondônia (Tidon et al. 2003), Minas Gerais (Kato et al. 2004), Mato Grosso and Rio de Janeiro (David et al. 2006).

The damage caused by *Z. indianus* results from the oviposition in the ostioles of the infrutescence and the subsequent penetration of the larvae, also turning fruits unsuitable for commercialization (Stein et al. 2003). It is usually associated with decomposing fruits, but it can be less frequently found in unripe fruits. It has been demonstrated that the thermal range of development of *Z. indianus* is between 14 and 32ºC, with total pupal lethality occurring at 12ºC or above 33ºC (Karan et al. 2000). When not control, yield losses due to this pest may reach 40-50%. Inadequate chemical control of these pests can result in the rejection of fruits for exportation to some countries, or even for local consumption, due to insecticide residues

(Haviland and Beers 2012). However, estimates of the economic losses caused by these two species pests are lacking worldwide, which limits the analysis of costs and benefits of protection measures (Kehlenbeck et al. 2012).

Preliminary evaluations showed the presence of *D. suzuki* and *Z. indianus* in southern Minas Gerais, where strawberry is extensively cultivated. In the context of Integrated Pest Management, the monitoring of pests and their natural enemies is an important tool to consider, and baseline information is relevant to decide upon the need to control a pest organism. The hypothesis of this study was that in strawberry cultivation in that region, these insects would only be found at low levels. The objective of this work was to evaluate their population dynamics in strawberry grown under two cultivation systems commonly adopted in the region, suspended and in low tunnels.

2.2. Material and Methods

The study was conducted in three strawberry fields, two in suspended cultivation, cultivar 'San Andreas' (Field 1: 600 m^2, 22°28'27"S 46°9'39"W and 1346 m of altitude; Field 2: 900 m^2, 22°33'21"S 46°11'54"W and 1510 m of altitude) and one in low tunnel cultivation, cultivar 'Albion' (Field 3: 675 m^2, 22°28'46"S 46°09'21"W and 1380 m of altitude). In the suspended cultivation, each field was covered with a single transparent plastic tent (Transparent Crystal Nortene®-100 Microns, with UV protection), whose walls were maintained always open. In the low tunnel cultivation, the whole field was constituted of 40 tunnels made of milky plastic with UV protection (Diffusor Film - 100 Microns - Shoplonas®), whose walls were raised daily, to allow aeration while preventing direct incidence of sunlight onto the plants and protecting them from the wind.

Fields 1 and 3 were located in Bom Repouso and Field 2, in Senador Amaral. In Fields 1 and 2 the seedlings were transplanted in mid-December 2016, in white plastic bags ("slabs"), 1.5 m long and 50 cm wide, about 30 cm in diameter when filled with a substrate composed of 70% carbonized rice husks and 30% ground pine bark. The planting lines were double, with a distance of 35 cm between plants in the line and between neighboring lines, and 50 cm between double lines. In Field 3, the seedlings were transplanted in mid-July 2016 in conventional beds about 20 cm high, each containing two strawberry lines, with plants spaced at 27 cm in the lines, 30 cm between lines and 50 cm between double lines.

In all fields, monitoring of the fly population started in February 2017, ending when growers removed the plants to renew the cultivation, in October 2018 in both suspended cultivations, and in April 2018 in the low tunnel cultivation. Monitoring was carried out with traps consisting each of a 2 L transparent, plastic PET bottle containing five holes of 5.0 mm in diameter, positioned equidistantly in a transverse line at the lower third of the bottle. Each bottle was partially filled with 500 ml of water, two spoons of biological yeast, two spoons of sugar, two drops of detergent (to break the surface tension of the water) and a spoons of vinegar (Walsh et al. 2011; Santos 2014).

Next to the central region of each plastic house of Fields 1 and 2, four traps were positioned about 10- 20 m away from each other and about 10 cm from the top of the plant below. In Field 3, four traps were also used, each placed in a different tunnel and also 10 to 20 m from each other. The traps were replaced every 30 days, bringing the removed ones to the laboratory, where the content of each was poured through a 60 mesh sieve to collect the flies, which were subsequently separated into three groups (*D. suzukii*, *Z. indianus* and other drosophilids), under a stereomicroscope (40x). Drosophilids were separated from flies of other families according to the standard characteristics of the family: presence of an incomplete subcostal vein; two ruptures in the costal vein; a small anal cell in the wing; converging postocellar setae; and generally with three frontal bristles on each side of the head, one facing forward and the other two facing backwards (Markow and O'Grady, 2006). Within this family, identification of *D. suzukii* and *Z. indianus* was done based on the characterization given respectively by Hauser (2011) and van der Linde (2010).

For the control of different pest organisms, growers of the three areas used the following biological products alternately and monthly, as insecticides and acaricides: Boveril® WP (*Beauveria bassiana* PL63) (200 g/100 L of water), Metarril® WP (*Metarhizium anisopliae* E9) (150 g/100 L of water), Neem oil (*Azadirachta indica* A. Juss) (1 kg/100 L of water); as fungicides: Trichodermil® SC (*Trichoderma harzianum* 1306) (50 g/100 L of water) and Serenade® (*Bacillus subtilis* QST713) (8 mL/L of water). Additionally, growers of fields 1 and 2 used the following chemical insecticides and acaricides: Ortus® 50 SC (fenpyroximate) (100 mL/100 L of water) and Karate Zeon® 50 CS (lambda-cyhalothrin) (80 mL/100 L of water); and Amistar Top® (azoxystrobin and difenoconazole) (45 mL/100 L of water), as fungicide. In October 2017, the predatory mite *Phytoseiulus macropilis* (Banks) (Phytoseiidae) was released in field 3, for the control of the two spotted spider mite (*Tetranychus urticae* Koch).

In the three fields, each plant was drip irrigated every 3 days, at a flow rate of 2 L/h and for 8 h at each day. Fertilization in Fields 1 and 2 was done via fertirrigation, in which a concentrated solution of macro and micronutrients was prepared, according to the recommendations of Silveira et al. (2016). In Field 3, fertilization was done alternately once every 20 days with Visa Fértil® (14N: 5P: 8K) (50 kg/ 10,000 plants at transplanting, by fertirrigation) and Adubos Real® (12N: 6P: 12K) (50 kg/10,000 plants, granulated, by fertirrigation). The temperature (°C) and relative humidity (%) were determined with a Datalogger HT-500 Instrutherm installed in each field, which collected data every six hours. Rainfall (mm) was determined from a pluviometer installed in each of the fields, the data being recorded daily.

Statistical analyses of the data densities of insects of three fields were fitted to a Poisson distribution, so the χ^2 test (Anova, test = 'Chisq') was used. Mean numbers of insects per trap were analyzed by Shapiro–Wilk normality test and as the data were not distributed normally, they were analyzed by the Kruskal–Wallis test, all tests were analyzed at 95% confidence intervals. Pearson's correlation coefficients were used to analyze the relation between mean number of insects and temperature (minimum, average and maximum), relative humidity (minimum, average and maximum) and rainfall. Student's t-test was used to compare the mean of insects per trap with the respective averages of meteorological variables to verify their significance at 95% confidence intervals. All analyzes were carried out in R (R Core Team 2018) to verify their significance at 95% confidence intervals.

2.3. Results

Overall average numbers of *D. suzukii* flies per trap were significantly higher in Fields 1 and 2, which did not differ significantly between themselves, and lower in field 3 (36.2 ± 13.7 a, 25.2 ± 6.2 a and 7.1 ± 1.8 b, respectively) (Df=2; $\chi^2 = 15.1$; $p < 0.001$). The highest mean numbers of *D. suzukii* per trap ranged only between 20.0 and 399.0 (Figure 1) as follows: field 1 - February 2017, January and February 2018 (41.5, 45.5 and 399.0, respectively); field 2 - December 2017, January, February and March 2018 (22.0, 158.0, 116.5 and 33.5, respectively); field 3 - February 2017, January and February 2018 (20.0, 32.5 and 37.5, respectively). In other sampling dates, the number of flies per trap ranged between 0.0 and 41.5, with averages for fields 1–3 of 3.2, 3.5 and 2.1, respectively.

Similar pattern of variation was observed for *Z. indianus*, with overall average numbers significantly higher in field 1 and 2, which did not differ significantly between themselves, and lower in field 3 (17.9 ± 2.7 a, 31.0 ± 5.6 a and 4.5 ± 0.9 b flies per trap, respectively) (Df=2; $\chi^2 = 32.7$; $p < 0.001$) (Figure 1). The highest mean numbers of adults of this insect only ranged between 20.0 and 170.0 (Figure 1) as follows: field 1 - June and July 2017 and February and March 2018 (34.2, 45.7, 28.2 and 67.5, respectively); field 2 - May and June 2017 and February

2018 (50.5; 67.5 and 170.0, respectively); field 3 - July 2017 (20.0). In other sampling dates, the number of flies per trap ranged between 0.0 and 37.5, with averages for fields 1–3 of 6.2, 9.9 and 4.5.

Almost invariably, in each sampling date, the number of other drosophilids as a group per trap were higher than the numbers of *D. suzukii* or *Z. indianus*. The exceptions were in February 2017 and February 2018 in field 1 and February 2018 in field 3, when the number of *D. suzukii* was higher, distinctly so in February 2018 in field 1 (Figure 1). The pattern of occurrence for the other drosophilids was quite different from the pattern of occurrence of the previous two species, with overall average numbers of significantly higher in field 2, followed by field 1 and 3 (140.8 ± 15.0 a; 81.2 ± 10.7 b and 34.6 ± 3.9 c flies per trap, respectively) (Df=2; χ^2= 43.5; $p < 0.001$) (Figure 1). In fields 1 and 2, the highest mean numbers ranged between 166.7 and 338.5, as follows: field 1: July 2017, February, March, August, September and October 2018 (201.2, 264.7, 172.7, 188.5, 166.7 and 201.0, respectively); field 2 - July 2017, January, February, March, September and October 2018 (292.0, 223.0, 264.5, 338.5, 268.2 and 250.0, respectively). In other sampling dates, the mean numbers ranged between 0.0 and 203.5, with averages for fields 1–2 of respectively 43.3 and 86.4. In field 3, the highest mean numbers ranged between 49.5 and 117.5, in June, September and October 2017 (49.5, 117.5 and 52.0, respectively). In this field, the number of flies ranged between 0.0 and 45.5, with an average of 25.0 in other sampling dates.

Temperature and humidity and total rainfall attained similar levels in all fields (Figure 1). Overall average temperature, average relative humidity and total rainfall throughout the study were: field 1: 16.3 ± 0.6°C, 85.9 ± 1.1% and 1910.8 mm; field 2: 15.2 ± 0.5°C, 84.3 ± 1.1% and 1862.7 mm; field 3: 16.3 ± 1.0°C, 85.1 ± 5.6% and 1422 mm. The highest values occurred in January 2018, as follows: field 1 - overall temperature, 19.4°C (average minimum and maximum of 13.7 and 27.8 °C); overall relative humidity, 93.7% (88.7 and 95.2%); and overall rainfall, 344 mm; field 2 – overall temperature, 18.1 °C (12.3 and 26.4), overall relative humidity, 93.7% (82.4 and 96.7%); overall rainfall, 338 mm; field 3 – overall temperature, 18.4 °C (13.2 and 28.4 °C); overall relative humidity, 92.8% (79.2 and 94.7%); overall rainfall, 339 mm.

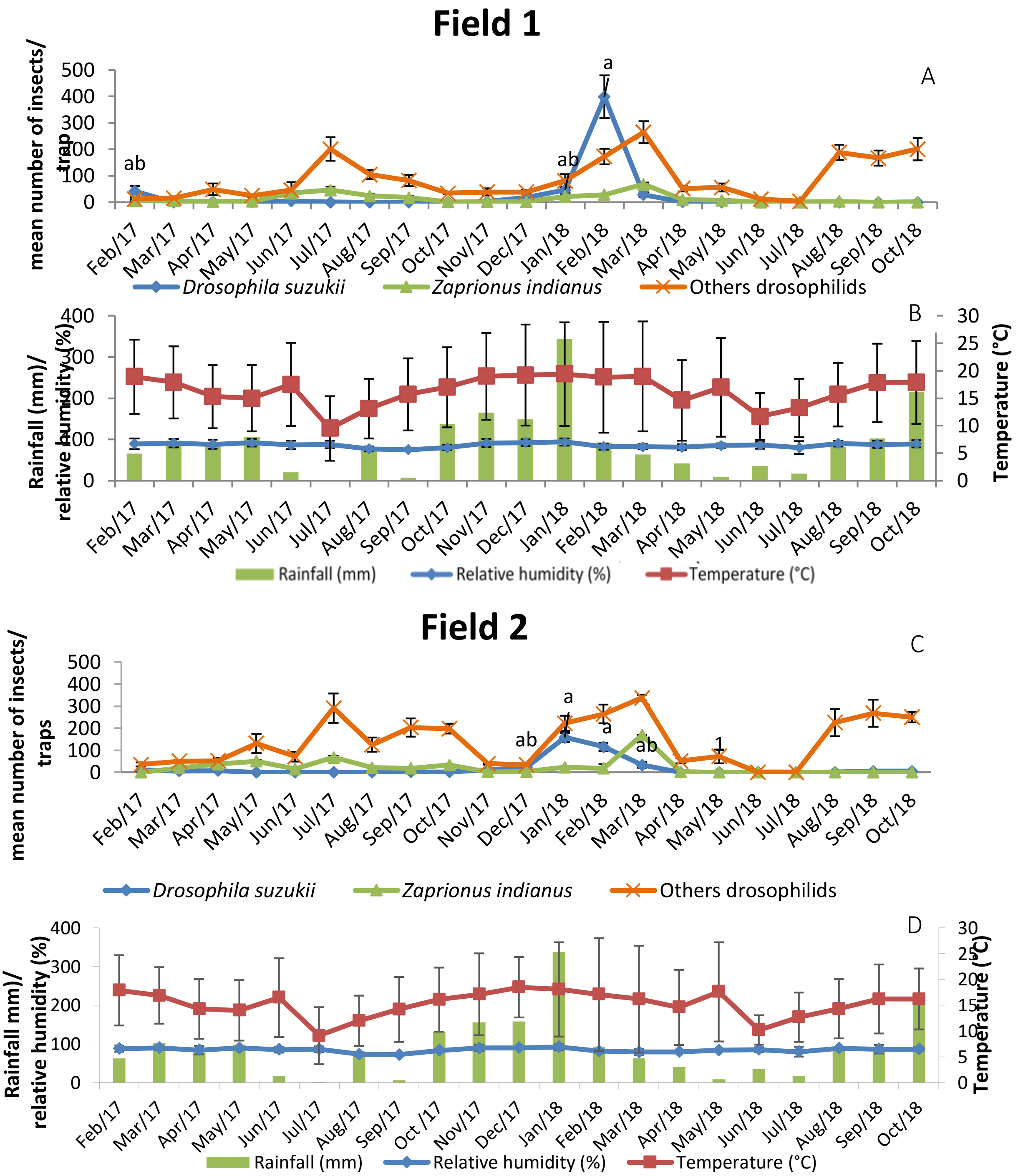

Figure 1. Mean number of insects/traps in strawberry plants under suspended cultivation, in Bom Repouso (Field 1, Fig. A) and Senador Amaral (Field 2, Fig. C), Minas Gerais. Temperature (°C) and humidity (%) data were obtained from a Datalogger and rainfall data (mm) were obtained from a pluviometer installed in each area (Fig. B, in Bom Repouso; Fig. D, in Senador Amaral). *The lines followed by the same letter did not differ by the Kruskal–Wallis ($p < 0.05$).
continue

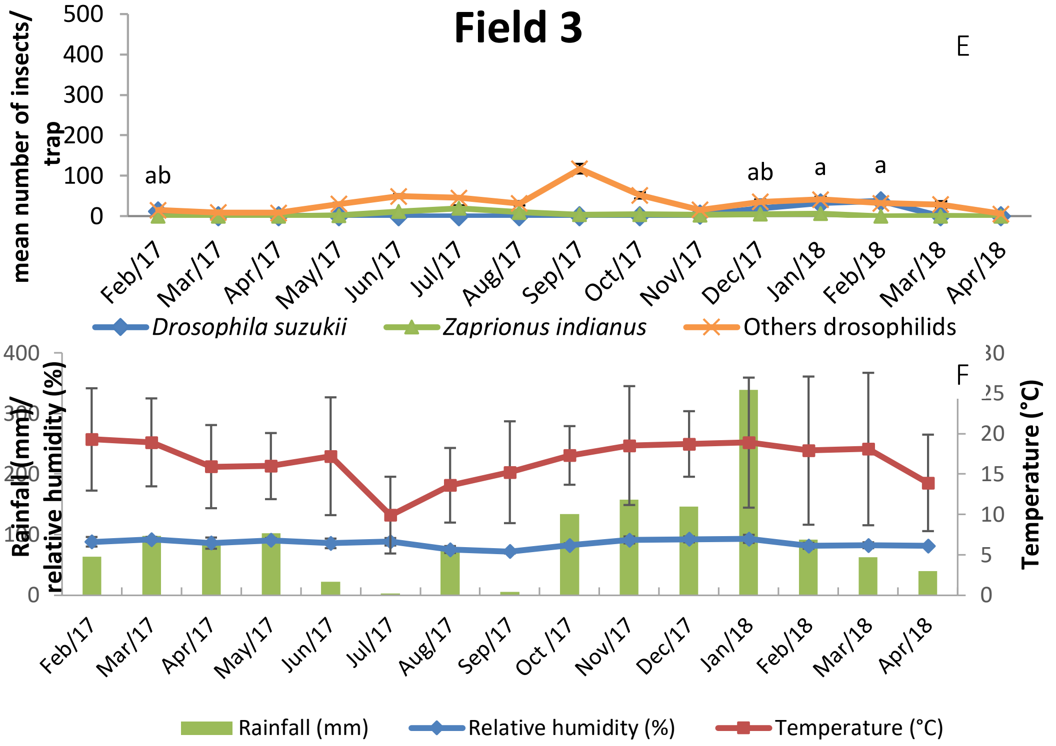

Figure 1. Mean number of insects/traps in strawberry plants under low tunnel (Field 3, Fig. E) in, Bom Repouso, Minas Gerais. Temperature (°C) and humidity (%) data were obtained from a Datalogger and rainfall data (mm) were obtained from a pluviometer installed in the area (Fig. F). *The lines followed by the same letter did not differ by the Kruskal–Wallis ($p < 0.05$). continuation

Using Pearson's correlation of *D. suzukii* densities as a function of climatic variables of temperature (minimum, average and maximum), relative humidity (minimum, average and maximum) and precipitation, significant positive relations were observed between the number of insects and the average temperature and maximum relative humidity levels in all fields (Table 1). For *Z. indianus*, average relative humidity was significantly and negatively related with the number of insects in all fields. For other drosophilids, none of the climatic variables were significantly correlated with the average number of insects.

Table 1. Pearson correlation coefficients (r) and probability (p) of the analysis among the average number of *Drosophila suzukii*, *Zaprionus indianus* and others drosophilids/ trap and the climatic variables of temperature (minimum, average and maximum), relative humidity (minimum, average and maximum) and precipitation of fields 1, 2 and 3.

	Drosophila suzukii						***Zaprionus indianus***						**Others drosophilids**					
	Field 1		**Field 2**		**Field 3**		**Field 1**		**Field 2**		**Field 3**		**Field 1**		**Field 2**		**Field 3**	
	r	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**
Temperature (°C)																		
Maximum	0,3	0,1	0,4	0,2	0,4	0,1	0,1	0,2	0	0,2	-0,2	0,1	0,2	0,2	0,1	0,2	0	0,2
Average	0,6	< 0,001	0,6	< 0,001	0,7	< 0,001	0	0	-0,1	0,2	-0,1	0,1	0	0,1	0	0,1	-0,2	0,1
Minimum	0	0,1	0,1	0,4	0,1	0,5	-0,4	0,2	-0,2	0,2	-0,3	0,1	-0,2	0,1	-0,2	0,1	-0,3	0,1
Relative Humidity (%)																		
Maximum	0,6	< 0,001	0,5	< 0,001	0,6	< 0,001	-0,5	< 0,001	-0,1	0,2	0,1	0,2	-0,1	0,2	-0,1	0,2	-0,5	0,2
Average	-0,1	0,5	0,2	0,6	0,3	0,6	-0,5	< 0,001	-0,5	< 0,001	-0,6	< 0,001	-0,1	0,1	-0,1	0,1	-0,5	0,1
Minimum	-0,1	0,2	0,2	0,2	0,2	0,2	-0,6	< 0,001	-0,2	0,1	-0,1	0,1	0,1	0,1	-0,1	0,1	-0,5	0,1
Rainfall (mm)																		
	0,3	0,1	0,1	0,1	0,3	0,2	0,2	0,2	-0,1	0,2	-0,2	0,1	0,1	0,1	0,2	0,1	-0,2	0,1

p < 0,001: significant Pearson correlation at 95% confidence intervals by Student's t test (p < 0,05).

2.4. Discussion

Finding *D. suzukii* in the three evaluated fields in the first assessment of the present work caused initial concern, as Senador Amaral and Bom Repouso are located about 600 km from the closest site where this insect had been previously reported, in Ervália, Minas Gerais state (Andreazza et al. 2016). However, for most of the duration of the study, the levels of occurrence of *D. suzukii* were low in the three fields, especially in field 3, where cultivation was under low tunnels.

Apparently, no economic damage resulted from the attack of *D. suzukii* or other species of the same family, as growers of the region where the study was conducted did not report reduction in revenue in recent years in comparison with revenues of previous years (personal communication with local growers). In contrast, in southern Brazil, losses were reported to reach about 30% (Santos 2014).

The pattern of occurrence of *D. suzukii* in this study indicated that the highest levels occurred from late spring to late summer (December through March), when temperature, relative humidity and rainfall were highest. This was confirmed with Pearson's correlation analysis (Table 1), except for the non-significant relation with the rainfall, most likely because in the three fields plants and insects were protected from it.

Shi et al. (2010) studied the potential distribution of *D. suzukii* in Brazil determining that more than 50% of the area considered favorable or highly favorable to the proliferation of this species was in the southeast, where the study sites of the present study were located, and where the average temperature is around 25°C (November through April), that is, higher than the overall average for the hottest month in the study sites (18.1–19.4°C). Tochen et al. (2015) studied the development of *D. suzukii* at 20, 33, 71, 82 and 94% RH and at 20.6 ± 0.2°C, concluding that the highest humidity level (94%) related to the highest net reproductive rate and highest intrinsic rate of population increase. Hence, humidity levels in the study sites (overall averages of 84.3 – 85.9 % for the three fields) seem close to level considered satisfactory to the fly.

But in addition to the temperature, which other factors could account for the relatively low levels of occurrence of *D. suzukii* in the region where the study was conducted?

Despite the continuous presence of strawberry fruits in the three fields in this study throughout the study, fruit production was highest from June to December 2017 in fields 1 and 2, and from March to September 2017 in field 3 (personal communication with growers). Those periods do not match the periods of highest fly catches in Fields 1 and 2 (December to March) in Field 3 (January and February), that is, after the period of highest availability of host substrate. That lack of synchrony could at least partially have prevented the insects from reaching higher levels, especially in field 3, where the peak fly levels occurred before the period of highest fruit production.

As the study was done in growers' fields, it was not possible to avoid the applications of chemical and biological control agents by the owners, to reduce other pest problems. In the three fields, acaricides and insecticides were used for the control of *T. urticae*, *Frankliniella occidentalis* (Pergande), *Trialeurodes vaporariorum* (Westwood) and *Neopamera bilobata* (Say). In a revision about the biological control of *D. suzukii*, Wang et al. (2020) reported high mortality levels of flies exposed to conidia of *B. bassiana* and *M. anisopliae* in laboratory investigations. Azadirachtin showed poor efficacy against this fly (Bruck et al. 2011), while lambda-cyhalothrin has demonstrated high toxicity to *D. suzukii* in laboratory assays (Rice et al. 2017). Thus, a possible effect of the biological and chemical products applied for the control of other insects on the drosophilids cannot be disregarded, preventing them from reaching higher levels.

It has been known that *D. suzukii* uses wild fruits along the edges of the field to build their populations before the cultivated hosts become available (Lee et al. 2011a; Cini et al. 2012; Ballman and Drumond 2017; Little et al. 2017). In the region where the present study was conducted, the cultivation of other common hosts of *D. suzuki* (raspberry, blackberry, blueberry, peach and plum) is quite limited. We have not investigated the role of native fruits as hosts to the drosophilids.

It should be asked whether natural enemies present in the area could have accounted for the lower population levels. Many parasitoids have already been reported parasitizing spotted-wing drosophila pupae, including *Pachycrepoideus vindemmiae* (Rondani) and *Trichopria drosophilae* Perkins (Diapriidae) in North America and Europe and *Trichopria anastrephae* Lima in Brazil (Wang et al. 2020). In addition to parasitoids, several predators this pest have been tested both in the laboratory and in the field, among these, terrestrial beetles (Carabidae) and field crickets (*Gryllus pennsylvanicus* Burmeister) showed predatory ability on pupae of this pest, with consumption of up to 60 pupae by *G. pennsylvanicus* under laboratory conditions (Ballman et al 2017). The effect of local parasitoids of the drosophilids has not been investigated, but they have never been observed to emerge from field collected insects occasionally brought to the laboratory. A study carried out in the same region where this study was conducted indicated the presence of a diverse fauna of predatory mites in the soil of strawberry fields and in nearby forest fragments (Esteca et al. 2018), with Blattisociidae, Melicharidae and Parasitidae (all of the order Mesostigmata) as the predominant families. A preliminary evaluation of the ability of those predators and other selected predatory mite species will be reported in a subsequent chapter. Mites of those families are generalist predators, and could conceivably prey on these insects (Castilho et al. 2015; Moraes et al. 2015).

In relation to *Z. indianus*, the observed peak population immediately after the peak population of *D. suzukii* is compatible with results of previous works indicating its attraction to fruits previously infested by *D. suzukii*, following the release of attractive volatiles from the damaged fruits (Van Timmeren and Isaacs 2013; Joshi et al. 2014; Abraham et al. 2015; Lasa and Tadeo 2015), despite the demonstrate ability of the fly to attack healthy strawberries, although at low rate (Bernardi et al. 2017).

Zaprionus indianus has shown high capacity to adapt to different hosts in temperate regions (Ramniwas et al. 2012). Some studies have reported the co-occurrence of *Z. indianus* and *D. suzukii* on grapes in the USA (Van Timmeren and Isaacs 2013, Joshi et al. 2014), in guava in Mexico (Lasa and Tadeo 2015) and in strawberries in southern Brazil (Nava et al. 2015). Therefore, the co-occurrence of both species is very disturbing, given that fruit damage by *D. suzukii*, can potentiate the problems generated by *Z. indianus*.

Although the other drosophilids occurred in relatively high numbers in relation to *D. suzukii* and *Z. indianus*, these are not cause for concern, as they are not able to attack fruits appropriated for human consumption. They are commonly found in rotting fruits and other decomposing organic matter, without being of economic importance (Yassin 2013).

The much lower numbers of *D. suzukii* in the field where strawberry was grown under low tunnel (field 3) may in part be related to the barrier provided by the tunnels to transiting insects, as opposed to the suspended cultivation, in which the sides of the greenhouse were fully open. As field 3 was located in a region subjected to wind of relatively high speed, the tunnels were daily managed so as to maintain closed even at day time their upwind face, which could have disrupted host finding by the insect. Reduced incidence of *D. suzuki* has been reported with the use of screens (Leach et al. 2016; Rogers et al. 2016). Certain plastic films have been related to disruption of the navigation process of the fly, by blocking UV transmission (Antignus 2000; Antignus et al. 2001; Doukas and Payne 2007; Leach et al. 2016; Rogers et al. 2016; Cramer et al. 2019; Fontain et al. 2020). The possibly different effects of the distinct types of plastic of the different fields was not evaluated in this study.

The lower levels of occurrence of *D. suzukii* in the tunnels could in principle also be considered to be due to varietal differences. However, this might not have been the case, as in a study conducted in Croatia, Živković et al. (2019) reported higher incidence of *D. suzukii* in fruits of ‘Albion’ (grown under tunnels in the present study), than in fruits of ‘San Andreas’ (grown in suspended cultivation).

In summary, *D. suzukii* and *Z. indianus* are presently found in southern Minas Gerais on strawberry, but they do not seem to cause significant losses to local growers.

REFERENCES

Abraham, J.; Zhang, A.; Angeli, S.; Abubeker, S.; Michel, C.; Feng, Y.; Rodriguez-Saona, C. (2015) Behavioral and antennal responses of *Drosophila suzukii* (Diptera: Drosophilidae) to volatiles from fruit extracts. Environmental Entomology, 44(2): 356-367.

Andreazza, F.; Haddi, K.; Oliveira, E.E.; Ferreira, J.A.M. (2016). *Drosophila suzukii* (Diptera: Drosophilidae) arrives at Minas Gerais State, a main strawberry production region in Brazil. Florida Entomologist, 99(4): 796-798.

Antignus, Y. (2000) Manipulation of wavelength-dependent behavior of insects: an IPM tool to impede insects and restrict epidemics of insect-borne viruses. Virus research, 71(1-2): 213-220.

Antignus, Y.; Nestel, D.; Cohen, S.; Lapidot, M. (2001) Ultraviolet-deficient greenhouse environment affects whitefly attraction and flight-behavior. Environmental Entomology, 30(2): 394-399.

Arnó, J.; Riudavets, J.; Gabarra, R. (2012) Survey of host plants and natural enemies of *Drosophila suzukii* in an area of strawberry production in Catalonia (northeast Spain). Survey of host plants and natural enemies of *Drosophila suzukii* in an area of strawberry production in Catalonia (northeast Spain), 80: 29-34.

Ballman, E.S.; Collins, J.A.; Drummond, F.A. (2017) Predation on *Drosophila suzukii* (Diptera: Drosophilidae) pupae in Maine wild blueberry fields. Journal of Economic Entomology, 110: 2308-2317.

Ballman, E.S.; Drummond, F.A. (2017) Infestation of wild fruit by *Drosophila suzukii* surrounding Maine wild blueberry fields. Journal of Agricultural and Urban Entomology, 33: 61-70.

Bernardi, D.; Andreazza, F.; Botton, M.; Baronio, C.A.; Nava, D.E. (2017). Susceptibility and interactions of *Drosophila suzukii* and *Zaprionus indianus* (Diptera: Drosophilidae) in damaging strawberry. Neotropical Entomology, 46(1): 1-7.

Bolda, M.P.; Goodhue, R.E.; Zalom, F.G. (2010) Spotted Wing Drosophila: Potential Economic Impact of Newly Established Pest. Agricultural and Resource Economics Update, California, 13(3): 5-8.

Bruck, D.J.; Bolda, M.; Tanigoshi, L.; Klick, J.; Kleiber, J.; DeFrancesco, J.; Gerdeman, B.; Spitler, H. (2011) Laboratory and field comparisons of insecticides to reduce infestation of *Drosophila suzukii* in berry crops. Pest Management Science, 67: 1375-1385.

Calabria, G.; Máca, J.; Bälich, G.; Serra, L.; Pascual, M. (2012) First records of the potential pest species *Drosophila suzukii* (Diptera: Drosophilidae) in Europe. Journal of Applied Entomology, 136: 139-147.

Castilho, R.C.; Venancio, R.; Narita, J.P.Z. (2015b) Mesostigmata as biological control agents, with emphasis on Rhodacaroidea and Parasitoidea. In: Prospects for Biological Control of Plant Feeding Mites and other Harmful Organisms; Carillo D.; Moraes, G.J.; Peña, J.E. (Eds.). Springer International Publishing: Cham, Switzerland, chap. 1, pp 1-32.

Castro, F.L.; Valente, V.L.S. (2001) *Zaprionus indianus* is invading Drosophilid communities in the southern Brazilian city of Porto Alegre. Drosophila Information Service, 84: 15-16.

Chassagnard, M.T.; Kraaijeveld, A.R. (1991) The occurrence of *Zaprionus* sensu stricto in the Palearctic region (Diptera: Drosophilidae). Annales de la Société entomologique de France (N.S.), 27: 495-496.

Cini, A.; Ioriatti, C.; Anfora, G. (2012) A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. Bulletin of Insectology, 65(1): 149-160.

Cramer, M.E.; Demchak, K.; Marini, R.; Leskey, T. (2019) UV-blocking high-tunnel plastics reduce Japanese beetle (*Popillia japonica*) in red raspberry. HortScience, 54(5): 903-909.

David, J.R.; Araripe, L.O.; Bitner-Mathé, B.C.; Capy, P.; Goñi, B.; Klaczko, L.B.; Legout, H.; Martins, M.B.; Voudibio, J.; Yassin, A.; Moreteau, B. (2006) Quantitative trait analysis and geographic variability of natural populations of *Zaprionus indianus*, a recent invader in Brazil. Heredity, 96(1): 53-62.

De Toni, D.C.; Hofmann, P.R.P.; Valente, V.L.S. (2001) First register of *Zaprionus indianus* (Diptera; Drosophilidae) in the state of Santa Catarina. Biotemas, 14(1): 71-85.

Deprá, M.; Poppe, J.L.; Schmitz, H.J.; Toni, D.C.; Valente, V.L.S. (2014) The first records of the invasive pest *Drosophila suzukii* in the South American continent. Journal of Pest Science, 87: 379-383.

Doukas, D.; Payne, C.C. (2007) The use of ultraviolet-blocking films in insect pest management in the UK; effects on naturally occurring arthropod pest and natural enemy populations in a protected cucumber crop. Annals of Applied Biology, 151(2): 221-231.

Esteca, F.C.N.; Rodrigues, L.R.; Moraes, G. J.; Delalibera Jr., I.; Klingen, I. (2018) Mulching with coffee husk and pulp in strawberry affects edaphic predatory mite and spider mite densities. Experimental and Applied Acarology, 76(2): 161-183.

Fachinello, J.C.; Pasa, M.S.; Schmtiz, J.D.; Betemps, D.L. (2011) Situação e perspectivas da fruticultura de clima temperado no Brasil. Revista Brasileira de Fruticultura, 33: 109-120.

Fartyal, R.S.; Sarswat, M.; Lhamo, N.; Sati, P.C.; Asha, L. (2014) Records of *Zaprionus indianus* and *Drosophila suzukii* indicus as invasive fruit pests from mid valley region of Garhwal Uttarakhand. India. Drosophila Information Service, 97: 119-123.

Fontain, M.T.; Badiee, A.; Hemer, S.; Delgado, A.; Mangan, M.; Dowding, C.; Davis, F.; Pearson, S. (2020) The use of light spectrum blocking films to reduce populations of *Drosophila suzukii* Matsumura in fruit crops. Scientific Reports, 10: 15358.

Goodhue, R.E.; Bolda, M.; Farnsworth, D.; Williams, J.C.; Zalom, F.G. (2011) Spotted wing drosophila infestation of California strawberries and raspberries: economic analysis of potential revenue losses and control costs. Pest Management Science, Oxford, 67(11): 1396-1402.

Grassi, A.; Giongo, L.; Palmieri, L. (2011) *Drosophila (Sophophora) suzukii* (Matsumura), new pest of soft fruits in Trentino (North-Italy) and in Europe. Integrated Plant Protection in Soft Fruits. IOBC wprs Bulletin, Budapest, 70: 121-128.

Hauser, M. (2011) A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. Pest Management Science, 67: 1352-1357.

Haviland, D.R.; Beers, E.H. (2012) Chemical control programs for *Drosophila suzukii* that comply with international limitations on pesticide residues for exported sweet cherries. Journal of Integrated Pest Management, 3(2): F1-F6.

Joshi, N.K.; Biddinger, D.J.; Demchak, K.; Deppen, A. (2014) First report of *Zaprionus indianus* (Diptera: Drosophilidae) in commercial fruits and vegetables in Pennsylvania. Journal of Insect Science, 14(1): 259.

Karan, D.; Dubey, S.; Moreteau. B.; Parkash, R.; David, J.R. (2000) Geographical clines for quantitative traits in natural populations of a tropical drosophilid: *Zaprionus indianus*. Genetica, 108: 91–100.

Kato, C. M.; Foureaux, L.V.; César, R. A.; Torres, M. P. (2004) Ocorrência de *Zaprionus indianus* Gupta, 1970 (Diptera: Drosophilidae) no Estado de Minas Gerais. Ciência e agrotecnologia, 28: 454-455.

Kehlenbeck, H.; Robinet, C.; Werf, W. Van Der; Kriticos, D.; Reynaud, P.; Baker, R. (2012) Modelling and mapping spread in pest risk analysis: a generic approach. EPPO Bulletin, 42: 74-80.

Lasa, R.; Tadeo, E. (2015) Invasive drosophilid pests *Drosophila suzukii* and *Zaprionus indianus* (Diptera: Drosophilidae) in Veracruz, Mexico. Florida Entomologist 98: 987-988.

Leach, H.; Van Timmeren, S.; Isaacs, R. (2016) Exclusion netting delays and reduces *Drosophila suzukii* (Diptera: Drosophilidae) infestation in raspberries. Journal of Economic Entomology, 109(5): 2151-2158.

Lee, J.C.; Bruck, D.J.; Curry, H.; Edwards, D.; Haviland, D.R.; Van Steenwyk, R.A.; Yorgey B.M. (2011 a) The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. Pest Management Science, 67: 1358-1367.

Lee, J.C.; Bruck, D.J.; Dreves, A.J.; Ioriatti, C.; Vogt, H.; Baufeld, P. (2011 b) In focus: spotted wing *Drosophila*, *Drosophila suzukii*, across perspectives. Pest Management Science, 67: 1349–1351.

Little, C.M.; Chapman, T.W.; Moreau, D.L.; Hillier, N.K. (2017) Susceptibility of selected boreal fruits and berries to the invasive pest *Drosophila suzukii* (Diptera: Drosophilidae) Pest Management Science, 73: 160-166.

Markow, T.A.; O'Grady, P.M. (2006) *Drosophila*: a guide to species identification and use (chapt. 1, p. 13). Academic, London, United Kingdom.

Moraes, G.J.; Venancio, R.; Santos, V.L.V.; Paschoal, A.D. (2015) Potential of Ascidae, Blattisociidae and Melicharidae (Acari: Mesostigmata) as biological control agents of pest organisms. In: *Prospects for biological control of plant feeding mites and other harmful organisms*; Carillo, D.; Moraes, G.J.; Peña, J.E. (Eds.). Springer International Publishing: Cham, Switzerland, chap. 2, pp. 33–76.

Nava, D.E.; Botton, M.; Bernardi, D.; Andreazza, F.; Baronio, C.A. (2015) Bioecologia, monitoramento e controle de *Drosophila suzukii* na cultura do morangueiro. Embrapa Clima Temperado, Pelotas, Rio Grande do Sul. 28p. (Série documentos 398).

Paula, M.A.; Lopes, P.H.S.; Tidon, R. (2014) First record of *Drosophila suzukii* in the Brazilian Savanna. Drosophila Information Service, 97: 113-115.

R Development Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online: https ://www.R-proje ct.org/ (accessed on 6 January 2021).

Ramniwas, S.; Kajla, B.; Parkash, R. (2012) Extreme physiological tolerance leads the wide distribution of *Zaprionus indianus* (Diptera: Drosophilidae) in temperate world. Acta Entomologica Sinica, 55: 1295–1305.

Rice, K.B.; Short, B.D.; Leskey, T.C. (2017) Development of an attract-and-kill strategy for *Drosophila suzukii* (Diptera: Drosophilidae): evaluation of attracticidal spheres under laboratory and field conditions. Journal of Economic Entomology, 110(2): 535-542.

Rogers, M.A.; Burkness, E.C.; Hutchison, W.D. (2016) Evaluation of high tunnels for management of *Drosophila suzukii* in fall-bearing red raspberries: Potential for reducing insecticide use. Journal of Pest Science, 89(3): 815-821.

Santos, J.F.; Rieger, T.T.; Campos, S.R.C.; Nascimento, A.C.C.; Felix, P.T.; Silva, S.V.O.; Freitas, F.M.R. (2003) Colonization of northeast region of Brazil by the drosophilid flies *Drosophila malerkotliana* and *Zaprionus indianus*, a new potential pest for Brazilian fruit culture. Drosophila Information Service, 86: 92-95.

Santos, R.S.S. (2014) *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) atacando frutos de morangueiro no Brasil. Enciclopédia Biosfera, 10: 4005 4011.

Shi, J.; Luo, Y.Q.; Zhou, F.; He, P. (2010) The relationship between invasive alien species and main climatic zones. Biodiversity and Conservation, 19: 2485-2500.

Silveira, C.A.P.; Martinazzo, R.; Pauletti, V. (2016) Fertilidade do solo e nutrição, *In:* Antunes, L.E.C.; Reisser Junior, C.; Schwengber, J.E. (eds). Morangueiro. Embrapa, Brasília. pp.151-199.

Stein, C.P.; Teixeira, É.P.; Novo, J.P.S. (2003) Aspectos biológicos da mosca do figo, *Zaprionus indianus* Gupta, 1970 (Diptera: Drosophilidae). Entomotropica, 18: 219-221.

Tidon, R.; Leite, D.F.; Leão, B.F.D. (2003) Impact of the colonization of *Zaprionus* (Diptera, Drosophilidae) in different ecosystems of the Neotropical Region: 2 years after the invasion. Biological Conservation, 112: 299-305.

Tochen, S.; Dalton, D.T.; Wiman, N.; Hamm, C.; Shearer, P.W.; Walton, V.M. (2014) Temperature-related development and population parameters for *Drosophila suzukii*

(Diptera: Drosophilidae) on cherry and blueberry. Environmental Entomology, 43: 501-510.

Tochen, S.; Woltz, J.M.; Dalton, D.T.; Lee, J.C.; Wiman, N.G.; Walton, V.M. (2015) Humidity affects populations of *Drosophila suzukii* (Diptera: Drosophilidae) in blueberry. Journal of Applied Entomology, 140: 47-57.

Van der Linde, K. (2010) *Zaprionus indianus*: species identification and taxonomic position. *Drosophila* Information Service, 93: 95.

Van Timmeren, S.; Isaacs, R. (2013) Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs. Crop Protection, 54:126-133.

Vilela, C.R. (1999) Is *Zaprionus indianus* Gupta, 1970 (Diptera, Drosophilidae) currently colonizing the Neotropical region? *Drosophila* Information Service, 82: 37-39.

Vilela, C.R.; Mori, L. (2014) The invasive spotted-wing *Drosophila* (Diptera, Drosophilidae) has been found in the city of São Paulo (Brazil). Revista Brasileira de Entomologia, 58(4): 371-375.

Walsh, D.; Bolda, M.; Goodhue, R.; Dreves, A.; Lee, J.C.; Bruck, D.J.; Walton, V.M.; O'Neal, S.D.; Zalom, F.G. (2011) *Drosophila suzukii* (Diptera: Drosophilidae): Invasive pest of ripening soft fruit expanding its geographic range and damage potential. Integrated Pest Management, 106(2): 289-295.

Wang, X.; Lee, J.C.; Daane, K.M.; Buffington, M.L.; Hoelmer, K.A. (2020). Biological control of *Drosophila suzukii*. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 15(54): 1-19.

Yassin, A. (2013) Phylogenetic classification of the Drosophilidae Rondani (Diptera): the role of morphology in the postgenomic era. Systematic Entomology, 38(2): 349-364.

Živković, I.P.; Duralija, B.; Barić, B.; Seljak, G.; Lemic, D.; Mešic, A. (2019) The development of drosophilid species (Diptera, Drosophilidae) in different strawberry cultivars. European Journal of Horticultural Science, 84(1): 48-52.

3. POTENTIAL PREDATION AND OVIPOSITION RATES OF GAMASINA MITES (ACARI: MESOSTIGMATA) ON DIFFERENT STAGES OF *Drosophila suzukii* (DIPTERA: DROSOPHILIDAE), A STRAWBERRY PEST

Abstract

The spotted-wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is a thin-skinned fruit pest, introduced to Brazil a few years ago. Although this pest has been found in southern Minas Gerais state since early 2017, no apparent economic damage to the crop has been reported, which could be due to the controlling effect of predatory mites. The objective of this work was to verify the potential of predation, oviposition and survivorship of Gamasina predatory mites, most of found in the area where the study was conducted, when offered different stages of *D. suzukii* in the laboratory, at 25 ± 2 ° C and 70 ± 10% RH. The following gamasine species were evaluated: Blatissociidae (*Lasioseius floridensis* Berlese); Laelapidae (*Gaeolaelaps queenslandicus* (Womersley) and *Stratiolaelaps scimitus* (Womersley)); Macrochelidae (*Macrocheles embersoni* Azevedo et al., *Macrocheles merdarius* (Berlese), *Macrocheles muscaedomesticae* (Scopoli) and *Macrocheles robustulus* (Berlese)); Melicharidae (*Proctolaelaps bickleyi* (Bram) and *Proctolaelaps pygmaeus* (Müller)); Parasitidae (*Parasitus* sp.); Phytoseiidae (*Neoseiulus californicus* (McGregor)) and Rhodacaridae (*Protogamasellopsis zaheri* Abo-Shnaf et al.). These were fed with either eggs, larvae or pupae of the pest. *Macrocheles embersoni*, *M. robustulus*, *P. bickleyi* and *P. zaheri* consumed the highest number of pupae (averages of 1.3, 0.7, 0.7 and 0.6 pupas/ female/ day, respectively), whereas *P. zaheri* and *L. floridensis* showed the highest oviposition rates (averages of 0.5 and 0.7 eggs/ female/ day, respectively). *Stratiolaelaps scimitus* was the only one among the evaluated predators that managed to prey on *D. suzukii* eggs inside the fruits. The results obtained with species commonly found in strawberry fields in southern Minas Gerais, *N. californicus*, *P. bickleyi* and *L. floridensis*, were not encouraging. Yet, some of the predators showing ability to attack and oviposit on this insect, such as *M. embersoni*, *M. robustulus P. zaheri* and *S. scimitus*, could be further investigated, as potentially useful for periodic releases.

Keywords: 1. Spotted-wing drosophila 2. Biological control 3. Predatory mites

3.1. Introduction

The spotted-wing drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), has been causing severe losses to strawberry (*Fragaria* x *ananassa* Duch.) cultivation in several countries (Goodhue et al. 2011; Andreazza et al. 2016), for its behavior to oviposit in unripe fruits. When feeding, the larvae damage the fruits and favor the entry of secondary microorganisms (Van Timmeren and Isaacs 2013). Direct losses in production due to this pest were estimated at 20-40% in the United States of America (Bolda et al. 2010; Goodhue et al. 2011; Gerdeman and Tanigoshi 2011) and at 30% in southern Brazil (Santos 2014). However, in southern Minas Gerais, although this pest was detected in February 2017 in strawberry crops grown under suspended and low tunnel cultivation systems (Chapter 2 of this thesis), economic damage has not been reported by the growers.

Some natural enemies have been reported associated with the spotted-wing drosophila, and initial evaluations have been conducted about their potential as control agents. Under laboratory condition, the beetle *Dalotia coriaria* Kraatz (Coleoptera, Staphylinidae) has demonstrated the ability to consume pest eggs and larvae, reducing their numbers by half (Renkema et al. 2015). Also, under laboratory condition, the hemipterans *Anthocoris nemoralis* (F.), *Orius insidiosus* (Say), *Orius laevigatus* (Fieber) and *Orius majusculus* (Reuter) were shown to be able to reduce pest numbers by 66–67% on strawberry and blueberry (Renkema and Cuthbertson 2018). Mortality of 16% has been shown by Alnajjar et al. (2017), with applications of the fungus *Metarhizium robertsii* Bisch., Rehner & Humber on spotted-wing drosophila adults. Pupal parasitoids have also been evaluated, such as *Pachycrepoideus vindemiae* (Rondani), *Trichopria drosophilae* Perkins and *Trichopria anastrephae* Lima in Brazil (Wang et al. 2020). However, while these enemies have shown potential as control agents of this pest in laboratory tests, none of them has so far been reported to be effective in the field (Wang et al. 2020).

Predatory mites are commonly found in the soil and on cultivated plants and plants of the natural vegetation. Most of these belong to the cohort Gamasina, of the order Mesostigmata. Although many of them have been demonstrated to have diverse feeding habits, most are known to prey on small invertebrates, including mites, nematodes and insects. For this reason, members of this group have been extensively studied as candidates for the control of aerial and edaphic pests (McMurtry et al. 2013; Carrillo et al. 2015). Gamasina have been reported in association with different groups of Diptera. *Stratiolaelaps scimitus* (Womersley), of the family Laelapidae, has been used commercially in Brazil and other countries for the control of fungus gnats (Sciaridae) in strawberry and several other cultures (Knapp et al. 2018). Also, many studies have been conducted on the role of gamasines of the family Macrochelidae as predators of Diptera of economic importance, especially of the family Muscidae (Azevedo et al. 2015; 2018).

Some authors reported the predation of *Drosophila melanogaster* Meigen by *Arctoseius cetratus* (Sellnick) (Ascidae) (O'Farrell and Butler 1948; Binns 1972), *Gaeolaelaps aculeifer* (Canestrini) (Laelapidae) (Ignatowicz 1974) and *Proctolaelaps regalis* De Leon (Melicharidae)

(Houck et al. 1991; Houck 1994). A crash in the population of *Drosophila* species was related with the increase in the population of *Proctolaelaps drosophilae* Karg et al., although actual feeding on the flies was not reported by the authors (Karg et al. 1995).

In a study carried out in southern Minas Gerais, several gamasine species were detected in soil-litter samples from a strawberry field (Esteca et al. 2018), the most common being *Blattisocius dentriticus* (Berlese) (Blattisociidae), *Proctolaelaps pygmaeus* (Müller) and the *Parasitus* sp. (Parasitidae). In an unpublished study, *Gaeolaelaps queenslandicus* (Womersley), *Macrocheles muscaedomesticae* (Scopoli) (Macrochelidae) and *Parasitus* sp. (Parasitidae) were the predominant gamasines collected in soil-litter samples from a forest fragment located next to a strawberry field in the same region.

Some publications have reported the temporary but apparently periodic migration of some mites from protected microhabitats to exposed plant parts, especially at night (Onzo et al. 2003; Fagan et al. 2006; Parecis et al. 2016). Esteca et al. (2018) reported the presence of the predominantly edaphic gamasines *P. pygmaeus* and *B. dentriticus* on strawberry leaflets mainly at night, indicating their possible periodic migration from the edaphic environment to the plants. In another study, unidentified species of *Proctolaelaps* and of the blattisociid *Lasioseius* were also reported to migrate from the soil to strawberry leaflets, mainly at night (Esteca et al. 2020). The reason for this behavior has yet to be properly studied. It could be related to search for food, in a period in which absence of light and/ or increase in humidity allow the presence of these predators (with relatively high locomotion capacity in relation to other mites) on plants. These findings suggest that above ground strawberry pests could theoretically be attacked by edaphic Gamasina at those occasions, which could go unnoticed, as most field direct observations by researchers are conducted at day time.

Neoseiulus californicus (McGregor) is a Gamasina of the family Phytoseiidae commonly used by strawberry growers of southern Minas Gerais and other countries for the control of spider mites (Antunes et al. 2016). Given the generalist behavior of *N. californicus* (McMurtry et al. 2013), the spotted-wing drosophila could also be preyed upon by this predator.

Hence, it is possible that the Gamasina found in strawberry crops of southern Minas Gerais could be helping to maintain the spotted-wing drosophila at relatively low levels. The hypothesis evaluated in the present study was that some of the gamasines found in this region are able to attack and reproduce on immature stages of the spotted-wing drosophila. This is the first step in the evaluation of the effect of these predators as control agents of spotted-wing drosophila in the study site.

3.2. Material and Methods

Origin and maintenance of the predators

As reported by Walsh et al. (2011), *D. suzukii* oviposits inside the fruits, where the larvae remain until reaching the third instar, when they move to the soil to pupate. Thus, the selection of predators to be evaluated was made in order to include a species that occurs almost exclusively on plants (*N. californicus*) and others present predominantly in the soil during the day, and that can rise to plants in the night time (Esteca et al. 2018; 2020); these were: Blattisociidae - *Lasioseius floridensis* Berlese; Laelapidae - *G. queenslandicus* and *S. scimitus*; Macrochelidae - *Macrocheles embersoni* Azevedo et al., *Macrocheles merdarius* (Berlese), *Macrocheles robustulus* (Berlese) and *M. muscaedomesticae*; Melicharidae - *Proctolaelaps bickleyi* (Bram) and *P. pygmaeus*; Parasitidae - *Parasitus* sp. and Rhodacaridae - *Protogamasellopsis zaheri* Abo-Shnaf et al..

The specimens of *N. californicus* were obtained from a colony established with mites collected in Piracicaba, São Paulo state, about five months before the beginning of the work. The colony was maintained in the laboratory on *Canavalia ensiformis* L. (Fabaceae) infested with the two-spotted spider mite (*Tetranychus urticae* Koch; Tetranychidae).

Gaeolaelaps queenslandicus, M. muscaedomesticae and *Parasitus* sp. were collected from the soil of a forest fragment located next to a strawberry field in Bom Repouso (22°28'29"S, 46°11'17"W, altitude 1380 m). The colonies were established about three months before the work was initiated. *Lasioseius floridensis, P. bickleyi* and *P. pygmaeus* were collected from fallen strawberry fruits and from coffee husk mulching of a strawberry cultivation in Bom Repouso (22° 28'22"S, 46°11'22"W, altitude 1410 m). The colonies of these species were also established about three months before the beginning of the work. *Macrocheles embersoni, M. merdarius, M. robustulus, P. zaheri* and *S. scimitus* were obtained from rearing units maintained in the laboratory, established with mites collected in different regions of the same state 12 - 24 months before the beginning of the work.

The colonies of all species of soil predators were maintained in the laboratory in plastic units (8 cm in diameter and 7 cm in height), the base of which was covered with a solidified layer (0.5 cm in height) of a paste consisting of plaster and activated charcoal (9v: 1v; Abbatiello 1965). The base was kept permanently humid by the daily addition of mineral water; the unit was sealed with a piece of transparent plastic film (Magipac®) to prevent the mites from escaping. These colonies were fed once every two days with a mixture of different stages of the free-living nematode *Rhabditella axei* (Cobbold), offered on pieces of putrefying bean pods used as a substrate for rearing the nematodes. In the case of the three *Macrocheles* species, in addition to the nematodes, eggs and larvae of the housefly were also offered as food.

All colonies were maintained at 25°C±2°C, 70±10% RH. The colony of *N. californicus* was maintained at a photoperiod of 12 h of light and 12 h of dark, while other colonies were maintained always in the dark.

Origin and maintenance of the prey

Immature stages of *D. suzukii* were obtained from a colony established five months before the beginning of the work, at the Laboratório de Ecologia e Entomologia Florestal at ESALQ / USP. To confirm the identity of the species, 10 adult females were taken from the colony weekly and identified with the help of a stereoscopic microscope (40x), according to the descriptions by Hauser (2011).

The colony was maintained in plastic cages (30 x 21 x 17 cm) screened with "voile" fabric, and fed an artificial diet (Simpson and Raubenheimer 1999). It was maintained at 25±1°C, 70±5% RH and photoperiod of 14 h of light and 10 h of dark.

Experimental procedures

Throughout the study, the experimental units were maintained at 25°C±2°C, 70±10% RH, in the dark.

Predators with free access to prey

All evaluated prey stage was taken from the stock colony, cleaning from the bits of adherent artificial diet and exposed to the predator on the experimental unit. The evaluated predator species were: a) prey pupae - *G. queenslandicus, L. floridensis, M. embersoni, M. merdarius, M. muscaedomesticae, M. robustulus, N. californicus, Parasitus* sp., *P. bickleyi, P. pygmaeus*, *P. zaheri* and *S. scimitus*; b) prey eggs and larvae - *M. embersoni. M. muscaedomesticae, P. bickleyi* and *S. scimitus*.

Each experimental unit consisted of a plastic container (2.5 cm in diameter and 1.0 cm high), whose base was covered with the same mixture of plaster and charcoal mentioned previously and sealed with a piece of transparent plastic film (Magipac®) to prevent the mites from escaping. In addition, cotton threads covered with a piece of coverslip were added to serve as shelter and oviposition site to the predator.

Either five pupae, 20 eggs, ten first instar larvae (L1) or second instar larvae (L2), or five third instar larvae (L3) were introduced into each unit, introducing soon afterward gravid, up to 2 days old, predatory mite into the same unit. Each experimental unit constituted one replicate, for a total of 20 replicates per predator. As a control treatment, ten units were set up with either five pupae, 20 eggs, ten L1, ten L2 or 5 L3 each, but no predator.

The units were examined daily under a stereomicroscope for 11 consecutive days to determine the number of preys attacked, the number of eggs laid by the predator and its survivorship. In each evaluation, the eggs laid were discarded and all prey of each unit (attacked or not) were replaced by new ones. Eggs laid on the first day were excluded from the analysis to avoid the possible influence of previous feeding.

Eggs as prey in fruits

This experiment was carried out to evaluate the ability of predators to access *D. suzukii* eggs inside strawberry fruits. The evaluated predators were: *M. embersoni*, *M. muscaedomesticae, P. bickleyi* and *S. scimitus*.

The eggs were obtained in oviposition chambers, each consisting of a plastic unit (5 cm high and 10 cm in diameter) containing a ripening strawberry and 10 adult females of spotted-wing drosophila, transferred with the help of a vacuum cleaner consisting of a micropipette tip (1mL) attached to a piece of flexible hose (0.5 cm in diameter). The fruit was taken from unsprayed strawberry plants of the cultivar 'Albion' maintained in a screen-house. The units were closed at the top with a 160 µm opening tissue “voil”, allowing gas exchange and preventing flies from escaping.

Two days later, the fruits were examined to discard those with less than 10 eggs, or to remove excess eggs (for a maximum of 10 eggs per fruit), with the aid of a fine needle. Oviposition sites were identified searching for the respiratory filaments externalized in the fruits.

The experimental units used were identical to the oviposition chambers, adding cotton threads under a small piece of coverslip that served as shelter and oviposition site for the predatory mites, sealing the opening of each unit with a piece of transparent plastic film (Magipac®), to prevent the mites from escaping. A fruit with 10 eggs and a gravid, up to 2 days old, predatory mite were transferred to each unit, representing one replicate, for a total of five replicates per predator species. As a control treatment, five units were set up, with one infested fruit but no predator.

Statistical analysis

Three separate analyses were done, for prey in the pupal stage, in the egg or larval stages, and for the ability to prey on eggs inside the fruits. For the three experiments, the mean numbers relative to the predation, oviposition and mite survivorship were adjusted to a Poisson distribution, using χ^2 tests (Anova, test = 'Chisq') to compare treatments. All studies were analyzed at 95% confidence intervals, using the statistical program R Development Core Team (2018).

3.3. Results

Pupae as prey

Adult emergence was 100% in all units without predators. In units containing ten of the twelve evaluated predatory mite species, emergence of adults was reduced, suggesting that to be due to predator attack (Table 1). However, the daily attack rate resulting in reduced emergence seemed to have been relatively low for all predators, the maximum reaching 1.3 pupae per predator (*M. embersoni*). For other predators, the rates were significantly lower (Df=12; χ^2 = 201.2; p< 0.001), ranging from 0.0 to 0.7 pupae per predator. Daily oviposition rates were also low, although significantly higher for *P. zaheri* and *L. floridensis* (0.6 to 0.7) (Df=12; χ^2 = 115.1; p< 0.001). Survivorship rates were generally high (at least 8.8 days during the experiment), except for *P. pygmaeus*, whose average rate was only 5.6 days (Df = 12; χ^2= 68.2; p< 0.001).

Table 1. Daily predation (dead prey ± SE), oviposition (eggs laid ± SE) and average survivorship (in days) of different predators for 10 days when pupae of *D. suzukii* were offered as prey at 25±2°C, 70±10% RH, in the dark.

Mites species	Predation	Oviposition	Survivorship
Blattisociidae			
Lasioseius floridensis Berlese	0.6 ± 0.0 cd	0.7 ± 0.2 a	9.1 ± 0.4 bc
Laelapidae			
Gaeolaelaps queenslandicus (Womersley)	0.2 ± 0.0 f	0.5 ± 0.1 bc	9.2 ± 0.5 abc
Stratiolaelaps scimitus (Womersley)	0.1 ± 0.0 g	0.2 ± 0.0 bc	10.0 ± 0.0 a
Macrochelidae			
Macrocheles embersoni Azevedo et al.	1.3 ± 0.0 a	0.0 ± 0.0 f	10.0 ± 0.0 a
Macrocheles merdarius (Berlese)	0.5 ± 0.1 de	0.2 ± 0.0 cd	8.9 ± 0.5 c
Macrocheles muscadomesticae (Scopoli)	0.0 ± 0.0 g	0.0 ± 0.0 ef	9.7 ± 0.3 ab
Macrocheles robustulus (Berlese)	0.7 ± 0.0 b	0.3 ± 0.0 b	8.9 ± 0.4 c
Melicharidae			
Proctolaelaps bickleyi (Bram)	0.7 ± 0.1 b	0.1 ± 0.0 de	10.0 ± 0.0 a
Proctolaelaps pygmaeus (Müller)	0.5 ± 0.1 de	0.3 ± 0.0 bc	5.6 ± 0.7 d
Parasitidae			
Parasitus sp.	0.5 ± 0.0 e	0.0 ± 0.0 ef	9.2 ± 0.5 abc
Phytoseiidae			
Neoseiulus californicus (McGregor)	0.0 ± 0.0 g	0.0 ± 0.0 ef	8.8 ± 0.1 c
Rhodacaridae			
Protogamasellopsis zaheri Abo-Shnaf et al.	0.6 ± 0.1 bc	0.6 ± 0.1 a	9.6 ± 0.4 ab

In the same column, treatments followed by the same letters do not differ by the χ^2 test (p <0.05).

Exposed eggs and larvae as prey

All the predators evaluated seem to have fed on eggs, as well as on the first, second and third larval stages (Table 2). In general, there was a tendency towards a reduction in the number of prey attacked as they approached the third larval instar, except for *P. bickleyi*, which attacked a very small number of eggs (approximately 2.1 prey eggs/ female/ day), much lower than observed for other predators (9.3 to 10.4 eggs) (Df=3; χ^2= 45.2; *p*< 0.001). For all predators, the number of attacked third instar larvae (1.1 to 1.9/ female/ day) was much lower than the number of larvae of younger instars (2.1 to 4.7 for *P. bickleyi* and 5.1 to 10.4 for the other species).

Regarding oviposition, erratic patterns of variation were observed in relation to the stage of prey (Table 2), but rates obtained when third instar prey were offered as food (0.4 to 0.6 eggs) were never higher than rates obtained on younger prey instars (0.6 to 1.8), for all predators (Df= 3; χ^2= 15.2; p = 0.45). Survivorship rates of predators were always very high, regardless of the stage offered as prey (minimum average of 7.2 days). Statistically, a significantly shorter duration was observed for *M. muscadomesticae* fed with second instar larvae and for *P. bickleyi* fed with third instar larvae (Df=3; χ^2 = 34.2; p< 0.01).

Table 2. Daily predation (average number of prey killed/ female/ day ± EP), daily oviposition (average number of eggs laid/ female/ day ± EP) and average survivorship (in days) of different predators for 10 days when offered differently *D. suzukii* stages as prey at 25 ± 2 ° C, 70 ± 10% RH, in the dark (L1 to L3: larvae from first to third instars).

Stages of *Drosophila suzukii*	*Macrocheles embersoni*	*Macrocheles muscaedomesticae*	*Proctolaelaps bickleyi*	*Stratiolaelaps scimitus*
		Predation		
Egg	9.4 ± 0.1 aA	10.4 ± 1.1 aA	2.1 ± 0.1 bB	9.3 ± 0.6 aA
L1	5.9 ± 0.2 bB	6.8 ± 0.2 aB	4.7 ± 0.1 bA	7.1 ± 0.5 aB
L2	5.1 ± 0.1 bB	5.1 ± 0.2 bB	3.4 ± 0.1 cAB	5.7 ± 0.1 aC
L3	1.4 ± 0.1 aC	1.1 ± 0.1 aC	1.1 ± 0.0 aC	1.9 ± 0.1 aD
		Oviposition		
Egg	1.1 ± 0.1 bA	0.5 ± 0.1 cA	1.2 ± 0.0 abA	1.8 ± 0.1 aA
L1	0.6 ± 0.1 bB	0.8 ± 0.1 bA	0.7 ± 0.0 bAB	1.5 ± 0.2 aA
L2	1.2 ± 0.1 bA	0.7 ± 0.1 bA	1.1 ± 0.0 bA	1.7 ± 0.1 aA
L3	0.6 ± 0.1 aB	0.5 ± 0.1 aA	0.4 ± 0.0 aB	0.6 ± 0.1 aB
		Survivorship		
Egg	10.0 ± 0.0 aA	9.9 ± 0.1 aA	9.7 ± 0.1 aA	9.7 ± 0.2 aA
L1	9.6 ± 0.0 aA	8.9 ± 0.6 bA	10.0 ± 0.1 aA	9.5 ± 0.4 aA
L2	9.6 ± 0.1 aA	7.2 ± 0.6 bB	9.6. ± 0.1 aA	10.0 ± 0.0 aA
L3	9.7 ± 0.1 aA	8.9 ± 0.6 bA	9.0 ± 0.0 aB	9.5 ± 0.4 aA

In the same row, mites followed by the same lower case letters do not differ from each other by the χ^2 test ($p < 0.05$), just as in the same column, mites followed by the same upper case letters do not differ from each other by the same test.

Eggs as prey in fruits

Stratiolaelaps scimitus seemed to have been the only predator able to access the pest eggs inside the fruits (Table 3), for which the daily predation rate was 3.3 eggs per female. Oviposition was 0.7 eggs/ predator/ day and survivorship average, 9.2 days. For other predators, the predation or oviposition rates were zero or nearly zero, with survivorship rates of 5.7–6.0 days.

Tabela 3. Daily predation (average number of dead prey/ female/ day ± EP), daily oviposition (average number of eggs laid / female / day ± EP) and average survivorship (in days) of different predators for 10 days when eggs from *D. suzukii* inside the fruits at 25 ± 2°C, 70 ± 10% RH, in the dark.

Species evaluated	Predation	Oviposition	Survivorship
Macrochelidae			
Macrocheles embersoni	0.0 ± 0.0	0.0 ± 0.1	6.0 ± 0.1
Macrocheles muscaedomesticae	0.0 ± 0.0	0.0 ± 0.1	6.0 ± 0.6
Melicharidae			
Proctolaelaps bickleyi	0.0 ± 0.0	0.1 ± 0.1	5.7 ± 0.5
Laelapidae			
Stratiolaelaps scimitus	3.3 ± 0.1	0.7 ± 0.3	9.2 ± 0.3

3.4. Discussion

From an ecological point of view, the pupa would be the stage most easily attacked by predators, as it is the only immature stage exposed in the environment and because it is found predominantly in the soil. From an applied point of view, pupal predation should receive most attention on the search for efficient predator mites, given that the spotted-wing drosophila is a primary pest.

In view of the emergence of adults from 100% of pupae not exposed to predators, it was assumed that the non-emergence of adults from exposed pupae was due to their action. Although it was not possible to observe ruptures in the pupae as a result of predation by mites, it was observed that predators often tried to insert the chelicerae in the spiracle of the insect, often causing the darkening of this region and the non-emergence of adults, which is extremely satisfactory. from the control point of view. In addition, some pupae became completely shriveled during exposure to predators, although it was not possible to observe other changes due to the attack. Also, in these cases, it was assumed that the non-emergence of adults from pupae exposed to predators was due to their action.

The adopted methodology allowed an estimation of the number of pupae attacked, but the actual number may have been underestimated, considering that the attack by the mite may not always have resulted in the non-emergence of prey adults. This phenomenon could be linked to the small volume of food consumed by each predator in relation to prey biomass, to be expected given the small size of each predator (length ranging from about 400 μm for *N. californicus* to 1400 μm for *Parasitus* sp.) in relation to the size of the pupa (length of about 3400 μm).

The results obtained were not very expressive when pupae of the pest were offered to Gamasina, with the maximum level of daily predation being only 1.3 pupae per predator, in this case, *M. embersoni*. This number is low compared to the number of eggs produced daily by spotted-wing drosophila (7-16 eggs; Walsh et al. 2011). However, it must be considered that the effect of predators would be at the end of the developmental phase of the pest, that is, after the possible controlling effect exerted by other environmental factors on the insect, adding to this.

On the other hand, as strawberry production is not concentrated in a restricted period, but in southern Minas Gerais spread along several months, the action of control agents of this primary pest, is certainly highly desirable even in the pre-pupal stages, due to the potential to reduce the pest population over the harvesting period, hypothetically gradually decreasing its population levels.

The greater impact of Gamasina on younger phases of their prey compared to later phases has been extensively cited in the literature, especially for phytoseiid predators (Filgueiras et al. 2020; Bazgir et al. 2020). This is often related to the lower biomass of the younger phases, forcing predators to consume a larger number of preys to satisfy their needs. But this would apparently not be the case in the present study, given the large biomass of each prey egg (600 µm in length) compared to the size of predators, even in the case of larger predators. Thus, the greater predation of the earliest stages of development could be due to other factors, such as the lower resistance of the egg chorion (to the penetration of the predator's chelicerae) compared to the resistance offered by the exoskeleton of the subsequent stages, in addition to other factors.

When eggs, first and second instar larvae were offered to predators, the results were more encouraging than when pupae were offered. This effect was observed especially when the predators were *M. embersoni* and *M. muscaedomesticae* that managed to prey on a relatively high number of prey eggs, while *S. scimitus* managed to prey on a significant number of first and second instar larvae.

When evaluating the potential of predators' access to pest eggs inside the fruits, *S. scimitus* was the only promising species. This ability could be related to the long chelicerae of this mite (almost 150 µm in length, from the region of articulation between the basal and median segments to the tip of the fixed digit). The chelicerae of other species were considerable shorter (52 µm for *P. bickleyi*, 95 for *M. muscadomesticae* and 114 µm for *M. embersoni*). However, the access potential could be related to other factors, not evaluated in the present study. Mites of the family Laelapidae have been collected on strawberry plants in Atibaia, state of São Paulo, mainly at night (Chapter 5), suggesting that *S. scimitus* (from this same family), may also have some positive action in the control of this pest on strawberry plants.

Some previous studies have reported the potential of *Proctolaelaps* species as predators of drosophilid species (Houck et al. 1991; Houck 1994; Karg et al. 1995), including one of the species evaluated in the present study, *P. pygmaeus*. Cantelo and Boswell (1973) and Ashburner (1989) reported that this predator caused a decrease in the population of *Drosophila* colonies maintained in the laboratory. However, in the present study, the two species of *Proctolaelaps* evaluated, *P. bickleyi* and *P. pygmaeus*, were not promising.

Nawar et al. (1990) showed that *D. melanogaster* allows the development and reproduction of *Lasioseius allii* Chant (= *bispinosus* Evans) in the laboratory. However, in the present study *L. floridensis* did not show promising results, although it preyed and oviposited when fed with spotted-wing drosophila pupae. The predation rate of *Parasitus* sp. was very low, and consequently oviposition did not occur. In the literature, *Pergamasus longicornis* (Berlese), of this family, has been reported as a predator of immature *D. melanog*aster, with a preference for third instar larvae (Bowman 1987).

Before the conduction of this work, it was expected that the predation of some stages of the pest by the phytoseid *N. californicus* could be observed, as it is considered a generalist predator (McMurtry et al. 2013), with proven ability to feed on thrips. The main interest in the inclusion of this predator in the study is due to its natural occurrence on strawberry plants and in its periodic release by the producers, for the biological control of mites. However, the results were not promising, even on eggs of the spotted-wing drosophila there was no predation or oviposition.

Although many studies report the association of natural enemies with spotted-wing drosophila, this is the first study to assess the predation, oviposition and survivorship of Gamasina when exposed to the different immature stages of the pest. The results of the study were not conclusive in showing that the low population level of spotted-wing drosohila in the south of Minas Gerais is due to the action of the predatory Gamasina.

The results obtained with species commonly found in strawberry fields in southern Minas Gerais, *N. californicus*, *P. bickleyi* and *L. floridensis*, were not encouraging. Yet, some of the predators showing ability to attack and oviposit on this insect, such as *M. embersoni*, *M. robustulus*, *P. zaheri* and *S. scimitus*, could be further investigated, as potentially useful for periodic releases. It is noteworthy that most of the study was conducted with pest insects produced on artificial diet, and that better results could be attained with insects developing in strawberry fruits. It has been shown in the literature that natural enemies are highly influenced by kairomones, that commonly results from the interaction of the pest and its host plant (Ayelo et al. 2021).

REFERENCES

Abbatiello, M.J. (1965) A culture chamber for rearing soil mites. Turtox News, Chicago, 43:162-164.

Alnajjar, G.; Drummond, F.A.; Groden, E. (2017) Laboratory and field susceptibility of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) to entomopathogenic fungal mycoses. Journal of Agricultural and Urban Entomology; 33: 111-32.

Andreazza, F.; Haddi, K.; Oliveira, E.E.; Ferreira, J.A.M. (2016) *Drosophila suzukii* (Diptera: Drosophilidae) arrives at Minas Gerais State, a main strawberry production region in Brazil. Florida Entomologist, 99(4): 796-798.

Antunes, L.E.C.; Reisser Junior, C.; Schwengber, J. E. (2016) Morangueiro. Embrapa Clima Temperado-Livro técnico (INFOTECA-E).

Ashburner, M. (1989) *Drosophila*: a laboratory handbook. Cold Spring Harbor Laboratory Press.

Azevedo, L.H.; Emberson, R.M.; Esteca, F.C.N.; Moraes, G.J. (2015) Macrochelid mites (Mesostigmata: Macrochelidae) as biological control agents. In: *Prospects for biological control of plant feeding mites and other harmful organisms*; Carillo, D.; Moraes, G.J.; Peña, J.E. (Eds.). Springer International Publishing: Cham, Switzerland, chap. 4, pp 103–132.

Azevedo, L.H.; Ferreira, M.P.; Castilho, R.C.; Cançado, P.H.D.; Moraes, G.J. (2018) Potential of *Macrocheles* species (Acari: Mesostigmata: Macrochelidae) as control agents of harmful flies (Diptera) and biology of *Macrocheles embersoni* Azevedo, Castilho and Berto on *Stomoxys calcitrans* (L.) and *Musca domestica* (L.) (Diptera: Muscidae). Biological Control, 123: 1-8.

Bazgir, F.; Shakarami, J.; Jafari, S. (2020) Prey-stage preferences, functional and numerical responses, and mutual interference of *Typhlodromus bagdasarjani* (Acari: Phytoseiidae) on *Eotetranychus frosti* (Tetranychidae). International Journal of Acarology, 46(3): 185-191.

Binns, E.S. (1972) *Arctoseius cetratus* (Sellnick) (Acarina: Ascidae) phoretic on mushroom sciarid flies. Acarologia, 14: 350-356.

Bolda, M.P.; Goodhue, R.E.; Zalom, F.G. (2010) Spotted Wing Drosophila: Potential Economic Impact of Newly Established Pest. Agricultural and Resource Economics Update, California, 13(3): 5-8.

Bowman, C.E. (1987) Studies on feeding in the soil predatory mite *Pergamasus longicornis* (Berlese) (Mesostigmata: Parasitidae) using dipteran and microarthropod prey. Experimental and Applied Acarology, 3(3): 201-206.

Cantelo, W.W.; Boswell, A.L. (1973) Mite control with chemicals: a *Drosophila* culture. *Drosophila* Information Service, 50: 163

Carrillo, D.; Moraes, G.J.; Peña, J.E. (2015) Prospects for Biological Control. of Plant. Feeding Mites and Other Harmful Organisms; Springer International Publishing: Cham, Switzerland, p. 337.

Esteca, F.C.N.; Rodrigues, L.R.; Moraes, G.J.; Delalibera Jr., I.; Klingen, I. (2018) Mulching with coffee husk and pulp in strawberry affects edaphic predatory mite and spider mite densities. Experimental and Applied Acarology, 76: 161-183.

Esteca, F.C.N.; Trandem, N.; Klingen, I.; Santos, J.C.; Delalibera Jr., I.; Moraes, G.J. (2020) Cereal straw mulching in strawberry – a facilitator of plant visits by edaphic predatory mites at night? Diversity, 12(6): 242.

Fagan, L.L.; Didham, R.K.; Winchester, N.N.; Behan-Pelletier, V.; Clayton, M.; Lindquist, E.E.; Ring, R.A. (2006) An experimental assessment of biodiversity and species turnover in terrestrial versus canopy leaf litter. Oecologia, 147: 335–347.

Filgueiras, R.M.C.; Mendes, J.D.A.; Da Silva, F.W.B.; Neto, E.P.D.S.; Melo, J.W.D.S. (2020). Prey stage preference and functional and numerical responses *of Neoseiulus barkeri* Hughes (Acari: Phytoseiidae) to eggs of *Raoiella indica* Hirst (Acari: Tenuipalpidae). Systematic and Applied Acarology, 25(6): 1147-1157.

Gerdeman BS, Tanigoshi LK (2011) Biology and management of spotted wing drosophila, *Drosophila suzukii* (Matsumura) in small fruits in the Pacific Northwest. IOBC/WPRS Bulletin, 70: 129-136.

Goodhue, R.E.; Bolda, M.; Farnsworth, D.; Williams, J.C.; Zalom, F.G. (2011) Spotted wing drosophila infestation of California strawberries and raspberries: economic analysis of potential revenue losses and control costs. Pest Management Science, Oxford, 67(11): 1396-1402.

Hauser, M. (2011) A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. Pest Management Science, 67: 1352-1357.

Houck, M.A. (1994) Mites as potential horizontal transfer vectors of eukaryotic mobile genes: *Proctolaelaps regalis* as a model. In: Houck, M.A. (ed) Mites: ecological & evolutionary analyses of the life-history patterns. Chapman & Hall, London, pp. 45-69.

Houck, M.A.; Clark, J.B.; Peterson, K.R.; Kidwell, M.G. (1991) Possible horizontal transfer of *Drosophila* genes by the mite *Proctolaelaps regalis*. Science, 253:1125-11297.

Ignatowicz, S. (1974) Observations on the biology and development of *Hypoaspis aculeifer* Canestrini, 1885 (Acarina: Gamsides). Zoologica Poloniae, 24: 41-59.

Karg, W.; Baker, R.A.; Jenkinson, L. (1995) Mite infestation in *Drosophila* cultures and description of a new mite species of the genus *Proctolaelaps* Berlese with notes on its control in the laboratory (Acarina, Parasitiformes). Deutsche Entomologische Zeitschrift, 42(1): 187-190.

Knapp, M.; van Houten, Y.; van Baal, E.; Groot, T. (2018) Use of predatory mites in commercial biocontrol: current status and future prospects. Acarologia, 58(Suppl): 72-82.

McMurtry, J.A.; Moraes, G.J.; Sourassou, N.F. (2013) Revision of the lifestyles of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. Systematic and Applied Acarology, 18: 297-320.

Nawar, M.S.; Rakha, M.A.; Ali, F.S. (1990) Laboratory studies on the predaceous mite, *Lasioseius bispinosus* Evans (Acari: Mesostigmata: Ascidae) on various kinds of food substances. Bulletin de la Société Entomologique d'Égypte, 69: 247-255.

O'Farrell, A.F.; Butler, P.M, (1948) Insects and mites associated with the storage and manufacture of foodstuffs in Northern Ireland. The Economic Proceedings of the Royal Dublin Society, 3: 343-407.

Onzo, A.; Hanna, R.; Zannou, I.; Sabelis, M.W.; Yaninek, J.S. (2003) Dynamics of refuge use: Diurnal, vertical migration by predatory and herbivorous mites within cassava plants. Oikos, 101: 59-69.

Parecis-Silva, P.V.; Nuvoloni, F.M.; Feres, R.J. (2016) Day vs. night: The importance of the circadian cycle over metacommunities and predator–prey densities. International Journal of Acarology, 42: 141-148.

Ayelo, P.M.; Pirk, C.W.W.; Yusuf, A.A.; Chailleux, A.; Mohamed; S.A.; Deletre, E. (2021) Exploring the Kairomone-based foraging behaviour of natural enemies to enhance biological control: a review. Fronteirs in Ecology Evolution, 9, Article 641974.

R Development Core Team (2018) R: A Language and Environment for Statistical Computing, v.3.0.1; R foundation for Statistical Computing: Vienna, Austria.

Renkema, J.M.; Cuthbertson, A.G.S. (2018) Impact of multiple natural enemies on immature *Drosophila suzukii* in strawberries and blueberries. BioControl, 63: 719-728.

Renkema, J.M.; Telfer, Z.; Gariepy, T.; Hallett, R.H. (2015) *Dalotia coriaria* as a predator of *Drosophila suzukii*: Functional responses, reduced fruit infestation and molecular diagnostics. Biological Control, 89: 1-10.

Santos, R.S.S. (2014) *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) atacando frutos de morangueiro no Brasil. Embrapa, Bento Gonçalves, Brazil, p 4 (Comunicado Técnico 159).

Simpson, S.J.; Raubenheimer, D. (1999) Assuaging nutritional complexity: a geometrical approach. Proceedings of the Nutrition Society, 58(4): 779-789.

Van Timmeren, S.; Isaacs, R. (2013) Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs. Crop Protection, 54: 126-133.

Walsh, D.; Bolda, M.; Goodhue, R.; Dreves, A.; Lee, J.C.; Bruck, D.J.; Walton, V.M.; O'Neal, S.D.; Zalom, F.G. (2011) *Drosophila suzukii* (Diptera: Drosophilidae): Invasive pest of ripening soft fruit expanding its geographic range and damage potential. Integrated Pest Management, 106(2): 289-295.

Wang, X.; Lee, J.C.; Daane, K.M.; Buffington, M.L.; Hoelmer, K.A. (2020). Biological control of *Drosophila suzukii*. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 15(54): 1-19.

4. EFFECT OF *Metarhizium humberi* LUZ ET AL. (ESALQ 1638) (HYPOCREALES: CLAVICIPITACEAE) IN PEST CONTROL AND IN THE PRODUCTIVITY OF STRAWBERRY CROP

Abstract

Recent studies with the fungus *Metarhizium humberi* Luz et al. (Hypocreales: Clavicipitaceae) (ESALQ 1638) have indicated this species as promising for pest control. The objective of this work was to evaluate the potential of *M. humberi* (ESALQ 1638): a) in the colonization of the planting substrate and endophytically to strawberry plants conducted under suspended cultivation; b) as an agent to control insects and mites, pests and pathogens; and c) as a promoter of increased strawberry productivity. The experiment was carried out in a commercial suspended strawberry field, located in Bom Repouso, Minas Gerais, Brazil. The experimental design was randomized in blocks, with two treatments (area where the *M. humberi* (ESALQ 1638) was applied and an area without application (control area)) and three replications (three greenhouses 40 m long x 15 m wide), each treatment consisted of 30 sampling points. The fungus was monthly inoculated (from May 2017 to March 2019) by dripping the conidia suspension onto the substrate through the irrigation system. At the end of the experiment, thirty samples of planting substrate (70 cm^3 each) and thirty whole strawberry plants were removed from each of the two treatments. Monthly, arthropods and disease symptoms were evaluated in 15 leaflets and 15 flowers randomly taken from neighboring plants at each sampling point. The harvest of fruits to evaluate productivity was carried out twice a week (May 2017 and ending in December 2018). This microorganism was recovered from samples of planting substrate, root, petiole and leaflet of inoculated plants, while in samples of fruits, the result was not clear. In some of the months evaluated, the inoculated plants had a statistically lower mean number: of the thrips/flower, of the two-spotted spider mite/leaflets and black flower/plant, while there was a higher mean number of Phytoseiidae/leaflets. Considering all evaluations, the average number of fruits/plant did not differ statistically between both treatments. Future work needs to investigate whether the results obtained here were only possible due to the monthly inoculation of beneficial fungi or whether the results would also be promising at more spaced intervals.

Keywords: 1. Beneficial fungi 2. Pest control 3. Endophytic colonization 4. Plant growth promotion

4.1. Introduction

Strawberry (*Fragaria* x *ananassa* Duch.) production is conducted in Brazil in temperate and subtropical regions, where the total amount of about 165 thousand tons is produced in nearly 4,500 ha (FAOSTAT 2019). About 50% of the production is done in southern Minas

Gerais (Antunes et al. 2020). As in other countries, strawberry plants are severely damaged in Brazil by pests and diseases.

The two-spotted spider mite (*Tetranychus urticae* Koch, Acari: Tetranychidae) has traditionally been considered one of its main pests in Brazil and elsewhere (Solomon et al. 2001). Additionally, several other pests are considered important to the crop worldwide, such as thrips (*Frankliniella occidentalis* (Pergande), Thysanoptera: Thripidae); aphids (*Chaetosiphon fragaefolli* (Cockerell), Hemiptera: Aphididae); whiteflies (*Trialeurodes vaporariorum* (Westwood), Hemiptera: Aleyrodidae); bugs (*Neopamera bilobata* (Say), Hemiptera: Rhyparochromidae); flies (*Drosophila suzukii* (Matsumura), Diptera: Drosophilidae) and beetles (*Maecolaspis* sp., reported as *Colaspis* sp., Coleoptera, Chrysomelidae-Eumolpinae) (Kuhn et al. 2014; Bernardi et al. 2015; Andreazza et al. 2016; Dara 2016, Montagnana et al. 2017).

Gray mold, *Botrytis cinerea* (Persoon: Fries), is the main fungus associated with fruits under field and postharvest conditions (Barakat and Al-Masri 2017). Micosferela leaf spot (*Mycosphaerella fragariae* (Tul.) Lindau) and dendrophoma (*Dendrophoma obscurans* (Ell & Ev.) HW Anderson) are the main foliar diseases and can be found in all regions where the crop is grown (Ueno et al. 2014). Anthracnose (*Colletotrichum acutatum* Simmonds) is one of the most important and destructive strawberry diseases in all production regions in Brazil (Dias et al. 2007; Ueno et al. 2014). Angular spot, also known as "bacterial spot", (*Xanthomonas fragariae* Kennedy and King), can cause high losses, especially where sprinkler irrigation is used (Tanaka et al. 2005; Ueno et al. 2014).

The application of agrochemicals has been the most common pest control method worldwide, but the effectiveness of this method has been markedly reduced by the emergence of resistant insects, mites and pathogens. Furthermore, this method can cause negative effects on the environment, producers and consumers (Van Lenteren 2012). Therefore, great pressure is currently exerted by the international market for the use of less aggressive methods of pest control in strawberry production.

Biological control agents accounts today for only about 2% of the total business related to pest control, but it has been estimated that the use of those organisms will increase at a yearly rate between 20 and 25% in Brazil (ABCBio 2019) and 16% worldwide (DunhamTrimmer's 2019) in the coming years. Two species of predatory mites of the family Phytoseiidae have been used to control the spider mite in Brazil, *Phytoseiulus macropilis* (Banks) and *Neoseiulus californicus* (McGregor). In addition, the predatory mite *Stratiolaelaps scimitus* (Womersley)

(Laelapidae) has been commercialized for the control of thrips and flies of the family Sciaridae (fungus gnats).

In addition to predatory mites, some species of beneficial fungi have also being marketed in Brazil for the control of various pests. *Metarhizium* (Hypocreales: Clavicipitaceae) is an important group of beneficial fungi of the phylum Ascomycota (Van Linteren et al. 2018). The best known type of action of this group refers to the infection of arthropods, acting as important natural enemies in different crops (Vega et al. 2009). Worldwide, approximately 31 species of this genus are recognized (Luz et al. 2019), 13 of which have been reported from Brazil. *Metarhizium robertsii* Bisch et al. and *Metarhizium anisopliae* (Metsch.) are the most abundant species found in Brazilian soils (Iwanicki et al. 2019).

Metarhizium humberi Luz et al. (ESALQ 1638) was collected from soil for the first time in 2001 in Goiás state (Luz et al. 2019). Evaluations of this fungus since then have indicated it as a promising species for pest control (Siqueira et al. 2020). However, until now *M. anisopliae* is the only species of this genus commercialized in Brazil for pest control (AGROFIT 2021). This fungus has been used on a large scale in this country for this purpose for many years; it is estimated that more than three million hectares are treated annually in Brazil with *M. anisopliae* for the control of leafhoppers (Hemiptera: Cercopidae) in sugarcane (Mascarin et al. 2019).

In addition to arthropod infection, other effects of *Metarhizium* species on agricultural production have been determined, such as: a) increase in availability of inorganic nutrients to plants, by promoting their solubilization in the rhizosphere; b) increase in nitrogen availability for the killing arthropods in the same micro-habitat, (Bixby-Brosi and Potter 2012); c) increase in plant root and shoot growth resulting from the production of phytohormones, during endophytic colonization (Steinwender et al. 2015; Canassa et al. 2019); and d) antagonism to phytopathogens (Sasan and Bidochka 2012).

Most studies on beneficial fungi as plant inoculants for pest and pathogen control have been carried out under laboratory conditions; few investigations have been conducted under field condition (Jaber and Ownley 2018). One field study was conducted in the United Kingdom, promising results were obtained with the submersion of strawberry roots in a solution of *Metarhizium brunneum* Petch [reported as *M. anisopliae*] for the control of black vine weevil larvae (*Otiorhyncus sulcatus* (Fabricius), Coleoptera: Curculionidae) (Ansari and Butt 2013).

Considering all the potential benefits of the application of beneficial fungi to a crop, and the positive result of the study of Ansari and Butt (2013), the present work was conducted to evaluate the following hypothesis: the application of the ESALQ 1638 isolate of *M. humberi* in strawberry cultivation leads to its colonization of the growing substrate and plants (endophytically), reducing the occurrence of pests and pathogens and increasing productivity.

4.2. Material and Methods

This experiment was carried out in a commercial strawberry field (latitude: 22°28’27” S, longitude: 46°9’39” W and altitude: 1346 m) cultivated by the suspended system in Bom Repouso, Minas Gerais. The seedlings (cultivar 'San Andreas') were planted in mid-December 2016 in white plastic bags (“slabs”) with dimensions of 150 x 50 cm, which were 30 cm in diameter when filled. The planting substrate was composed of 70% carbonized rice husk and 30% ground pine husk. The bags were lined on benches about 1.0 m apart, 15 m long and 1.25 m high. Each row of substrate had two plant lines 35 cm from each other and with plants 0.5 cm apart on each line. The experimental design was completely randomized blocks, with two treatments (with and without application of the fungus) and three blocks (each a greenhouse 64 m long x 15 m wide), about 200 m from each other. In each block, 6000 plants were subjected to each treatment. In the area corresponding to each treatment, 10 sampling areas were demarcated in the beginning of the study, 10 – 20 m from each other.

The general management of the crop was carried out homogeneously in the whole greenhouses. Plants were drip irrigated every 3 days, at a flow rate of 170 mL/plant/day. The application of macro and micronutrients was carried out via irrigation (fertirrigation), according to recommendations of Silveira et al. (2016). In addition, the foliar fertilizer Raizal® (100g/100L) was applied 10 and 25 days after transplanting and, according to the need identified by the foliar chemical analysis, the following fertilizers were applied in foliar applications: Kinglife K® 400 (200 g/100 L), GelyFlow-Cab® (50mL/100L) and Wuxal CA CS® (100mL/100L).

Temperature and relative humidity were determined every 6 hours with a Datalogger HT-500 Instrutherm® installed in one of the greenhouses. Rainfall (mm) was determined with a pluviometer installed at an equidistant point from the three greenhouses, recording the data daily.

Obtaining the fungus and confirming its viability

The fungus used in the study, *M. humberi* (ESALQ 1638), was obtained from a soil sample collected in 2001 in Goiás state and since then stored at “Prof. Sérgio Batista Alves”

Entomopathogen Collection, Laboratory of Pathology and Microbial Insect Control of ESALQ/USP, Piracicaba, at -80°C. It was cultured for 7–10 days in Potato Dextrose Agar medium (BDA, Difco, USA), in a B.O.D. (Biology Oxygen Demand) chamber, at 26°C and 12 h of daily photophase. After that period, the conidia were harvested by adding 10 ml of sterile 0.05% Tween 80 to the culture, scraping them with a sterile spatula. Conidia concentrations were estimated using a Neubauer chamber and adjusted to 1×10^8 conidia ml^{-1}. Subsequently, 10 ml of the suspension was inoculated with a pipette into individual polypropylene bags (35 cm long x 22 cm wide) containing 300 g of autoclaved parboiled rice (121°C, 20 min), in an aseptic flow chamber laminar.

Rice grains inoculated with the fungus were mixed in plastic bags and incubated in the dark at 25°C. The bags were gently shaken every 2 days to ensure evenly distribution of the fungus. After 10 days, the bags were vacuum sealed and frozen at -20°C until use (Jaronski and Jackson 2012).

To confirm the viability of each volume of fungus to be used throughout the experiment, the proportion of conidium germination was evaluated 5 days before use, according to the methodology of Oliveira et al. (2015). For this, a suspension of conidia was prepared by adding 1 g of rice with sporulating fungi taken from the plastic bag to 10 ml of sterile 0.05% Tween 80. From the third dilution, 150 µl of the conidia suspension were transferred with a pipette to Rodac® Petri dishes containing 4 ml of PDA culture medium plus the fungicide Derosal® 500 SC (10 microliters/L) (active ingredient Carbendazim, Bayer CropScience, São Paulo, Brazil). The plates were kept in a B.O.D chamber for 24 h at 26°C and 12 h of daily photophase. The numbers of germinated and non-germinated conidia were determined under an optical microscope with at 400x magnification.

Application of the fungus to plants

Monthly, the original concentration of conidia per gram of rice grains varied between $3.8 \times 10^8 g^{-1}$ and $5.5 \times 10^8 g^{-1}$. The concentration was adjusted to 1.5×10^{12} conidia in 3.0 to 4.0 kg of rice, depending on the original conidia concentration.

Rice grains colonized with the fungus were added to well water plus 0.01% Tween 80 (0.1 mL 0.01% Tween 80/1L water) in a reservoir, where they were mixed until the rice grains turned completely white, indicating that the conidia had detached from them, resulting in a solution with 1.5×10^6 conidia ml^{-1}. The conidia solution was sieved and transferred to a reservoir from where it was sent to the plants' irrigation system.

Fungus inoculation was performed monthly (May 2017 to March 2019) by dripping the conidia suspension onto the substrate through the irrigation system at a flow rate of 2 L/h for 1 h (each pulse of irrigation lasting 5 minutes, with a volume applied in each pulse of 7.5 mL, generating a total of 90 mL per plant). The fungus was applied by itself, with no other additional products.

Parameters evaluated

The following parameters were evaluated: a) ability of the fungus to colonize the planting substrate and the plant (endophytically); b) levels of occurrence of arthropods and pathogens; c) crop productivity in different treatments.

Determination of fungus colonization in the planting substrate and on the plant

Isolation of the fungus was carried out using selective agar medium, composed of Potato Dextrose Agar (BDA, Difco, USA) with 0.5 g/L of 65% Dodine (Dodex 450 SC – Sipcam Isagro, Brazil), 0.5 g /L of Cyclohexamide, 0.2 g/L of Chloramphenicol and 0.01 g/L of crystal violet. These products were added to the PDA as recommended by Behie et al. (2015), to reduce contaminating fungi and bacteria that normally occur in the soil.

Isolation of the fungus from the planting substrate

Thirty days after the last inoculation of the fungus (by irrigation), a sample of about 70 cm^3 of the planting substrate were collected from each sampling area treatment (total of 30 samples per treatment). Each sample consisted of two sub-samples of 35 cm^3 of planting substrate collected 10 cm from each side of a given plant. Each sample was stored in a sterile plastic bag, and the bags were kept in a Styrofoam box with freezing gel (Gelox®), keeping the temperature at approximately 15°C, for transportation to the laboratory. Each sample was homogenized, transferring 1 g of the substrate to a test tube containing 90 mL of sterile distilled water and 0.05% Tween 80 (Oxiteno, Brazil). The contents of the tubes were homogenized in a Vortex and diluted in series (10^{-1}, 10^{-2}, 10^{-3}). A volume of 0.1 ml of each dilution was then inoculated in duplicate into Petri dishes (90 x 15 mm) containing the selective PDA medium (as described above), and these were kept for 15 days in a chamber at 25 ± 1°C, 70 ± 10% relative humidity and 12 h daily photoperiod. In this time, the plates were evaluated daily to quantify the presence of fungus colonies, by observing the morphological characteristics described by Luz et al. (2019).

Isolation of the fungus from the root, petiole, leaflet and fruit

On the same date substrate samples were collected, a strawberry plant was taken from each sampling area of both treatments to assess the endophytic colonization of the fungus. Each plant was stored in a sterile plastic bag, and the bags were kept and transported as described above.

Roots, petioles, leaflets and fruits were washed with distilled and sterilized water and cut into small sections (approximately 7 cm). For each plant organ, a subsample of 10 g was separated and superficially sterilized (Greenfield et al. 2016), dipping it for 2 minutes in 70% ethanol, for 3 minutes in 2% sodium hypochlorite, for 2 minutes in 70% ethanol, and finally washed three times in sterile distilled water.

Sterilization efficiency was confirmed by plating 100 µl of the last sterilization rinse water on PDA (Parsa et al. 2013). In addition, prints from the sterilized leaflets were used as an additional method to confirm the efficiency of sterilization. To do this, the cut edge of each section of leaflets was gently pressed into the middle of selective PDA (Greenfield et al. 2016).

Then, three sections from each part of the plant were transferred to different Petri dishes (90 x 15 mm) containing the selective medium described above. Petri dishes were incubated at 25°C for 15 days before visually examining the growth of the fungus isolate from each plant organ. The frequency of occurrence was determined as the proportion of samples of each plant organ with the fungus.

Monitoring of arthropods and phytopathogens

Monthly, 15 leaflets and 15 flowers were randomly taken from plants at each sampling area for analysis. Each was removed and immediately examined with the help of a pocket magnifying glass to count the number of mites, separating them at the family level (Phytoseiidae or Tetranychidae), and the number of insects, also separating them at the family level (Aleyrodidae, Aphididae, Chrysomelidae, Rhyparochromidae and Thripidae). Leaflets containing predatory mites were collected in 70% ethanol and transported to the Laboratory of Acarology (ESALQ/USP) for species identification. All predatory mites found were mounted in Hoyer medium, identifying the species using the original descriptions and redescriptions available in the literature. Insect identification wase confirmed by experts of ESALQ/USP. Leaflets with disease symptoms were counted and photographed for later comparison with the symptoms described by Ueno et al. (2014).

Crop productivity

Fruit harvesting for productivity evaluation was carried out twice a week, starting in May 2017 and ending in December 2018. The fruits of all plants in the area corresponding to each treatment were harvested and placed in plastic boxes. They were later transferred to commercial plastic trays (19 x 11 x 6 cm), and the number of trays was multiplied by 1.2 (average weight in each tray in kg) to estimate total productivity. This total was divided by the total number of plants in each treatment (18000 plants, 30 plants/replicate).

Statistical analysis

The numbers of thrips, spider mite, predatory mites and leaflets with disease symptoms per leaflet or per flower of two treatments were fitted to a Poisson distribution, so the χ^2 test (Anova, test = 'Chisq') was used. Mean numbers of thrips, spider mite, predatory mites and leaflets with symptom of phytopathogen per leaflet or per flower of two treatments were analyzed by Shapiro–Wilk normality test and as the data were not distributed normally, they were analyzed by the Kruskal–Wallis test, all tests were analyzed at 95% confidence intervals. Data on fruit productivity were compared using the Kruskall-Wallis test ($p<0.05$), considering the accumulated production per plant at each sampling area as replicates. All these analyzes were performed using the R Core Team (2018) program.

4.3. Results

Fungus colonization in the planting substrate and in the plants

The fungus was recovered from samples of strawberry planting substrate, root, petiole and leaflet (Figure 1 a-d). In fruits, recovery could not be proved due to the presence of many contaminants, making it difficult to visualize the colonies of the target fungus (Figure 1 e).

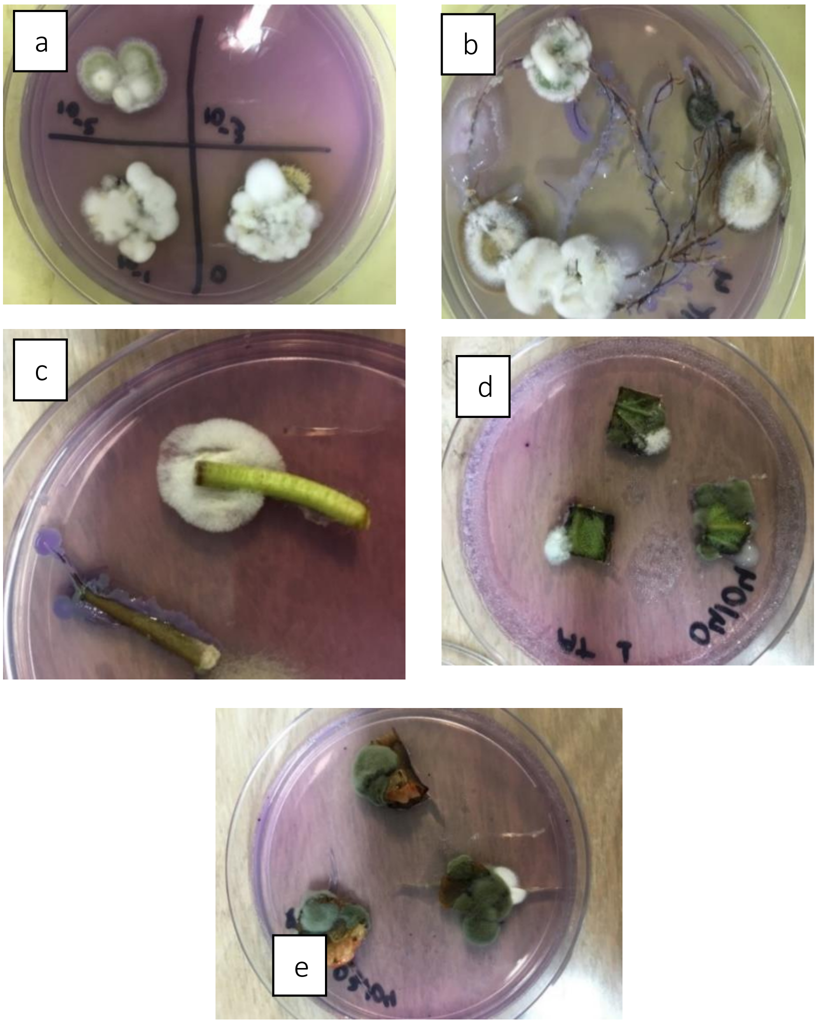

Figure 1. Plates with specific culture medium for isolation of the fungus *Metarhizium humberi* (ESALQ 1638) (inoculated via irrigation system) containing a suspension of the 10^{-3} dilution of the planting substrate (a), parts of the root (b), of the petiole (c), leaflet (d) and strawberry fruit (e).

The highest recovery frequency was observed in the substrate (70% of the samples), followed by root (66.6%), petiole (33.3%) and leaflet (26.6%) samples). The fungus was not found in the samples of the control treatment; in which it was not applied (Table 1).

Table 1. Frequency of occurrence (%) of the isolate of *Metarhizium humberi* (ESALQ 1638) in samples of planting substrate and organs of strawberries inoculated or not with the fungus (% of plots with detection of the fungus), based on plating in selective medium (Behie et al. 2015) (N= 30 samples/ part of the plant in each treatment).

	Metarhizium humberi	**Control area**
Planting substrate	70.0	0
Root	66.6	0
Petiole	33.3	0
Leaflet	26.6	0
Fruit	0	0

Monitoring of arthropods and pathogens

Considering all assessments together, 7693 phytophagous arthropods and 1806 beneficial arthropods were found in the plots where the fungus was applied (Table 2), while in the control treatment, totals were respectively 11431 and 1034.

In both treatments, most of the pest arthropods were represented by thrips and two-spotted spider mites. On the other hand, except for a few parasitoids, predatory mites were the main beneficial arthropods found. In both treatments, the most abundant predatory mite species were *N. californicus* and *P. macropilis*. Other pest insects and mites occurred sporadically, with very similar numbers in both treatments.

Table 2. Total number of insects and mites collected monthly from May 2017 to January 2019 in strawberry leaflets and flowers (N=15 leaflets and flowers/sample point) in the area inoculated with the fungus *Metarhizium hum*beri (ESALQ 1638) and in the control area, in Bom Repouso, Minas Gerais.

	Metarhizium humberi	**Control area**
Phytophagous		
Hemiptera: Aphididae		
Chaetosiphon fragaefolli (Cockerell)	14	41
Thysanoptera: Thripidae		
Frankliniella occidentalis (Pergande)	3560	5659
Coleoptera: Chrysomelidae		
Maecolaspis sp.	33	129
Hemiptera: Rhyparochromidae		
Neopamera bilobata (Say)	29	22
Acari: Tetranychidae		
Tetranychus urticae Koch	3941	5468
Hemiptera: Aleyrodidae		
Trialeurodes vaporariorum (Westwood)	116	112
Total	**7693**	**11431**
Beneficial		
Hymenoptera		
Unidentified parasitoids	6	4
Acari: Phytoseiidae		
Amblydromalus limonicus (Garman & McGregor)	122	38
Neoseiulus californicus (McGregor).	723	511
Phytoseiulus macropilis (Banks)	696	454
Proprioseiopsis cannaensis (Muma)	259	27
Total	**1806**	**1034**

In both treatments, thrips occurred in highest numbers in October 2017 and 2018 and December 2018 (Figure 2A). When pooling the data obtained at all evaluation dates, no significant difference was observed between treatments in relation to the average numbers of thrips/flower (1.3 ± 0.3 and 1.9 ± 0.4 thrips/ flower, respectively; Df = 1, $\chi^2 = 0.98$; p = 0.31). However, when comparison was done separately at each monthly evaluation, the mean number of thrips/flower was statistically lower in area inoculated with the fungus in May, July, August, October, December 2017, and August, October and December 2018; differences were not significantly different in other evaluations (Table 3).

In both treatments, the highest mean numbers of the two-spotted spider mite/leaflet occurred in August and October 2017 and October 2018 (Figure 2B) in both treatments. Comparing the mean number of this mite/leaflet by pooling all data, no statistical difference was observed between treatments (1.2 ± 0.3 and 1.7 ± 0.4 two-spotted spider mite/ leaflets, respectively for fungus treated and control treatments; Df = 1, $\chi^2 = 2.1$; $p = 0.14$). However, considering the month evaluations separately, the average number of the two-spotted spider mite/leaflet was statistically lower in the area that received the application of the fungus in May, June, August, September, October 2017, January, May, October 2018), with no statistical difference between treatments in the other months.

Also, for the predaceous phytoseiid mites, no significant difference between treatments were observed when all data were pooled (0.6 ± 0.2 and 0.3 ± 0.1 Phytoseiidae/ leaflet, respectively in fungus treated and control treatments; Df = 1, $\chi^2 = 3.04$; $p = 0.08$) (Figure 2C). However, if it was evaluated individually per month, it is concluded that in five of the 19 months evaluated, the number of phytoseiids/ leaflets was higher in the area where the fungus was applied, with no statistical difference between treatments in the other months.

The mean temperature and mean relative humidity and total precipitation throughout the study were: 16.3 ± 0.6° C, 85.9 ± 1.1% and 2314.8 mm (Figure 2D). The highest values occurred in January 2018, with an average temperature of 19.4°C (minimum and maximum average of 13.7 and 27.8°C), the average relative humidity of 93.7% (88.7 and 95.2%) and total precipitation of 344 mm. Average relative humidity varied relatively little throughout the observation period. However, rainfall increased progressively from July 2017, decreasing dramatically from January 2018.

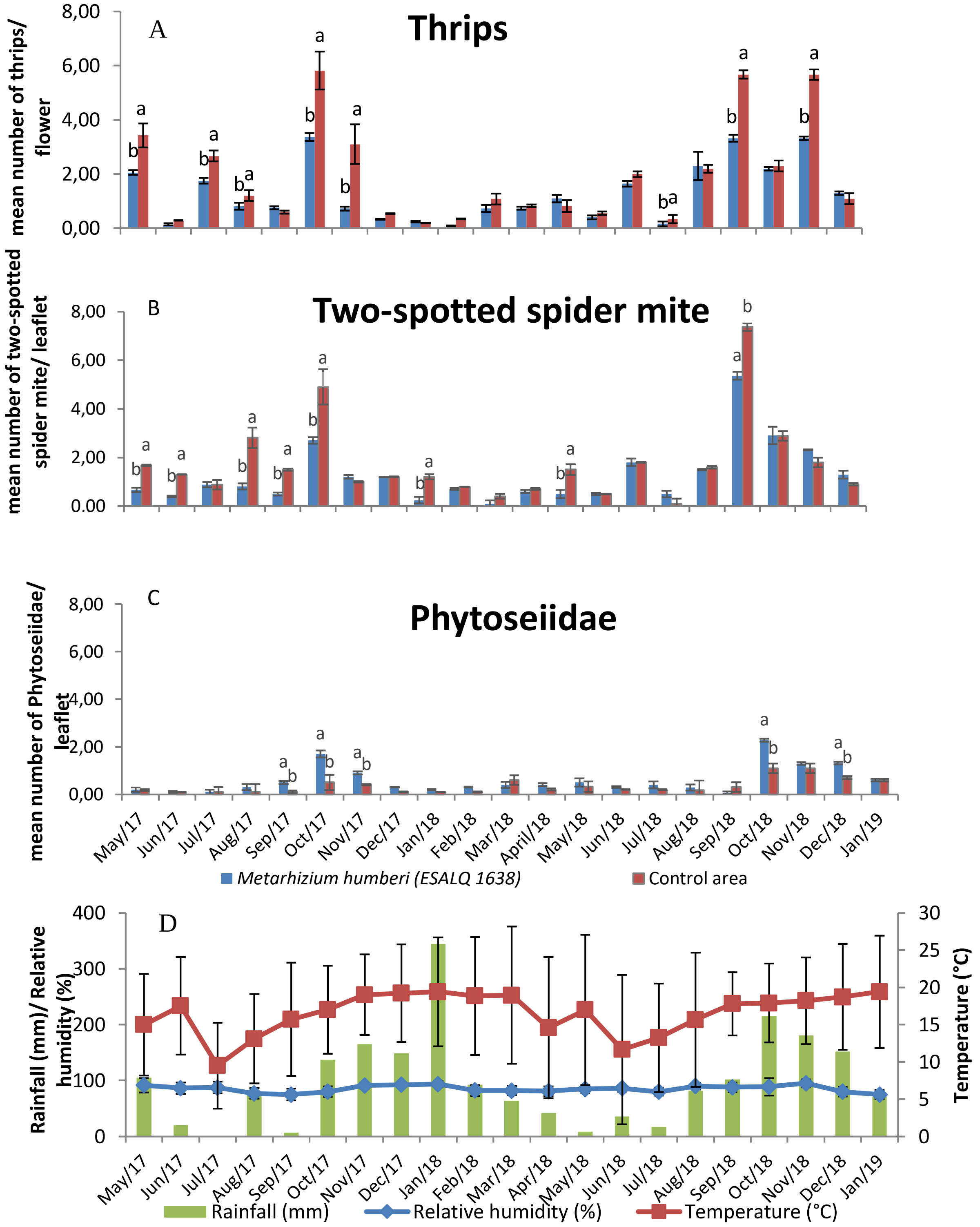

Figure 2. A) Mean number of thrips/flower; B) Mean number of two-spotted spider mite/ leaflet; C) Mean number of phytoseiids/ leaflets in the area treated with *Metarhizium humberi* (ESALQ 1638) and in the control area; D) Temperature (°C) and humidity (%) data were obtained from a Datalogger and rainfall data (mm) were obtained from a pluviometer installed in the experimental area, in Bom Repouso, Minas Gerais.

*At each month, bars accompanied by the same letters do not differ statistically from each other by the Kruskal-Wallis test ($p < 0.05$), both for $p < 0.05$.

The diseases detected in both treatments were: angular leaf spot, micosferela, anthracnose and gray mold. Throughout the experiment, the mean number of leaflets with symptoms of angular leaf spot, micosferela and gray mold per sampling point did not differ statistically between inoculated area and control area (angular leaf spot: 1.1 ± 0.1 and 1.4 ± 0.1; Df = 1; χ^2 = 1.5; p = 0.13; micosferela: 0.7 ± 0.3 and 1.2 ± 0.8; Df = 1; χ^2 = 0.9; p = 0.34; gray mold: 1.5 ± 0.4 and 2.2 ± 0.9; Df = 1; χ^2 = 0.5; p = 0.47). In December 2017 and January 2018, the average number of leaflets/plant with anthracnose symptoms was statistically lower in the inoculated area (December 2017: 3.9 ± 0.8 leaflets/plant; January 2018: 3.8 ± 1.1 leaflets/plant, respectively) than in the control area (December 2017: 10.7 ± 1.6 leaflets/plant; January 2018: 14.2 ± 1.0 leaflets/ plant) (December 2017: Df = 1; χ^2 = 7.5; $p < 0.01$ and January 2018: Df = 1; χ^2 = 9.8; $p < 0.01$).

Crop productivity

Considering the monthly productivity, no significant difference was observed between treatments (Df = 1, χ^2 = 0.17; p = 0.6743), with average monthly production of 0.15 ± 0.03 and 0.13 ± 0.02 kg/strawberry plant for fungus treated and control treatments, respectively. At the end of the experiment, each inoculated plant produced 3.0 kg of strawberry, while in the control area each plant produced 2.7 kg.

4.4. Discussion

Fungus colonization in the planting substrate and in the plant

The results showed that the fungus *M. humberi* was recovered from the planting substrate, root, petiole and strawberry leaflets at the end of the experiment, suggesting that the inoculations made by the irrigation system enabled the persistence and endophytic colonization of the fungus in the strawberry cultivation.

Several works have reported endophytic colonization by fungi. In a laboratory study in Ontario, Canada, Sasan and Bidochka (2013) submerged *Panicum virgatum* L. (Poaceae) and *Phaseolus vulgaris* L. (Fabaceae) seeds in a fungal solution containing *M. robertsii* and concluded that it endophytically colonized both plants. They also concluded that the fungus was closely associated with plant roots and that it induced root hair proliferation and plant root growth when compared to control plants. In a semi-field experiment carried out in Norway, Klingen et al. (2015) reported the persistence of *Beauveria pseudobassiana* (Balsamo)

Vuillemin and *M. brunneum* (Norwegian isolates and an Austrian isolate) for over a year in the soil and the rhizosphere. In another study carried out in experimental strawberry fields in Inconfidentes, Minas Gerais, the application of two fungal isolates (*M. anisopliae* (ESALQ1037) and *M. robertsii* (ESALQ1426)) led the authors to conclude that both persisted in the soil and in the rhizosphere by up to 12 months (Castro et al. 2016). Canassa et al. (2020) submerged strawberry plant roots for 2 minutes before transplanting in a solution containing two fungal treatments (*M. robertsii* - ESALQ 1622 and *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin ESALQ 3375) in four strawberry commercial plantations in Atibaia, São Paulo and Senador Amaral, Minas Gerais. At the end of the growing season, they recovered both fungi from strawberry leaflets and soil adjacent to the roots.

In the present study, the highest frequency of fungus recovery was observed in the planting substrate and strawberry roots. Previous work has led to the conclusion that *B. bassiana* is a more efficient colonizer of leaf tissues than *Metarhizium* species (Akutse et al. 2013; Behie et al. 2015). It has also been reported that endophytic establishment can be influenced by several factors, such as climatic conditions, type of substrate and soil, host plant and fungal strain (Sánchez-Rodríguez et al. 2018). Parsa et al. (2018) reported that the successful establishment of fungal isolates is significantly greater when inoculations are performed on unnatural cultivation substrates, as was the case in the present study, in which the fungus was inoculated via an irrigation system into the planting substrate. Perhaps this is also one of the reasons why it was possible to determine in this study high frequencies of occurrence of the fungus in the planting substrate and in roots, and even, at lower frequencies, in petioles and leaflets.

Monitoring the level of occurrence of arthropods and pathogens

The hypothesis of this work was partially proved, in what refers to the reduction of levels of occurrence of arthropod pests and pathogens with the application of isolate ESALQ 1638 of *M. humberi*. This was true when considering the levels of occurrence of thrips, two-spotted spider mite, angular leaf spot, micosferela, anthracnose and gray mold.

The results of this work were compatible with what has been reported in the literature in similar studies, concerning other plants, pests and fungi. Castillo-Lopez et al. (2014) conducted a field study about cotton pests in Texas, USA, in which they subjected cotton seeds to inoculation of *B. bassiana* based product, showing that the inoculated plants developed lower densities of *Aphis gossypii* Glover (Hemiptera: Aphididae). In a study conducted in China, Dash et al. (2018) demonstrated that on bean (*P. vulgaris*) plants produced from seeds treated with suspensions of three *B. bassiana* isolates, one *Isaria fumosorosea* Wise isolate and one

Lecanicillium lecanii (Zimmermann) isolate, spider mites had reduced larval development, adult longevity and fecundity. Similar results were obtained by Canassa et al. (2019) in a greenhouse study in Denmark, in which bean plants generated from seeds inoculated with strains ESALQ 1622 of *M. robertsii* and strain ESALQ 3375 of *B. bassiana* had lower population levels of spider mite populations in comparison with untreated plants. Canassa et al. (2020) also concluded that there was a lower number of spider mites in strawberry plants inoculated with those fungi, in addition to lower proportions of leaflets with symptoms of micosferela and pestalotia spot.

The mechanisms behind the negative impacts on pests and pathogens by beneficial fungi still remain largely unknown. Cherry et al. (2004) and Vega et al. (2008) proposed that these could result from antibiosis and impediment in food uptake mediated by the production of secondary metabolites as a result of the internal plant colonization by the fungi. Some works reported that beneficial fungi directly affect insects and mites because they are related to the increase of jasmonic acid in plants, improving their defense mechanisms (Van Wees et al. 2008).

Other studies have suggested that by endophytically colonizing plants, beneficial fungi can trigger the process of plant recognition of invasive organisms, leading to the expression of immune responses, with the synthesis of specific regulatory elements (Brotman et al. 2013, McKinnon et al. 2017). In addition, some authors have discussed that compounds produced by the plant, when associated with the fungus, cause lethal and sublethal effects to such organisms (Vidal and Jaber 2015; McKinnon et al. 2017). Shrivastava et al. (2015) detected terpenoids in tomato plants endophytically colonized by *B. bassiana*. These compounds are secondary plant metabolites with anti-herbivory properties.

In addition to these mechanisms, another indirect effect of the fungus in the present study could refer to the effect of natural enemies of thrips and spider mites, given the higher levels of occurrence of predatory mites, especially *N. californicus* and *P. macropilis*, both marketed for the spider mite control. It has been reported that *N. californicus* can also feed on thrips (Mizobe et al. 2005; Rahmani et al. 2009).

The higher incidence of predatory mites on plants inoculated with the fungus may be related to greater release of volatile kairomones generated when plants are attacked by the two-spotted spider mite or thrips in the presence of the fungus. Seiedy et al. (2013) and Dogan et al. (2017) studied the combined inoculation of *Metarhizium* spp. and *B. bassiana* on predators, including predatory mites, but so far little is known about the effects of beneficial fungi on predators. Schausberger et al. (2012) showed an increase in the production of the volatile sesquiterpenes β-omicene and β-caryophyllene in the presence of the two-spotted spider mite on bean plants colonized by the beneficial fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, resulting in greater attraction of the predatory phytoseiid *Phytoseiulus persimilis* Athias-Henriot.

In a laboratory study, Pappas et al. (2018) showed that volatile mixtures naturally produced by tomato plants are altered when they are inoculated with the K strain of *Fusarium solani* (Mart.). The authors also demonstrated that the endophytic presence of this fungus increased indirect defense ability of this plant, which became more attractive to predators such as *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae). Canassa et al. (2019) found no difference in the predation rate of spider mites by *P. persimilis* on plants inoculated or not with ESALQ 1622 strain of *M. robertsii* or with ESALQ 3375 strain of *B. bassiana*, however, they observed greater feeding on this prey in the initial phase of the study on inoculated plants. On the other hand, Canassa et al. (2020) found no effect on the number of predatory mites, mainly *N. californicus*, on strawberry plants inoculated with *M. robertsii* ESALQ 1622 or *B. bassiana* ESALQ 3375, in comparison with non-inoculated plants.

Crop productivity

The results of the study did not confirm the second part of the hypothesis, i.e., the application of the fungus did not result in higher plant productivity. Although this result could be contradictory, given the determined higher levels of pest organisms on the control treatment (no fungus application), a possible explanation could be that in both treatments the population levels of the pests were lower than the economic damage level. Botton et al. (2018) have proposed 6 thrips/ flower and 7 mites/ leaflet as the economic damage levels of those organisms. In the present study, the levels of those organisms in was almost invariably lower than the numbers mentioned by those authors. In addition, the level of occurrence of anthracnose was punctual and although the inoculated area had a smaller number of leaflets with symptoms, in both treatments the occurrence was low. Differences between productivity of fungus treated and control plants would have been significant, had the population levels of those species and these pathogens been higher.

The results of the present work were slightly different from a few other studies. Dash et al. (2018) showed that the inoculation of common bean plants with *B. bassiana, I. fumosorosea* or *L. lecanii* resulted in greater plant growth. In an experiment conducted in Texas, an increase in dry biomass and number of nodes were reported in plants generated from seeds inoculated with *B. bassiana* and *Purpureocillium lilacinum* (Thom.) (= *Paecilomyces lilacinus*) (Castillo-

López and Sword 2015). In a similar study carried out in greenhouses in Jordan, inoculation of *Vicia faba* L. bean seeds with *M. brunneum* and *B. bassiana* led to a significant increase in the percentage of seedling emergence and development (Jaber and Enkerli 2016). In Denmark, Canassa et al. (2019) observed that inoculation of common bean seeds with suspensions of ESALQ 1622 strain of *M. robertsii* and ESALQ 3375 strain of *B. bassiana* improved plant growth and increased pod production.

Although the mechanism responsible for the increase in plant growth is not fully elucidated, some works cite that beneficial fungi produce siderophores (organic molecules that form stable bonds with soil iron and transport it to plants) and organic acids that can alter the bioavailability of various nutrients to plants (Jirakkakul et al. 2015). Furthermore, studies on plant interactions with endophytic fungi revealed that the positive effects could be due to the fixation of soil nutrients and production of bioactive metabolites by the fungi or the increased production of growth hormones such as auxin, ethylene, etc. (Berg 2009). Another factor that can influence the higher productivity of plants treated with the fungus could be indirect, and linked to the reduction in the pest population. Beneficial fungi of the genus *Metarhizium* and *Beauveria* have been previously reported as plant growth promoters (eg Jaber and Enkerli 2016; Jaber and Enkerli 2017), reducing damage related to pest infestation. Although the underlying mechanism(s) is not yet clear, those studies provide evidence that *M. brunneum* and *B. bassiana*, in addition to colonizing endophytically, can also act as growth promoters (Gathage et al. 2016; Jaber and Araj 2018).

In summary, the present study demonstrated that ESALQ 1638 strain of *M. humberi* can be applied via the irrigation system to strawberry plants cultivated under the suspended system, reducing the occurrence of the two-spotted spider mite, thrips and anthracnose, although in both treatments the population levels of the pests were lower than the economic damage level, moreover, promoting an increase in the population of predatory mites. Future work needs to investigate whether the results obtained here were only possible due to the monthly inoculation of beneficial fungi or whether the results would also be promising at a lower frequency of applications. In addition, it seems warranted to repeat this study under conditions of higher levels of pest incidence, under the hypothesis that greater productivity can be obtained from plants inoculated with the fungus.

REFERENCES

ABCBio, 2019: Associação Brasileira das Empresas de Controle Biológico. Available online: https://www.abcbio.org.br/ (accessed on 06 May 2021).

AGROFIT (2021) Sistema de agrotóxicos fitossanitários - Ministério da Agricultura, Pecuária e Abastecimento. Available online: http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. (accessed on 10 May 2021).

Akutse, K.S.; Maniania, N.K.; Fiaboe, K.K.M.; Van Den Berg, J.; Ekesi, S. (2013) Endophytic colonization of *Vicia faba* and *Phaseolus vulgaris* (Fabaceae) by fungal pathogens and their effects on the life-history parameters of *Liriomyza huidobrensis* (Diptera: Agromyzidae). Fungal Ecology, 6: 293-301.

Andreazza, F.; Haddi, K.; Oliveira, E.E.; Ferreira, J.A.M. (2016) *Drosophila suzukii* (Diptera: Drosophilidae) arrives at Minas Gerais State, a main strawberry production region in Brazil. Florida Entomologist, 99(4): 796-798.

Ansari, M.A.; Butt, T.M. (2013) Influence of the application methods and doses on the susceptibility of black vine weevil larvae *Otiorhynchus sulcatus* to *Metarhizium anisopliae* in field-grown strawberries. Biocontrol, 58: 257-267.

Antunes, L.E.C.; Bonow, S.; Reisser Jr., C. (2020) Morango: Crescimento constante em área e produção. Campo e Negócios, 1: 88-92.

Barakat, R.M.; Al-Masri, M.I. (2017) Effect of *Trichoderma harzianum* in combination with fungicides in controlling gray mould disease (*Botrytis cinerea*) of strawberry. American Journal of Plant Sciences, 8(04): 651.

Behie, S.W.; Jones, S.J.; Bidochka, M.J. (2015) Plant tissue localization of the endophytic insect pathogenic fungi *Metarhizium* and *Beauveria*. Fungal Ecology, 13: 112-119.

Berg, G. (2009). Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Applied microbiology and biotechnology, 84(1): 11-18.

Bernardi, D.; Botton, M.; Nava, D.E.; Zawadneak, M.A.C. (2015) Guia para a identificação e monitoramento de pragas e seus inimigos naturais em morangueiro. Embrapa Uva e Vinho-Livro científico, Brasília. (ALICE).

Bixby-Brosi, A.J.; Potter, D.A. (2012) Endophyte-mediated tritrophic interactions between a grass-feeding caterpillar and two parasitoid species with different life histories. Arthropod Plant Interact, 6: 27-34.

Botton, M.; Nava, D.; Zawandneak, M.; Bernardi, D. (2018) Manejo integrado de pragas. Embrapa Clima Temperado-Capítulo em livro técnico (INFOTECA-E).

Brotman, Y.L.; Landau, U.; Cuadros-Inostroza, A.; Takayuki, T.; Fernie, A.R.; Chet, I.; Viterbo, A.; Willmitzer, L. (2013) Trichoderma-plant root colonization: escaping early plant defense responses and activation of the antioxidant machinery for saline stress tolerance. PLOS Pathogens, 9: e1003221.

Canassa, F.; Esteca, F.C.N.; Moral, R.A. Meyling, N.V.; Klingen, I.; Delalibera Jr., I. (2020) Root inoculation of strawberry with the entomopathogenic fungi *Metarhizium robertsii* and *Beauveria bassiana* reduces incidence of the twospotted spider mite and selected insect pests and plant diseases in the field. Journal of Pest Science, 93: 261-274.

Canassa, F.; Tall, S.; Moral, R.A.; Lara, I.A.R.D.; Delalibera Jr., I.; Meyling, N.V. (2019) Effects of bean seed treatment by the entomopathogenic fungi *Metarhizium robertsii* and *Beauveria bassiana* on plant growth, spider mite populations and behavior of predatory mites. Biological Control, 132: 199-208.

Castillo-López, D.; Sword, G.A. (2015) The endophytic fungal entomopathogens *Beauveria bassiana* and *Purpureocillium lilacinum* enhance the growth of cultivated cotton (*Gossypium hirsutum*) and negatively affect survival of the cotton bollworm (*Helicoverpa zea*). Biological Control, 89: 53-60.

Castillo-Lopez, D.; Zhu-Salzman, K.; Ek-Ramos, M.J.; Sword; G.A. (2014) The entomopathogenic fungal endophytes *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*) and *Beauveria bassiana* negatively affect cotton aphid reproduction under both greenhouse and field conditions. PLOS ONE, 9: e103891.

Castro, T.; Mayerhofer, J.; Enkerlib, J.; Eilenberg, J.; Meyling, N.V.; Moral, R.A.; Demétrio, C.G.B.; Delalibera Jr., I. (2016) Persistence of Brazilian isolates of the entomopathogenic fungi *Metarhizium anisopliae* and *M. robertsii* in strawberry crop soil after soil drench application. Agriculture, Ecosystems & Environment, 233: 361-369.

Cherry, A.J.; Banito, A.; Djegui, D.; Lomer, C. (2004) Suppression of the stem-borer *Sesamia calamistis* (Lepidoptera; Noctuidae) in maize following seed dressing, topical application and stem injection with African isolates of *Beauveria bassiana*. International Journal of Pest Management, 50(1): 67-73.

Dara, S.K. (2016) Managing strawberry pests with chemical pesticides and non-chemical alternatives. International Journal of Fruit Science, 16: 129-141.

Dash, C.K.; Bamisile, B.S.; Keppanan, R.; Qasim, M.; Lin, Y.; UlIslam, S.; Hussain, M.; Wang, L. (2018) Endophytic entomopathogenic fungi enhance the growth of *Phaseolus vulgaris* L. (Fabaceae) and negatively affect the development and reproduction of *Tetranychus urticae* Koch (Acari: Tetranychidae). Microbial Pathogemesis, 125: 385-392.

Dias, M.S.C.; Silva, J.J.C.; Pacheco, D.D.; Rios, S.A.; Lanza, F.E. (2007) Produção de morangos em regiões não tradicionais. Informe Agropecuário, Belo Horizonte, 8(236): 24-33.

Dogan, Y.O.; Hazir, S.; Yildiz, A.; Butt, T.M.; Cakmak, I. (2017) Evaluation of entomopathogenic fungi for the control of *Tetranychus urticae* (Acari: Tetranychidae) and the effect of *Metarhizium brunneum* on the predatory mites (Acari: Phytoseiidae). Biological Control, 111: 6-12.

DunhamTrimmer's (2019) The market research company. Data presented in BioControl Latan 2019 conference.

FAOSTAT (2019) Food and Agriculture Organization of the United Nations Statistics. Available online: http://faostat.org/. (accessed on 10 January 2021).

Gathage, J.W.; Lagat, Z.O.; Fiaboe, K.K.M.; Akutse, K.S.; Ekesi, S.; Maniania, N.K. (2016) Prospects of fungal endophytes in the control of *Liriomyza leafminer* flies in common bean *Phaseolus vulgaris* under field conditions. Biocontrol, 61: 741-753.

Greenfield, M.; Gomez-Jimenez, M.I.; Ortiz, V.; Vega, F.E.; Kramer, M.; Parsa, S. (2016) *Beauveria bassiana* and *Metarhizium anisopliae* endophytically colonize cassava roots following soil drench inoculation. Biological Control, 95: 40-48.

Iwanicki, N.S.A.; Pereira, A.A.; Botelho, A.B.R.Z.; Rezende, J.M.; Moral, R.D.A.; Zucchi, M.I.; Delalibera Jr., I. (2019) Monitoring of the field application of *Metarhizium anisopliae* in Brazil revealed high molecular diversity of *Metarhizium* spp in insects, soil and sugarcane roots. Scientific Reports, 9: 4443.

Jaber, R. and Araj, S.E. (2018) Interactions among endophytic fungal entomopathogens (Ascomycota: Hypocreales), the green peach aphid *Myzus persicae* Sulzer (Homoptera: Aphididae), and the aphid endoparasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae). Biological Control, 116: 53-61.

Jaber, L.R.; Enkerli, J. (2016) Effect of seed treatment duration on growth and colonization of *Vicia faba* by endophytic *Beauveria bassiana* and *Metarhizium brunneum*. Biological Control, 103: 187-195.

Jaber, L.R.; Enkerli, J. (2017) Fungal entomopathogens as endophytes: can they promote plant growth? Biocontrol Science and Technology, 27(1): 28-41.

Jaber, L.R.; Ownley, B.H. (2018) Can we use entomopathogenic fungi as endophytes for dual biological control of insect pests and plant pathogens? Biological Control, 116: 36-45.

Jaronski, S.T.; Jackson, M.A. (2012) "Mass production of entomopathogenic Hypocreales," in Manual of techniques in insect pathology. ed. Lacey L. (Ed). Academic Press, London. 2° ed, pp 317-328.

Jirakkakul, J.; Cheevadhanarak, S.; Punya, J.; Chutrakul, C.; Senachak, J.; Buajarern, T.; Tanticharoen, M.; Amnuaykanjanasin, A. (2015) Tenellin acts as an iron chelator to prevent iron-generated reactive oxygen species toxicity in the entomopathogenic fungus *Beauveria bassiana*. FEMS Microbiology Letters, 362(2): 1-8.

Klingen, I.; Westrum, K.; Meyling, N. (2015) Effect of Norwegian entomopathogenic fungal isolates against *Otiorhynchus sulcatus* larvae at low temperatures and persistence in strawberry rhizospheres. Biological Control, 81: 1-7.

Kuhn, T.M.D.A.; Loeck, A.E.; Zawadneak, M.A.C.; Garcia, M.S.; Botton, M (2014) Parâmetros biológicos e tabela de vida de fertilidade de *Neopamera bilobata* (Hemiptera: Rhyparochromidae) em morangueiro. Pesquisa Agropecuária Brasileira, 49(6): 422-427.

Luz, C.; Rocha, L.F.N.; Montalva, C.; Souza, D.A.; Botelho, A.B.R.Z.; Lopes, R.B.; Faria, M.; Delalibera Jr., I. (2019) *Metarhizium humberi* sp. nov. (Hypocreales: Clavicipitaceae), a new member of the PARB clade in the *Metarhizium anisopliae* complex from Latin America. Journal of Invertebrate Pathology, 166: 107216.

Mascarin, G.M.; Lopes, R.B.; Delalibera Jr., Í. Fernandes, É.K.K.; Luz, C.; Faria, M. (2019) Current status and perspectives of fungal entomopathogens used for microbial control of arthropod pests in Brazil. Journal of Invertebrate Pathology, 165: 46-53.

McKinnon, A.C.; Saari, S.; Moran-Diez, M.E.; Meyling, N.V.; Raad, M.; Glare, T.R. (2017) *Beauveria bassiana* as an endophyte: a critical review on associated methodology and biocontrol potential. Biocontrol, 62: 1-17.

Mizobe, M.; Kashio, T.; Morita, S.; Takagi, M. (2005) Predation rate of *Neoseiulus californicus* (McGregor) on three species of greenhouse thrips. Kyushu Plant Protection Research, 51: 73-77.

Montagnana, J.M.; Costa, A.L.; Pereira, J.M.; Tonelli, M.; Leite, L.G. (2017) Eficiência de *Steinernema Brazilense* no controle de *Colaspis* sp. em morangueiro. In: 9ª Jornada Científica e Tecnológica e 6º Simpósio da Pós-graduação do IFSULDEMINAS, Machado.

Oliveira, D.G.P.; Pauli, G.; Mascarin, G.M.; Delalibera Jr., I. (2015) A protocol for determination of conidial viability of the fungal entomopathogens *Beauveria bassiana* and *Metarhizium anisopliae* from commercial products. Journal of Microbiological Methods, 119: 44-52.

Pappas, M. L.; Liapoura, M.; Papantoniou, D.; Avramidou, M.; Kavroulakis, N.; Weinhold, A.; Broufas, G.D; Papadopoulou, K.K. (2018) The beneficial endophytic fungus fusariumsolani strain k alters tomato responses against spider mites to the benefit of the plant. Frontiers in Plant Science, 9: 1603.

Parsa, S.; Ortiz, V.; Gómez-Jiménez, M.I.; Kramer, M.; Vega, F.E. (2018) Root environment is a key determinant of fungal entomopathogen endophytism following seed treatment in the common bean, *Phaseolus vulgaris*. Biological Control, 116: 74-81.

Parsa, S.; Ortiz, V.; Vega, F.E. (2013) Establishing fungal entomopathogens as endophytes: towards endophytic biological control. Journal of Visualized Experiments, 74: e50360.

R Core Team, (2018). R: A Language and Environment for Statistical Computing R Foundation for Statistical Computing, Vienna, Austria (2018) Available online: https://www.R-project.org/ (accessed on 12 May 2019).

Rahmani, H.; Fathipour, Y.; Kamali, K. (2009) Life history and population growth parameters of *Neoseiulus californicus* (Acari: Phytoseiidae) fed on *Thrips tabaci* (Thysanoptera: Thripidae) in laboratory conditions. Systematic and Applied Acarology, 14(2): 91-100.

Sánchez-Rodríguez, A.R.; Raya-Díaz, S.; Zamarreño, Á.M.; García-Mina, J.M.; Campillo, M.C.; Quesada-Moraga, E. (2018) An endophytic *Beauveria bassiana* strain increases spike production in bread and durum wheat plants and effectively controls cotton leafworm (*Spodoptera littoralis*) larvae. Biological Control, 116: 90-102.

Sasan, R.K.; Bidochka, M.J. (2012) The insect-pathogenic fungus *Metarhizium robertsii* (Clavicipitaceae) is also an endophyte that stimulates plant root development. American Journal of Botany, 99: 101-107.

Sasan, R.K.; Bidochka, M.J. (2013) Antagonism of the endophytic insect pathogenic fungus *Metarhizium robertsii* against the bean plant pathogen *Fusarium solani* f. sp. *phaseoli*. Canadian Journal of Plant Pathology, 35: 288-293.

Schausberger, P.; Peneder, S. Jürschik, S.; Hoffmann, D. (2012) Mycorrhiza changes plant volatiles to attract spider mite enemies. Functional Ecology, 26(2): 441-449.

Seiedy, M.; Saboori, A.; Zahedi-Golpayegani, A. (2013) Olfactory response of *Phytoseiulus persimilis* (Acari: Phytoseiidae) to untreated and *Beauveria bassiana*-treated *Tetranychus urticae* (Acari: Tetranychidae). Experimental and Applied Acarology, 60(2): 219-227.

Shrivastava, G.; Ownley, B.H.; Augé, R.M.; Toler, H.; Dee, M.; Vu, A.; Köllner, T.G.; Chen, F. (2015) Colonization by arbuscular mycorrhizal and endophytic fungi enhanced terpene production in tomato plants and their defense against a herbivorous insect. Symbiosis, 65(2): 65-74.

Silveira, C.A.P.; Martinazzo, R.; Pauletti, V. (2016) Fertilidade do solo e nutrição, *In:* Antunes, L.E.C.; Reisser Junior, C.; Schwengber, J.E. (eds). Morangueiro. Embrapa, Brasília. pp.151-199.

Siqueira, A.C.O.; Mascarin, G.M.; Gonçalves, C.R.N.C.B.; Marcon, J.; Quecine, M.C.; Figueira, A.; Delalibera Jr., Í. (2020) Multi-trait biochemical features of *Metarhizium* species and their activities that stimulate the growth of tomato plants. Frontiers in Sustainable Food Systems, 4: 137.

Solomon, M.G.; Jay, C.N.; Innocenzi, P.J.; Fitzgerald, J.D.; Crook, D.; Crook, A.M.; Easterbrook, M.A.; Cross, J.V. (2001) Review: natural enemies and biocontrol of pests of strawberry in Northern and Central Europe. Biocontrol Science and Technology, 11: 165-216.

Steinwender, B.M.; Enkerli, J.; Widmer, F.; Eilenberg, J.; Kristensen, H.L.; Bidochka, M.J.; Meyling, N.V. (2015) Root isolations of *Metarhizium* spp. from crops reflect diversity in the soil and indicate no plant specificity. Journal of Invertebrate Pathology, 132: 142-148.

Tanaka, M.A.S.; Betti, J.A.; Kimati, H. (2005) Doenças Do Morangueiro. In: Kimati, H.; Amorim, L.; Rezende, J.A.M.; Bergamin Filho, A.; Camargo, L.E.A. (Ed.). Manual de Fitopatologia, São Paulo: Agronômica Ceres, p.489-499.

Ueno, B.; Costa, H.; Dias, M.S.C.; Jesus, A.M. (2014) Manejo integrado das principais doenças do morangueiro. Informe Agropecuário, Belo Horizonte, 35(279): 82-91.

Van Lenteren, J.C. (2012) The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. BioControl, 57(1): 1-20.

Van Lenteren, J.C.; Bolckmans, K.; Köhl, J.; Ravensberg, W.J.; Urbaneja, A. (2018) Biological control using invertebrates and microorganisms: plenty of new opportunities. BioControl, 63(1): 39-59.

Van Wees, S.C.; Van der Ent, S.; Pieterse, C.M. (2008) Plant immune responses triggered by beneficial microbes. Current opinion in plant biology, 11(4): 443-448.

Vega, F.E. (2018) The use of fungal entomopathogens as endophytes in biological control: a review. Mycologia, 110: 4-30.

Vega, F.E.; Goettel, M.S.; Blackwell, M.; Chandler, D.; Jackson, M.A.; Keller, S.; Koike, M.; Maniania, N.K.; Monzón, A., Ownley, B.H.; Pell, J.K.; Rangel, D.E.N.; Roy, H.E. (2009) Fungal entomopathogens: new insights on their ecology. Fungal Ecology, 2: 149-159.

Vidal, S.; Jaber, L.R. (2015) Entomopathogenic fungi as endophytes: plant-endophyte-herbivore interactions and prospects for use in biological control. Current Sciemce, 109: 46-54.

5. MITE DIVERSITY IN COFFEE HUSK AND PULP, POTENTIALLY USEFUL FOR MULCHING IN STRAWBERRY CROPS, AND IN FOREST FRAGMENTS IN THE MAIN STRAWBERRY PRODUCTION REGION IN BRAZIL

Abstract

Southern Minas Gerais is the main strawberry (*Fragaria* x *ananassa* Duch.) and coffee (*Coffea arabica* L.) producing region in Brazil. Soil covering is an important practice in strawberry cultivation, as it prevents the development of spontaneous plants and prevents the fruit from coming into contact with the soil. Many producers use polyethylene plastic as mulching, but it is expensive and non-biodegradable, making it necessary to look for alternative materials that can be used for this purpose, especially organic. At the same time, it is expected that forest fragments could pragmatically serve as valuable reservoirs of natural enemies. The objectives of this work were: a) to evaluate the composition of the mite fauna and variation in their density in coffee husk and pulp available for sale in southern Minas Gerais and in forest fragments in the main strawberry production region in Brazil, in southern Minas Gerais state. For the study of the mites in the coffee husk a pulp, 11 monthly samples (each approximately 400 cm^3: cylinder 5 cm high and 10 cm in diameter) were obtained and placed in Berlese funnels for mite extraction. For the study of mites in the forest fragments, 11 soil-litter samples (the same dimension) were taken from each of four forest fragments close to strawberry cultivation and the mites were extracted as previously described. We concluded that the highest number of predatory mites can be found in coffee husk and pulp at the beginning of the year, coincidentally the period of greatest strawberry planting in southern Minas Gerais. The density and composition of mites varied among the forest fragments studied. Therefore, further studies should be conducted to better understand the variation of Gamasina composition and densities in different fragments along the year, to determine the most appropriate combinations of sites and periods for eventual enrichment, and to evaluate the effect of each predatory mite on the main pest species amenable to attack.

Keywords: 1. Edaphic predatory mites 2. Organic cover 3. Biological control

5.1. Introduction

The state of Minas Gerais is the main strawberry producing region in Brazil, accounting for about 50% of the strawberry production the country (Antunes et al. 2020). In this type of cultivation, mulching is commonly used, to reduce erosion, the development of spontaneous plants, water evaporation, and to avoid contact of the fruits with the soil (Cook et al. 2006). In Brazil, polyethylene film is widely used for mulching in strawberry crop (Morra et al. 2016), despite being costly and non-biodegradable.

In the search for a more ecologically acceptable and economically viable product, an alternative could be the use of organic material, such as sawdust, rice husk or by-products from coffee processing (Castilho et al. 2015a; Esteca et al. 2018; Esteca et al. 2020). This would reduce environmental problems related to their disposal after use, in addition to bringing other benefits, such as the availability of nutrients to the plants along time (Oliveira et al. 2008), better moisture retention inside growing tunnels (Resende et al. 2005), in addition to improving the conservation of soil microbiota, increasing the levels of occurrence of beneficial soil organisms such as fungi and predatory mites (Mathews et al. 2002).

Most edaphic predatory mites belong to the Gamasina cohort of the mite order Mesostigmata. These feed on small invertebrates, including other mites, small insects and nematodes, often contributing significantly to the control of pest species (Krantz and Walter 2009; Carrillo et al. 2015). In southern Minas Gerais, strawberry artrhopod pests attacked by predatory mites are mostly the two-spotted spider mite (*Tetranychus urticae* Koch, Tetranychidae), as well as the western flower thrips (*Frankliniella occidentalis* (Pergande), Thripidae). In other regions, also other mite species, as well as nematodes are important pests of this crop (Solomon et al. 2001; Patenaude et al. 2020).

Coffee husk and pulp is a by-product that has been evaluated for use as mulching in southern Minas Gerais, given its availability at affordable prices in this region (Durán et al. 2017). On the other hand, it has been hypothesized (Esteca et al. 2018) that the prior placement of this material in forest fragments in the vicinity of these crops for a certain period could contribute to its enrichment with local populations of beneficial mites (similar to the production of bokashi), which could later contribute to biological pest control in the crop. But nothing is known about the diversity and abundance of edaphic mites in forested areas in southern Minas Gerais, where the predominant natural vegetation is classified Atlantic Forest (Fundação SOS Mata Atlântica and INPE 2011). Climatic conditions in most of this area are rather mild, given the elevation, that in most strawberry producing sites range between 1000 and 1500 m above sea level. The climate in this area is classified as Cwa (humid subtropical climate), with a characteristic of Cwb (altitude subtropical climate) (Köppen 1948; Peel et al. 2007), with annual average temperature of 16.1ºC and total rainfall of about 1698 mm (Alvares et al. 2014).

Taking into account the possible benefits related to presence of predatory mites, it is considered convenient to study the mites that already exist naturally in commercialized coffee husk and pulp and in forest fragments of strawberry production areas of southern Minas Gerais.

The hypotheses to be evaluated are: a) that mites are present in the coffee husk and pulp in density and specific composition that vary along the year, including no detrimental mite species; b) that representative forest fragments in southern Minas Gerais have variable diversity and density of Gamasina, some of which may contribute to pest control.

The objectives of this work were: a) to evaluate the composition of the mite fauna and variation in their density in coffee husk and pulp recently acquired along the year in southern Minas Gerais; b) to evaluate the composition of the mite fauna in four forest fragments located in the municipality of Senador Amaral and Bom Repouso, within the strawberry growing areas of southern Minas Gerais.

5.2. Material and methods

Mites fauna in coffee husk and pulp

Ten kilograms of coffee husk and pulp were purchased monthly from a company (Primos Indústria e Comércio de Café) located in Ouro Fino, Minas Gerais. Upon receiving this material in the laboratory, 11 samples (each approximately 400 cm^3: a cylinder 5 cm high and 10 cm in diameter) were taken and placed in Berlese funnels to extract the mites they eventually contained. Each funnel had a 40 W lamp positioned about 20 cm from the surface of the material subjected to extraction, increasing its intensity slowly with the use of a dimmer for five days, until reaching a temperature of approximately 45-50°C (Oliveira et al. 2001). The extraction was done for seven days, collecting the mites in containers with 70% ethanol. The extracted material was screened under a stereomicroscope, mounting the mites in Hoyer's medium on glass slides. Identifications of the mites were later done at family level using taxonomic keys provided by Krantz and Walter (2009), at genus level using unpublished keys provided by the Ohio Summer Program, Agricultural Acarology, Columbus, Ohio, USA, and at species level using descriptions and redescriptions available in the literature.

Mites in forest fragments

Eleven samples of approximately 400 cm^3 (5 cm high and 10 cm diameter cylinder) of upper soil layer and litter were taken on March 19, 2018 from each of four forest fragments (1: 22°33'14"S, 46°13'53"W, altitude: 1514 m; 2: 22°33'10.4"S, 46°14'47.6"W, altitude: 1530 m; 3: 22°28'16"S, 46°11'17"W, altitude: 1404 m; 4: 22°28'29.8"S, 46°11'09.7"W, altitude: 1427 m), in Senador Amaral (1 and 2) and Bom Repouso (3 and 4). The sampling time was toward the end of the rainy season. Average temperature and total rainfall in the three months immediately before the sampling date were respectively 20.7°C and 510 mm (INMET 2020).

Fragments 2 and 4 were found in lowland areas in relation to strawberry crop. Each sample was put in a polyethylene bag, in turn put in a cool box for transport to the laboratory. Mites were extracted from the samples as described in the previous item.

Analysis

The predominant Gamasina species were calculated as proposed by Pinzón and Spence (2010). Average numbers of mites per sample were compared using the *glht* function of the multicomp package in the R program (R Development Core Team 2018).

5.3. Results

Mites in coffee husk and pulp

In total, 171 mites were collected (Table 1). Of these, 87 (50.9%), 61 (35.7%) and 23 (13.4%) belonged respectively to the orders Trombidiformes, Mesostigmata and Sarcoptiformes.

All Thrombidiformes belonged to Cheyletidae, of the suborder Prostigmata, while all Sarcoptiformes belonged to Acaridae. The Mesostigmata were represented by four families, with mites of the family Blattisociidae representing 91.8% of all Mesostigmata. All blatisocids belonged to the genus *Blattisocius*, represented by two species (based on adults), *Blattisocius dentriticus* (Berlese) and *Blattisocius keegani* Fox.

The cheyletids were found in the first five months of the evaluation (January to May) and also in August. Other mites were found only in either or both of the first two months (January and February).

Table 1. Mites extracted from eleven samples (400 cm^3 each) of coffee husk and pulp from Ouro Fino, Minas Gerais, Brazil, between January and December 2018.

	Month of collection									
Taxa	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Sarcoptiformes, Oribatida, Astigmatina										
Acaridae	23	0	0	0	0	0	0	0	0	0
Trombidiformes, Prostigmata										
Cheyletidae	7	11	9	24	35	0	0	1	0	0
Parasitiformes, Mesostigmata, Gamasina										
Blattisociidae										
Blattisocius dentriticus (Berlese)	8*	2	0	0	0	0	0	0	0	0
Blattisocius keegani Fox	7*	0	0	0	0	0	0	0	0	0
Male	28	0	0	0	0	0	0	0	0	0
Immature	11	0	0	0	0	0	0	0	0	0
Melicharidae										
Proctolaelaps pygmaeus (Müller)	0	1	0	0	0	0	0	0	0	0
Parasitidae										
Parasitus sp.	0	1	0	0	0	0	0	0	0	0
Phytoseiidae										
Neoseiulus californicus (McGregor)	3	0	0	0	0	0	0	0	0	0
Total	**87**	**15**	**9**	**24**	**35**	**0**	**0**	**1**	**0**	**0**

(*) Predominant Gamasina species.

Mites in forest fragments

The number of mites found in each fragment ranged from 210 to 1340, with Gamasina densities in fragments 2 and 4 being significantly higher than in the other fragments, which did not differ from each other (Table 2). In all, the most abundant mites were oribatids (non-Astigmatina Sarcoptiformes), which represented 81.9 to 89.7% of all mites in fragments 1, 3 and 4, but only 26.5% in fragment 2. The latter was the only fragment in which Astigmatina were represented by more than one individual (28 mites). Trombidiformes were not very abundant, and represented by three families of Prostigmata (Cheyletidae, Cunaxidae and Pseudocheylidae), and found in only two fragments.

The Mesostigmata (all, Gamasina) were most abundant in fragments 2 and 4. They constituted the most diverse mite group, being represented by eight families, each with 1–4 species, including the two species of *Blattisocius* found in coffee husk and pulp samples. Of those, the most represented were Macrochelidae (*Macrocheles* sp.), Parasitidae (*Parasitus* sp.) and Ologamasidae (*Neogamasellevans* sp.), the first two mainly in fragment 1 and the last in fragments 3 and 4.

Table 2. Mites extracted from eleven samples (400 cm^3) of the upper soil layer and litter of four natural forest fragments in Senador Amaral (1 and 2) and Bom Repouso (3 and 4), Minas Gerais, Brazil.

	Fragments			
Taxa	**1**	**2**	**3**	**4**
Sarcoptiformes, Others Oribatida	**172**	**111**	**526**	**1180**
Sarcoptiformes, Oribatida, Astigmatina (Acaridae)	**0**	**28**	**1**	**0**
Trombidiformes, Prostigmata	**0**	**3**	**0**	**6**
Cheyletidae	0	3	0	0
Cunaxidae	0	0	0	2
Pseudocheylidae	0	0	0	4
Parasitiformes, Mesostigmata, Gamasina	**38**	**277**	**59**	**154**
Ascidae				
Iphidozercon sp.	0	0	0	1
Male	2	0	0	0
Blattisociidae				
Blattisocius dentriticus (Berlese)	0	0	0	4
Blattisocius keegani Fox	0	1	0	0
Lasioseius sp.	0	7	0	1
Platyseius sp.	0	2	0	1
Immature	0	0	0	5
Digamasellidae				
Digamasellus sp.	0	0	3	7
Immature	0	0	0	4
Laelapidae				
Stratiolaelaps sp.	1	0	0	0
Macrochelidae				
Glyptholaspis sp.	0	3	2	3
Holostapella sp.	0	1	0	3
Macrocheles sp.	5	22*	0	2
Male	0	14	0	0
Immature	3	52	0	5
Ologamasidae				
Neogamasellevans sp.	6*	10	11*	40*
Male	7	11	24	34
Immature	6		7	15
Parasitidae				
Parasitus sp.	2	55*	3	3
Male	0	29	0	0
Immature	4	69	8	21
Podocinidae				
Podocinela sp.	1	0	1	2
Podocinum sp.	0	0	0	3
Immature	1	0	0	0
Total	**210**	**418**	**586**	**1340**
Densities (mean number of Gamasina/ sample ± SE)**	**3.4 ± 2.5 a**	**25.1 ± 5.7 b**	**5.4 ± 4.3 a**	**14.1 ± 9.7 b**

(*) Predominant Gamasina species. (**) Different letters indicate statistical difference. The means were compared using the *glht* function of the multicomp package in the R program.

5.4. Discussion

Mites fauna in coffee husk and pulp

The mites present in the samples of coffee husk and pulp were relatively few, and varied in density and composition along the year, confirming part of our initial hypothesis. The low mite densities were probably due to the way this material is obtained as a by-product, given that coffee grains are only processed after the moisture content is lowered to a maximum of 11%. This level of humidity is below what is usually tolerated by mites, as shown by a previous work, in which representatives of the edaphic Gamasina community (*Lasioseius floridensis* Berlese (Blattisociidae), *Cosmolaelaps* sp. (Laelapidae), *Proctolaelaps bickleyi* (Bram) (Melicharidae), *Protogamasellopsis posnaniensis* Wisniewski & Hirschmann (Rhodacaridae) and *Stratiolaelaps scimitus* (Womersley) (Laelapidae)) were observed to perished within hours when subjected to levels of up to 70% (our unpublished observations).

The higher number of mites at the beginning of the year is probably related to the higher rainfall at this time (INMET 2020), to the fact that climatic conditions in the storage area are not controlled and the fact that material commercialized in this period had been brought to storage months before. In southern Minas Gerais, coffee is harvested in June – September (Revista Cafeicultura 2018). In this period, environmental conditions are less favorable for mite survivorship and development, due to the low rainfall and the consequently low air humidity (INMET 2020), which may explain the very low numbers of mites found in this period. However, infestations could take place in storage, allowing mite population to increase even if slightly

Among the mites found, none belongs to groups known to cause regular damage to strawberry plants, and this also proves the second part of the first hypothesis of this work. Quite the opposite, most of them were predatory mites, with a predominance of Cheyletidae and Blattisociidae species, commonly found in stored products and occasionally found in soil or on plants (Athanassiou and Palyvos 2015).

The relatively higher frequency of cheyletids was already expected, as their presence in stored products has been extensively reported (Hughes 1976, Athanassiou et al. 2003 and 2011, Athanassiou and Palyvos, 2015). Species of this family are predominantly predators of various micro-arthropods in stored products, including mites (Hernandes et al. 2015) and insect eggs (Žd'árková 1979 and 1998).

In a work carried out by Esteca et al. (2018), the authors found several species of Prostigmata and Gamasina in the soil of a strawberry crop, after using of coffee husk and pulp (from the same origin as that used in the present work) as mulching. Among the predominant mites, cheyletids and the gamasine *B. dentriticus*, *P. pygmaeus* were found in that work. From the results of the present work, it could be expected that those mites could have been added to the strawberry crop together with the coffee husk and pulp then used for mulching.

Moraes et al. (2015) presented a review of the feeding habits of *B. dentriticus* and *P. pygmaeus* highlighting that these develop and reproduce on astigmatine mites, especially of the family Acaridae, also found in this work. This suggests that in the present work the predators could be feeding on the latter. In fact, acarid mites have been released on purpose in crops, to promote the establishment of commercially released predatory mites (OConnor 2009; Grosman et al. 2011; Munoz Cardenas, 2017).

In practice, the presence of blattisociid and melicarid mites in the coffee husk and pulp to be used as strawberry mulching is potentially advantageous, as *P. pygmaeus* has already been reported to feed on the two-spotted spider mite (Mathys and Tencalla 1959), key pest of this crop (Bernardi et al. 2015). Further investigation should be conducted about the importance of *P. pygmaeus* as a predator of the two-spotted spider mite on strawberry plants. As this predator was suspected to cause the elimination of a *Drosophila* laboratory colony by feeding on its eggs, as summarized by Moraes et al. (2015), it could be hypothesized to have some important effect as predator of *Drosophila suzukii* (Matsumura), recently found in southern Minas Gerais on strawberry (chapter 1 of this thesis) and a serious strawberry pest in southern Brazil (Santos 2014) and in Europe (Kinjo et al. 2014). However, the results of a laboratory study (chapter 3 of this thesis) could not support that suspicion.

Mites in forest fragments

The largest proportions of oribatids in comparison with other mite groups was expected, as extensively reported in in recent studies of edaphic mites in other regions in Brazil (Barros 2020; Yamada 2020; Azevedo 2021). These mites are mostly involved in the decomposition of organic matter in the soil (Wickings and Grandy 2011), although some may also prey on other organisms, especially nematodes (Muraoka and Ishibashi 1976; Rockett 1980; Oliveira et al. 2007).

The second hypothesis of this work was proven, given the variation in composition and density of Gamasina among the four evaluated forest fragments. The higher densities in fragments 2 and 4 could be related to the following factors: these fragments were found in lowland spots, with seemingly higher humidity levels, favoring the development of edaphic Gamasina. Fragment 2 was sided by a pasture area, which seemingly favored the occurrence of

Gamasina, mainly Macrochelidae and Parasitidae (Azevedo et al. 2015). Additionally, fragments 2 and 4 were larger than the others (about 40.000 and 48.000 m^2, in comparison with about 36.000 and 14.000 m² of fragments 1 and 3) which could favor the presence of edaphic mites given that forest fragments with larger areas generally have greater density and diversity of arthropods, when compared to smaller forest fragments (Turner 1996; Souza and Brown 1994; Didham 1998).

The Family Blattisociidae was quite well represented, including the two *Blattisocius* species found in samples of coffee husk and pulp, *B. dentriticus* and *B. keegani*, as well as *Lasioseius* and *Platyseius* species. However, blattisociids were only found in fragments 2 and 4, in which gamasine density was higher.

Macrochelids and parasitids, the predominant Gamasina groups in fragment 2, are the most abundant among the groups of predatory mites in excrements or decaying animals (Braig and Perotti 2009; Castilho et al., 2015b). Filipponi (1964) divided the macrochelids into three main groups, fimicolous, humicolous and insecticolous, referring respectively to their association with excreta, humus or insects (mainly as carriers of these mites). These, together with the parasitids, have potential as agents for the biological control of harmful organisms, including different types of flies and nematodes (Azevedo et al. 2015). In chapter 3 of this thesis, macrochelids were able to feed and lay eggs on pupae of *D. suzukii*, but the same was not observed with *Parasitus* sp. evaluated in that same chapter.

It also seems interesting the fact that Ologamasidae was the only family well represented in all fragments, despite being represented by mites of the genus *Neogamasellevans*. Esteca et al. (2018) also found ologamasids in strawberry cultivation covered with coffee straw that had previously been deposited in a forest fragment. Studies conducted in other parts of Brazil have reported ologamasids as a common group in natural vegetation in the Atlantic Forest biome (Mineiro and Moraes 2001; Silva et al. 2004; Junqueira 2017). Mites of this family have also been found in natural vegetation in other Brazilian biomes, as the Cerrado (Marticorena 2017; Azevedo et al. 2020), Caatinga (Santos 2013; Barros 2020) and Pampa (Duarte et al. 2020). Castilho et al. (2015b) summarized the predatory habits of ologamasids, concluding that they feed on other mites, small insects, nematodes and Collembola. Although in reduced number and diversity, mites of the family Laelapidae were also found in this study. These mites have been commercially used for the control of edaphic pests (Moreira and Moraes 2015). In chapter 3 of this thesis, the laelapid species found in this study (*S. scimitus*) was the only predator observed to prey on *D. suzukii* eggs inside the fruits. Ascidae and Digamasellidae were also found, but in low numbers. These mites have not been reported to have high potential as biological control agents, but they deserve further research attention (Moraes et al. 2015; Castilho et al. 2015b). As for Podocinidae, these have been reported as predators, but their biology is poorly known (Lindquist et al. 2009; Rueda-Ramírez et al. 2019). Esteca et al. (2018) did not find ascids, digamasellids and podocinids in strawberry cultivation mulching with coffee husk and pulp previously deposited in a forest fragment, most likely because they were not present in that fragment.

The predominant Gamasina of this study were not common in soil of strawberry crops in a previous study conducted in southern Minas Gerais (Esteca et al. 2018). The reason for that is unclear, but could be due to the use of chemicals in the crop or to the unsuitable environmental conditions in the strawberry fields. The critical evaluation of the true reasons could help in determining the suitability of the attempt to enrich the material for mulching with predatory mites in forest patches prior to its use. In the study by Esteca et al. (2018), little differences in terms of composition and density of edaphic mites were found between treatments with and without previously deposition of the mulching material in a forest fragment. But this could be due to the low densities of predatory mites in the chosen forest fragment, at least in the period "enrichment" attempt was conducted (June 2015) a possibility suggested by the results of the present study. That fragment was only 500 m^2, and in the period the material was in the fragment, rainfall was only 60 mm (Esteca et al., 2018). Hence the importance of additional studies of the soil mites in forest fragments along the year. Information from such studies might help in choosing most suitable forest fragments to eventually deposit the coffee husk and pulp for enrichment with predatory mites for subsequent use as strawberry mulching. In the present study, preference would be given to fragments 2 or 4, as in these the largest number and diversity of Gamasina were found.

In summary, based on the results of this study, it can be concluded that the highest number of predatory mites can be found in coffee husk and pulp at the beginning of the year, coincidentally the period of greatest strawberry planting in southern Minas Gerais (Gonçalves et al. 2016). This is most probably due to the fact that the storage site is not hermetic, allowing access of mites from the surroundings, and variation of environmental conditions along the year. The density and composition of mites varied among the forest fragments studied. Therefore, further studies should be conducted to better understand the variation of Gamasina composition and densities in different fragments along the year, to determine the most appropriate combinations of sites and periods for eventual enrichment, and to evaluate the effect of each predatory mite on the main pest species amenable to attack.

REFERENCES

Alvares, C.A.; Stape, J.L.; Sentelhas, P.C.; Gonçalves, J.L.M.; Sparovek, G. (2014) Koppen's climate classification map for Brazil. Meteorologische Zeitschrift, 22(6): 711-728.

Antunes, L.E.C.; Bonow, S.; Reisser Júnior, C. (2020) Morango: Crescimento constante em área e produção. Campo e Negócios, 1: 88-92.

Athanassiou, C.G.; Kavallieratos, N.G.; Palyvos, N.E.; Buchelos C.T. (2003) Three-dimensional distribution and sampling indices of insects and mites in horizontally-stored wheat. Applied Entomology and Zoology, 38: 413-426.

Athanassiou, C.G.; Kavallieratos, N.G.; Sciarreta, A.; Palyvos N.; Trematerra, P. (2011) Spatial associations of insects and mites in stored wheat. Journal of Economic Entomology, 104: 1752-1764.

Athanassiou, C.G.; Palyvos, N.E. (2015) The Cheyletoidea (Prostigmata), with special reference to the potential of *Cheyletus malaccensis* Oudemans as biological control agent of post-harvest pests. In Prospects for Biological Control of Plant Feeding Mites and Other Harmful Organisms (pp. 241-249). Springer, Cham.

Azevedo, E.B. (2021) Efeito dos cultivos agrícolas na diversidade de ácaros predadores Mesostigmata e prospecção para uso no controle biológico. Doctor's Thesis, Faculdade de Ciências Agrárias e Veterinárias, Paulista State University, Jaboticabal. Retrieved 2021-07-12, from https://repositorio.unesp.br/handle/11449/204502.

Azevedo, E.B.; Azevedo, L.H.; Moreira, G.F.; Santos, F.A.D.; Carvalho, M.A.F.D.; Sarmento, R.D.A.; Castilho, R.C. (2020) Diversity of soil gamasine mites (Acari: Mesostigmata: Gamasina) in an area of natural vegetation and cultivated areas of the Cerrado biome in Northern Brazil. Diversity, 12(9): 331.

Azevedo, L.H.; Emberson, R.M.; Esteca, F.C.N.; Moraes, G.J. (2015) Macrochelid mites (Mesostigmata: Macrochelidae) as biological control agents. In: *Prospects for biological control of plant feeding mites and other harmful organisms*; Carillo, D.; Moraes, G.J.; Peña, J.E. (Eds.). Springer International Publishing: Cham, Switzerland, chap. 4, pp 103–132.

Barros, A.R.A. (2020) Diversidade de ácaros edáficos Gamasina (Mesostigmata) no bioma Caatinga no Estado de Alagoas, com descrição de uma espécie nova, e elaboração de um catálogo para a família Podocinidae. Master's Dissertation, Faculdade de Ciências Agrárias e Veterinárias, Paulista State University, Jaboticabal. Retrieved 2021-07-12, from https://repositorio.unesp.br/handle/11449/192835.

Bernardi, D.; Botton, M.; Nava, D. E.; Zawadneak, M.A.C. (2015) Guia para a identificação e monitoramento de pragas e seus inimigos naturais em morangueiro. Embrapa Uva e Vinho- Livro científico (ALICE).

Braig, H.R.; Perotti, M.A. (2009) Carcases and mites. Experimental and Applied Acarology, 49: 45-84.

Carrillo, D.; Moraes, G.J.; Peña, J.E. (2015) Prospects for Biological Control of Plant Feeding Mites and Other Harmful Organisms; Springer International Publishing: Cham, Switzerland, p. 337.

Castilho, R.C.; Duarte, V.S.; Moraes, G.J.; Westrum, K.; Trandem, N.; Rocha, L.C.D.; Delalibera, Jr., I.; Klingen, I. (2015a) Two-spotted spider mite and its natural enemies on strawberry grown as protected and unprotected crops in Norway and Brazil. Experimental and Applied Acarology, Amsterdam, 66(4): 509-528.

Castilho, R.C.; Venancio, R.; Narita, J.P.Z. (2015b) Mesostigmata as biological control agents, with emphasis on Rhodacaroidea and Parasitoidea. In: *Prospects for Biological Control of Plant Feeding Mites and other Harmful Organisms*; Carillo D.; Moraes, G.J.; Peña, J.E. (Eds.). Springer International Publishing: Cham, Switzerland, chap. 1, pp 1-32.

Cook, H.F.; Valdes, G.S.B.; Lee, H.C. (2006) Mulch effects on rainfall interception, soil physical characteristics and temperature under *Zea mays* L. Soil Tillage Research, Amsterdam, 91: 227.

Didham, R.K. (1998) Altered leaf-litter decomposition rates in tropical forest fragments. Oecologia, 115: 397-406.

Duarte, A.D.F.; Moreira, G.F.; Cunha, U.S.D.; Siqueira, P.R.E.; Moraes, G.J. (2020) Edaphic mesostigmatid mites (Parasitiformes, Mesostigmata) in a region of the Pampa biome of the state of Rio Grande do Sul, Brazil. Ciência Rural, 50(2): e 20190043.

Durán, C. A.; Tsukui, A.; Santos, F. K. F.; Martinez, S. T.; Bizzo, H. R.; Rezende, C. M. (2017) Café: aspectos gerais e seu aproveitamento para além da bebida. Revista Virtual de Química, 9(1): 107-134.

Esteca, F.C.N.; Rodrigues, L.R.; Moraes, G.J.; Delalibera Júnior, I; Klingen, I. (2018) Mulching with coffee husk and pulp in strawberry affects edaphic predatory mite and spider mite densities. Experimental and Applied Acarology, 76(2): 161-183.

Esteca, F.C.N.; Trandem, N., Klingen, I., Cruz, J. S.; Delalibera Júnior, I.; Moraes, G.J. (2020) Cereal straw mulching in strawberry—a facilitator of plant visits by edaphic predatory mites at night? Diversity, 12(6): 242.

Filipponi, A. (1964) The feasibility of mass producing macrochelid mites for field trials against houseflies. Bulletin of the World Health Organization, 31(4): 499–501.

Fundação SOS Mata Atlântica; Instituto Nacional De Pesquisas Espaciais (INPE) (2011) Atlas dos Remanescentes Florestais da Mata Atlântica Período 2008-2010.

Gonçalves, M.A.; Picolotto, L.; Cocco, C.; Vignolo, G.K.; Antunes, L.E.C. (2016) Crescimento e desenvolvimento. In Morangueiro; Antunes, L.E.C., Reisser Júnior, C., Schwengber, J.E., Eds.; Embrapa: Brasília, Brazil, pp. 47-66.

Grosman, A.H.; Messelink, G.J.; Groot, E.B. (2011) Combined use of a mulch layer and the soil-dwelling predatory mite *Macrocheles robustulus* (Berlese) enhance the biological control of sciarids in potted plants. IOBC/WPRS Bulletin, 68: 51-54.

Hernandes, F.A.; Castro, T.M.M.; Venancio, R. (2015) Prostigmata (Acari: Trombidiformes) as biological control agents. In: *Prospects for biological control of plant feeding mites and other harmful organisms*; Carillo, D.; Moraes, G.J.; Peña, J.E. (Eds.). Springer International Publishing: Cham, Switzerland, chap. 6, pp 151-184.

Hughes, A.M. (1976) The mites of stored food and houses, 2nd edition, Technical Bulletin 9. Ministry of Agriculture, Fisheries and Food. Her Majesty's Stationery Office, London, p 400.

INMET (2020) Instituto Nacional de Meteorologia. Estações Automáticas—Gráficos. Available online: http://www.inmet.gov.br/portal/index.php?r=home/page&page=rede_estacoes_auto_graf. (Accessed on 02 July, 2021)

Junqueira, B.R. (2017) Diversidade de ácaros edáficos em um fragmento de Mata Atlântica e três cultivos agrícolas, em Jaboticabal/SP, com ênfase nos Gamasina (Mesostigmata). Master's Dissertation, Faculdade de Ciências Agrárias e Veterinárias, Paulista State University, Jaboticabal. Retrieved 2021-07-12, from https://repositorio.unesp.br/handle/11449/152291.

Kinjo, H.; Kunimi, Y.; Nakai M. (2014) Effects of temperature on the reproduction and development of *Drosophila suzukii* (Diptera: Drosophilidae). Applied Entomology and Zoology, 49: 297-304.

Köppen, W. (1948) Climatologia: con un estúdio de los climas de la tierra. Fondo de Cultura Econômica, México, D.F., 479 pp.

Krantz, G.W.; Walter, D.E. (2009) A Manual of Acarology, 3rd edition; Texas Tech University Press: Lubbock, TX, USA, p. 411.

Lindquist, E.E.; Krantz, G.W.; Walter, D.E. (2009) Order Mesostigmata. In: Krantz GW, Walter DE (Eds.) A Manual of Acarology. Third Edition, Lubbock, Texas: Texas Tech University Press, p. 124-232.

Marticorena, J.L.M. (2017) Alteration of the faunistic composition of edaphic mites according to land use in the central-southern region of Brazil. Doctored's Thesis, Faculdade de Ciências Agrárias e Veterinárias, Paulista State University, Jaboticabal. Retrieved 2021-07-12, from https://repositorio.unesp.br/handle/11449/150882.

Mathews, C.R; Bottrell, D.G; Brown, M.W. (2002) A comparison of conventional and alternative understory management practices for apple production: multi-trophic effects. Applied Soil Ecology, Amsterdam, 21: 221-231.

Mathys, G.; Tencalla, Y. (1959) Note préliminaire sur la biologie et la valeur prédatice de *Proctolaelaps hypudaei* Oudms (Acarien: Mesostigmata: Aceosejidae). Stations Fédérales D'Essais Agricoles, 600: 645-654.

Mineiro, J.L.C.; Moraes, G.J. (2001) Gamasida (Arachnida: Acari) edáficos de Piracicaba, Estado de São Paulo. Neotropical Entomology, 30: 379-385.

Moraes, G.J.; Venancio, R.; Santos, V.L.V.; Paschoal, A.D. (2015) Potential of Ascidae, Blattisociidae and Melicharidae (Acari: Mesostigmata) as biological control agents of pest organisms. In: *Prospects for biological control of plant feeding mites and other harmful organisms*; Carillo, D.; Moraes, G.J.; Peña, J.E. (Eds.). Springer International Publishing: Cham, Switzerland, chap. 2, pp. 33–76.

Moreira, G.F.; Moraes, G.J. (2015) The potential of free-living laelapid mites (Mesostigmata: Laelapidae) as biological control agents. In: *Prospects for biological control of plant feeding mites and other harmful organisms*; Carillo, D.; Moraes, G.J.; Peña, J.E. (Eds.). Springer International Publishing: Cham, Switzerland, chap. 3, pp. 77-102.

Morra, L.; Bilotto, M.; Cerrato, D.; Coppola, R.; Leone, V.; Mignoli, E.; Pasquariello, M.S.; Petriccione, M.; Cozzolino, E. (2016) The Mater-Bi® biodegradable film for strawberry (*Fragaria* x *ananassa* Duch.) mulching: effects on fruit yield and quality. Italian Journal of Agronomy, Pavia, 11: 731.

Munoz Cardenas, K.A. (2017) What lies beneath? Linking litter and canopy food webs to protect ornamental crops. Department of Population Biology, Institute for Biodiversity and Ecosystem Dynamics University of Amsterdam, Amsterdam, The Netherlands, p. 144.

Muraoka, M.; Ishibashi, N. (1976) Nematode-feeding mites and their feeding behaviour. Applied Entomology and Zoology, 11: 1-7.

OConnor, B.M. (2009) Astigmatid mites (Acari: Sarcoptiformes) of forensic interest. Experimental and Applied Acarology, 49: 125-133.

Oliveira, A.D.; Moraes, G.D.; Demétrio, C.D.; Nardo, E.A.B. (2001) Efeito do vírus de poliedrose nuclear de *Anticarsia gemmatalis* sobre Oribatida edáficos (Arachnida: Acari) em um campo de soja. Boletim de pesquisa, 13: 31-37.

Oliveira, A.R.; Moraes, G.J.; Ferraz, L.C. (2007) Consumption rate of phytonematodes by *Pergalumna* sp. (Acari: Oribatida: Galumnidae) under laboratory conditions determined by a new method. Experimental and Applied Acarology, 41(3): 183-189.

Oliveira, F.F.; Guerra, J.G.M.; Almeida, D.L.; Ld Ribeiro, R.; Espindola, J. A.A.; Ricci, M.; Ceddia, M.B. (2008) Avaliação de coberturas mortas em cultura de alface sob manejo orgânico. Horticultura Brasileira, 26: 216-220.

Patenaude, S.; Tellier, S.; Fournier, V. (2020) Cyclamen mite (Acari: Tarsonemidae) monitoring in eastern Canada strawberry (Rosaceae) fields and its potential control by the predatory mite *Neoseiulus cucumeris* (Acari: Phytoseiidae). The Canadian Entomologist, 152(2): 249-260.

Peel, M.C.; Finlayson, B.L.; McMahon, T.A. (2007) Updated world map of the Köppen-Geiger climate classification. Hydrology and Earth System Sciences, 11: 1633-1644.

Pinzón, J.; Spence, J.R. (2010) Bark-dwelling spider assemblages (Araneae) in the boreal forest: dominance, diversity, composition and life-histories. Journal of Insect Conservation, 14(5): 439-458.

R Core Team, (2018). R: A Language and Environment for Statistical Computing R Foundation for Statistical Computing, Vienna, Austria (2018) Available online: https://www.R-project.org/ (accessed on May 12, 2019).

Resende, F.V.; Souza, L.S.D.; Oliveira, P.S.R.D.; Gualberto, R. (2005) Uso de cobertura morta vegetal no controle da umidade e temperatura do solo, na incidência de plantas invasoras e na produção da cenoura em cultivo de verão. Ciência e Agrotecnologia, 29(1): 100-105.

Revista De Cafeicultura (2018). Produtores preparam lavouras visando o início da colheita do café no Sul de Minas. Available online: http://revistacafeicultura.com.br/?mat=66132 (accessed on November 15, 2018).

Rockett, C.L. (1980) Nematode predation by oribatid mites (Acari: Oribatida). International Journal of Acarology, 6: 219-224.

Rueda-Ramírez, D.; Santos, J.C.; Sourassou, N.F.; Demite, P.R.; Puerta-González, A.; Moraes, G.J. (2019) Complementary description of *Africoseius lativentris* and placement of *Africoseius* in Podocinidae (Acari, Mesostigmata) based on molecular and morphological evidences. Systematic and Applied Acarology, 24: 2369-2394.

Santos, J.C. (2013) Ácaros (Arthropoda: Acari) edáficos do estado do Alagoas, com ênfase nos Mesostigmata. Master’s Dissertation, Faculdade de Ciências Agrárias e Veterinárias, Paulista State University, Jaboticabal. Retrieved 2021-07-12, from https://repositorio.unesp.br/handle/11449/91310.

Santos, R.S.S. (2014) *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) atacando frutos de morangueiro no Brasil. Embrapa, Bento Gonçalves, Brazil, p. 4 (Comunicado Técnico 159).

Silva, E.S.; Moraes, G.J.; Krantz, G.W. (2004) Diversity of edaphic rhodacaroid mites (Acari: Mesostigmata: Rhodacaroidea) in natural ecosystems in the State of São Paulo, Brazil. Neotropical Entomology, 33: 547-555.

Solomon, M.G.; Jay, C.N.; Innocenzi, P.J.; Fitzgerald, J.D.; Crook, D.; Crook, A.M.; Easterbrook, M.A.; Cross, J. V. (2001) Natural enemies and biocontrol of pests of strawberry in northern and central Europe. Biocontrol Science and Technology, 11(2): 165-216.

Souza, O.F.F.; Brown, V.K. (1994) Effects of habitat fragmentation on Amazonian termite communities. Journal of Tropical Ecology, 10: 197-206.

Turner, I.M. (1996) Species loss in fragments of tropical rain forest: a review of the evidence. Journal of Applied Ecology, 33: 200-209.

Wickings, K.; Grandy, A.S. (2011) The oribatid mite *Scheloribates moestus* (Acari: Oribatida) alters litter chemistry and nutrient cycling during decomposition. Soil Biology and Biochemistry, 43: 351-358.

Yamada, M. (2020). Diversidade de ácaros edáficos no bioma Pantanal e potencial de algumas espécies para o controle biológico de pragas de solo. Master's Dissertation, Escola Superior de Agricultura “Luiz de Queiroz”, University of São Paulo, Piracicaba. doi:10.11606/D.11.2020.tde-30042020-171813. Retrieved 2021-07-12, from www.teses.usp.br.

Žd'árková, E. (1979) Cheyletid fauna associated with stored products in Czechoslovakia. Journal of Stored Products Research, 15: 11-16.

Žd'árková, E. (1998) Biological control of storage mites by *Cheyletus eruditus*. Integr Pest Management, 3: 111-116.

6. USE OF COFFEE HUSK AND PULP AS MULCHING IN STRAWBERRY CULTIVATION IN LOW TUNNEL AND OPEN FIELD PRODUCTION SYSTEMS

Abstract

An important practice in strawberry (*Fragaria* x *ananassa* Duch.) cultivation is mulching. In southern Minas Gerais and the neighboring region in São Paulo state, the main producing regions, most producers use polyethylene as a ground cover, but this product is not biodegradable and is expensive. Hence the evaluation of the possibility to look for an alternative, as coffee husk and pulp, is warranted. The objectives of this work were to evaluate: a) whether the use of coffee husk and pulp as mulching could lead to the introduction of harmful mites into strawberry cultivations; b) whether the maintenance of this material in a forest fragment would allow the movement of predatory mites into it; c) populations of arthropod strawberry pests; d) temperature, relative humidity and dew point; e) the weight and Brix° of the strawberries and the macro and micronutrient levels of strawberry leaflets; e) the ability of edaphic Gamasina, to climb on the strawberry plants at night, and to prey on *Tetranychus urticae* (Koch, Tetranychidae). Two experiments were carried out: one in Bom Repouso, Minas Gerais and another in Atibaia, São Paulo. Both consisted of three treatments: 1) coffee husk and pulp maintained for 2 months on the floor of a forest fragment for possible enrichment with predatory mites; 2) coffee husk and pulp without such "enrichment"; 3) polyethylene film. The results showed: a) the absence of harmful mites in the coffee husk and pulp, and that its maintenance in a forest fragment increased the number and diversity of edaphic predatory mites; b) that the organic mulching maintained a microclimate, close to the plants, favorable to predatory mites and harmful to spider mites; c) that edaphic Gamasina can move to strawberry plants at night; d) that the use of coffee husk and pulp resulted in the production of heavier and sweeter strawberries; d) that some edaphic Gamasina were able to attack and oviposit on *T. urtice*. Future studies should include the evaluatikon of cost/benefit of using coffee husk and pulp compared to polyethylene for mulching.

Keywords: 1. Organic mulching 2. Two-spotted spider mite 3. Edaphic predatory mites 4. Biological control

6.1. Introduction

Southeastern Minas Gerais state and the neighboring region in São Paulo state comprise an important strawberry (*Fragaria* x *ananassa* Duch.) producing area in Brazil, accounting for more than 50% of the national production (Cruz 1999; Antunes et al. 2020). An important management practice in the strawberry cultivation around the world is the use of ground cover (*mulching*), to reduce the incidence of weeds, to protect the fruits from direct contact with the soil and to maintain soil moisture (Strassburger et al. 2009). Polyethylene film is widely used for that purpose universally, including in that area (Morra et al. 2016), despite being expensive and not biodegradable. Still, in many other countries, mulching consists of some type of organic material locally available, often a byproduct of agriculture (Castilho et al. 2015; Esteca et al. 2018; Esteca et al. 2020). Given the extensive cultivation of coffee in southern Minas Gerais, a mixture of coffee husk and pulp (a byproduct of the processing of coffee beans) is widely available at prices that make it affordable for use as mulching in strawberry cultivation.

The use of organic mulching can offer numerous advantages over polyethylene, stabilizing temperature, humidity and soil structure, while serving as a refuge for pest natural enemies, including insects of the order Coleoptera and spiders (Johnson et al. 2004), in addition to predatory mites (Esteca et al. 2018; 2020). Mites of the order Mesostigmata have been shown to benefit from the use of organic mulching (Hoddle et al. 2002; Jensen et al. 2002; Jamieson and Stevens 2006; Sánchez-Moreno and Ferris 2007). Within this order, Gamasina is the largest group (cohort), found in different habitats, but mainly in the soil, where these mites feed on small invertebrates, including mites, nematodes and insects, as well as on fungi (Carrillo et al. 2015). In this context, the use of organic mulching in strawberry production could be helpful in reducing the level of pest organisms attacked by those predators (Solomon et al. 2001; Bernardi et al. 2015; Dara 2016; Montagnana et al. 2017).

Esteca et al. (2018) hypothesized that the temporary exposure of coffee husk and pulp in forest fragments in the vicinity of strawberry fields for a certain period could contribute to their enrichment with local populations of beneficial mites, in a similar way as reported in the production of *bokashi* (Lasmini et al. 2018), which could later contribute to the biological pest control in the crop. However, up to the present, little is known about the diversity and abundance of soil mites in forested areas of southern Minas Gerais. Esteca et al. (2018) showed evidence that in plots covered with that material, the population of the two-spotted spider mite (*Tetranychus urticae* Koch) reached lower densities, being conceived by those authors to be due to increase in humidity, although that parameter was not measured in their study. This mite is considered to be a strawberry pest worldwide (Solomon et al. 2001). Development of *T. urticae* has been shown to be inhibited by free water or high humidity (Mori and Chant 1966; Duso et al. 2004), as also suggested by Castilho et al. (2015) in strawberry cultivation. Another possible factor mentioned by Esteca et al. (2018) was the effect of edaphic predatory mites, when they demonstrated that some of the edaphic Gamasina, including *Proctolaelaps pygmaeus* (Muller) (Melicharidae) and *Blattisocius dentriticus* (Berlese) (Blattisociidae), were found on strawberry leaflets, mainly at night. However, also in this case, the authors did evaluate the actual attack of those predators on *T. urticae*.

The purpose of this work was to answer some of the questions raised by Esteca et al. (2018). Thus, the hypotheses proposed for study were: a) the maintenance of the organic material (coffee husk and pulp) destined for later use as *mulching* on the floor of a forest fragment for a given period allows local predatory mites to move into it, what could be considered “an enrichment” of that material; b) organic *mulching* causes changes in the microclimate close to the plants, favoring the presence of predatory mites and/ or disfavoring the presence of the two-spotted spider mite; c) *mulching* with coffee husk and pulp influences the quality of strawberry pseudofruits; d) predators that climb the plants at night can act as biological control agents of the two-spotted spider mite, which is considered important as management of mulching may directly impact the edaphic mites.

The objectives of this work were to evaluate: a) whether the use of coffee husk and pulp as *mulching* could lead to the introduction of harmful mites for strawberry crop; b) if the maintenance of this material in a forest fragment would allow the movement of predatory mites to this material. Furthermore, to evaluate the effect of coffee husk and pulp on: c) populations of strawberry pest arthropods; d) temperature, relative humidity and dew point; e) the weight and Brix° of the pseudofruits and the macro and micronutrient levels of the strawberry leaflets. Finally, evaluate the ability of edaphic Gamasina, which climbs on plants at night, to prey on *T. urticae*.

6.2. Material and Methods

Two experiments were carried out, one in Bom Repouso, Minas Gerais (22º28'"S; 46º11'"W, altitude: 1442 m) and another in Atibaia, São Paulo (23°04"S; 46°40'"W, altitude: 766 m). Both consisted of three treatments, each corresponding to the use of following materials as mulching: 1) coffee husk and pulp maintained for two months on the floor of a forest fragment (for “enrichment” with predatory mites); 2) coffee husk and pulp not exposed in the forest fragment, that is, “not enriched”; 3) polyethylene film.

Storage of coffee husk and pulp and preliminary mite evaluation

Forty bags (about 20 L each) of coffee husk and pulp were purchased from “Primos Indústria e Comércio de Café”, at Ouro Fino, Minas Gerais, on July 17, 2018. The content of the half bags was distributed in a layer of approximately 10 cm in height onto the surface of the floor of the lowest (wet) part of a natural forest fragment (22°28'29.16"S; 46°11'17"W, altitude:1380 m; extension about 48,000 m^2) located near the strawberry field where the study would be conducted. This corresponded to fragment 4 of Chapter 4 of this thesis, selected for presenting the highest predatory mite density. On the day the organic material was deposited in the fragment and every third day thereafter, it was moistened with well water (about 2 L/bag). This material was used for “treatment 1”. Half of these were kept piled up in a shed in Bom Repouso, with no control of the environmental conditions. This material was used for “treatment 2”.

To assess the mites that could be introduced into the strawberry field, samples were taken from the recently acquired coffee husk and pulp as well as from the floor (soil and litter) of the forest fragment, from which mites were expected to move to the exposed layer of coffee husk and pulp. In total, 12 samples were taken randomly from the bags of coffee husk and pulp and 12 from the forest floor, each sample consisting of a volume of approximately 400 cm^3. To extract the mites, each sample was maintained in a modified Berlese funnel (Oliveira et al. 2001) for a period of seven days. The mites were collected in a solution of 70% ethanol, sorted under a stereomicroscope and mounted on slides in Hoyer's medium. Mite identification was later done at the family level using taxonomic keys provided by Krantz and Walter (2009), at the genus level using unpublished keys provided by the Ohio Summer Program, Agricultural Acarology, Columbus, Ohio, USA, and at the species level using descriptions and redescriptions available in the literature.

The same number of bags of coffee husk and pulp were purchased from the same company mentioned for the study in Bom Repouso, transporting them to Atibaia on February 13, 2020. Again, half of these were kept piled up in a shed in Atibaia, with no control of the environmental conditions. This material was used for “treatment 1”. The content of the other bags was distributed in a layer of approximately 10 cm in height onto the surface of the floor of the lowest (wet) part of a natural forest fragment (23°04'14"S 46°40'49"W, altitude: 780 m; about 21,000 m^2) located near the strawberry field where the study would be conducted. On the day the organic material was deposited in the fragment and every third day thereafter, it was moistened with well water (about 2 L/bag). This material was used for “treatment 2”.

Periodic wetting of the material and the evaluation of the mites were carried out following the same procedure mentioned for Bom Repouso.

Crop management

The total area of this field was 1.8 ha, the cultivar was 'Aromas' and plantlets were transplanted in mid-July 2018. Cultivation was done in beds covered by low tunnels, each about 50 m long, with three rows of plants spaced 35 cm between and within the rows and 50 cm between beds.

The mulching corresponding to each treatment was placed in the field on September 27, 2018, in a randomized block experimental design, with 12 replicates. Each experimental plot corresponded to a 7.0 m long bed section (approximately 50 plants), keeping a distance of 2 m between plots on the same bed and 1 m between neighboring experimental beds. Mulching of treatments 1 and 2 was about 5 cm thick, as used in previous studies (Filgueira, 2000; Esteca et al. 2018, 2020).

As the study was conducted in a grower's field, we could not prevent the use of chemicals, done at his will. The following biological products were used for pest control, alternately and about monthly: Boveril® WP (*Beauveria bassiana* PL63) (200 g/100 L of water), Metarril® WP (*Metarhizium anisopliae* E9) (150 g/100 L of water), Neem Oil (*Azadirachta indica* A. Juss) (1 L/100 L of water). In December 2018, Ortus® 50 SC (fenpyroximate) (100 mL /100 L of water) was used for the control of *T. urticae*.

Temperature, humidity and dew point were determined every 6 hours with a Datalogger HT-500 Instrutherm® installed in the central region (about 10 cm above the mulching) in a randomly determined plot of each treatment. Temperature and humidity were determined likewise outside the tunnel, in a shaded shed, about 10 m from the margin of the crop. In addition, rainfall was recorded daily with a rain gauge installed about 10 m from the margin of the crop.

In Atibaia, the total area of the field was 0.2 ha, the cultivar was 'Festival', and plantlets were transplanted on March 29, 2020. The plants were cultivated in open beds (without aerial protection), each about 40 m long, with three rows of plants spaced 35 cm between and within the rows and 50 cm between the beds.

The procedure adopted was the same mentioned for Bom Repouso. The only differences are subsequently mentioned. The mulching corresponding to each of the three different treatments was placed in the field on May 19th. Plants were drip irrigated every 2 days. Again, by being a grower´s field, the use of chemicals was done at the owner´s will, with Neem oil (*Azadirachta indica* A. Juss) (1 kg/100 L of water) used about monthly. Amistar Top® (azoxystrobin and difenoconazole) (45 mL/100 L of water) was applied once, in November 2020, as fungicide. Metarril® WP (*Metarhizium anisopliae* E9) (150 g/100 L of water) was used in June and July.

Monitoring mites at the surface of strawberry beds

For both experiments, three evaluations of mite density were carried out: on the first day (Bom Repouso: September 27, 2018; Atibaia: May 19, 2020), in the middle (Bom Repouso:

January, 08, 2019; Atibaia: September 3, 2020) and at the end (Bom Repouso: April 10, 2019; Atibaia: December 1, 2020) of the experiment. In each evaluation, a sample was collected from the central region of each experimental plot, each corresponding to a volume of approximately 400 cm^3 collected with the aid of a metallic cylinder, 5 cm in height and 10 cm in diameter. Each sample was placed in a plastic bag, in turn placed in a thermal box for transport to the laboratory. The procedure adopted for the extraction of mites, mounting and identification was the same mentioned before.

Monitoring arthropods on plants at daytime

In both experiments, a monthly evaluation was carried out, starting 40 days after the beginning of the experiment, for a total of seven evaluations. In each evaluation, 18 fully developed leaflets were randomly taken from the central line of each plot between 10 and 12 A.M. The leaflets from each plot were placed in a “ziplock” plastic bag (40 x 30 cm) containing 70% ethanol and transported to the laboratory for screening and identification of the arthropods.

Edaphic mites on whole strawberry plants

This activity was carried out only in Atibaia, about four months from the beginning of the experiment. For that purpose, four whole plants (approximately 30 leaflets each) were removed from the central line of each plot at around 10 AM and four whole plants were removed from the same row at around 10 PM. Each plant was immediately placed in a “ziplock” plastic bag (40 x 30 cm) containing 70% ethanol for transport to the laboratory, where the liquid was poured through a 160 µm sieve to extract the mites. The plant material was then washed with jets of 70% ethanol, onto the same sieve, collecting the trapped mites with a brush and mounting them in Hoyer's medium for subsequent identification.

Strawberry quality

In both experiments, 40 days after placing the organic coverings in the beds and monthly afterward, 18 ripe strawberries taken randomly from each plot were placed in a plastic bag and transported to the laboratory, where they were weighed with a precision electronic digital scale (Sf-400, up to 10kg).

For the experiment conducted in Atibaia, the same strawberries were used for the quantification of soluble solids. They were crushed to form a homogeneous pulp, which was then filtered through a 200 µm sieve. Two drops of the sieved material of each sample were placed on the lens of a KASVI® digital refractometer, for determination of the °Brix.

Leaf contents of macro and micronutrients

For the experiment conducted in Atibaia, leaflet samples were collected on December 01, 2020, at the end of the experiment, to evaluate the levels of macro and micronutrients. Following the methodology proposed by Raij et al. (1996), leaflets were taken from the third or fourth leaves from the plant top. A sample was taken from each other experimental plot of each treatment, each sample consisting of 30 leaflets, each from a different plant. Each sample was placed in a paper bag and transported to a commercial laboratory (Pirasolo Laboratório Agrotécnico Piracicaba Ltda) for the analysis.

***Predator attack rate and oviposition on* Tetranychus urticae**

The following predators were collected in Atibaia for the establishment of colonies to be evaluated: Blattisociidae - *Blattisocius dentriticus* (Berlese) and *Lasioseius* sp.; Melicharidae - *Proctolaelaps bickleyi* (Bram) and *Proctolaelaps pygmaeus* (Müller); Laelapidae - Gaeolaelaps *queenslandicus* (Womersley). They were collected from samples of coffee husk and pulp, about three months before the beginning of this experiment.

The colony of each species was maintained in a plastic unit (8 cm in diameter and 7 cm in height), whose base was covered by a solidified layer (0.5 cm in height) of a paste consisting of plaster and activated charcoal (9v:1v; Abbatiello 1965), which was maintained permanently moistened by daily addition of mineral water. Each unit was sealed with a piece of transparent plastic film (Magipac®) to prevent mites from escaping. Colonies were fed every other day with a mixture of different stages of the free-living nematode *Rhabditella axei* (Cobbold), offered on pieces of rotting bean pods used as substrate for nematode rearing, and maintained at 25°C ± 2°C, 70 ± 10% RH and permanently in the dark. The prey to be offered in the test was obtained from infested *Canavalia ensiformis* L. (Fabaceae) plants maintained in a screenhouse.

Each experimental unit consisted of plastic containers (2.5 cm in diameter and 1.0 cm in height), whose base was covered with a layer of caragenin, onto which a disk of strawberry leaflet was placed (top side down), and sealed with a piece of transparent plastic film (Magipac®) to prevent mites from escaping. In addition, cotton threads covered with a piece of coverslip were added to each unit, to serve as shelter and oviposition site for the predator.

Thirty spider mite eggs were introduced to each experimental unit together with a pregnant female that was up to two days old. Each unit constituted one of a total of 30 replicates per predator, all maintained at 25°C ± 2°C, 70 ± 10% RH and in the dark.

The units were examined daily under a stereomicroscope for 11 consecutive days, to determine the number of preys attacked and the number of eggs laid by each predator, as well as predator survivorship. At each evaluation, the eggs laid were discarded and all prey of each unit (attacked or not) were replaced by new ones. Eggs laid on the first day were excluded from the analysis to avoid the possible influence of previous feeding.

Statistical analysis

In all experiments, the predominant species of Gamasina were calculated as proposed by Pinzón and Spence (2010). The average numbers of mites per sample (soil, coffee husk and pulp, leaflets or whole plants from treatments 1, 2 and 3 were compared using the *glht* function of the multicomp package in the R program (R Development Core Team 2018). Mean numbers for mite predation, oviposition and survivorship were fitted to a Poisson distribution, using χ^2 tests (Anova, test = 'Chisq') to compare predatory mites. All studies were analyzed at 95% confidence intervals, using the statistical program R Development Core Team (2018).

6.3. Results

Preliminary mite evaluations in Bom Repouso

In the recently acquired samples of coffee husk and pulp, a total of 290 mites was collected (Table 1). The most abundant mite group was Gamasina, representing 60.6%, followed by Astigmatina and Prostigmata (24.8 and 14.4%, respectively). The predominant Gamasina were *B. dentriticus, P. bickleyi* and *P. pygmaeus*.

A total of 1569 mites was collected from the soil + litter samples from the forest fragment (Table 1). Oribatida was by far the most abundant group, corresponding to 81.8% of all mites collected, followed by Gamasina (Mesostigmata) and Prostigamata (Trombidiformes) (16.4 and 1.6 %, respectively). The most abundant gamasines belonged to Ologamasidae, with *Neogamasellevans* as the predominant genus.

Table 1. Total mites from 12 samples (400 cm^3 each) of coffee husk and pulp acquired in Ouro Fino, Minas Gerais and of soil + litter from a forest fragment located in Bom Repouso, Minas Gerais. All collected on July 17, 2018.

Taxa	Coffee husk and pulp	Forest Fragment
Sarcoptiformes (Oribatida Astigmatina: Acaridae**)**	**72**	**0**
Sarcoptiformes (Other Oribatida)	**0**	**1285**
Trombidiformes (Prostigmata)	**42**	**26**
Cheyletidae	42	0
Cunaxidae	0	12
Pseudocheylidae	0	14
Mesostigmata (Gamasina)	**176**	**258**
Blattisociidae		
Blattisocius dentriticus (Berlese)	115*	4
Lasioseius sp.	0	1
Platyseius sp.	0	1
Immature		5
Digamasellidae		
Digamasellus sp.	0	17
Immature	0	4
Laelapidae		
Stratiolaelaps sp.	0	12
Macrochelidae		
Glyptholaspis sp.	0	4
Holostapella sp.	0	9
Macrocheles sp.	0	12
Male	0	11
Immature	0	9
Melicharidae		
Proctolaelaps bickleyi (Bram)	21*	0
Proctolaelaps pygmaeus (Müller)	30*	0
Proctolaelaps sp. Immature	1	0
Proctolaelaps sp. Male	9	0
Ologamasidae		
Neogamasellevans sp.	0	49*
Male	0	32
Immature	0	19
Parasitidae		
Parasitus sp.	0	13
Male	0	0
Immature	0	41
Podocinidae		
Podocinela sp.	0	2
Podocinum sp.	0	13
Total	**290**	**1569**
Gamasina/ sample ± SE	**14.6 ± 6.1**	**21.5 ± 3.5**

(*) Predominant Gamasina species.

Preliminary mite evaluations in Atibaia

In the recently acquired samples of coffee husk and pulp, 312 mites were collected, a number similar to what was observed for the evaluation conducted in the material for Bom Repouso (Table 2). The most abundant mite group was Gamasina representing 58.6%, followed by Astigmatina and Prostigmata (29.1 and 12.1%, respectively). The predominant Gamasina mites were the same determined on samples of the material to be used in Bom Repouso, namely *B. dentriticus, P. bickleyi* and *P. pygmaeus*.

A total of 931 mites were collected in the soil + litter samples from the forest fragment (Table 2). Differently from Bom Repouso, Gamasina was the most abundant group, representing 36.9% of the mites collected, followed by Oribatida, Trombidiformes, Astigmatina and Uropodina (a cohort of Mesostigmata) (36.9, 29.4, 12.5, 12.3 and 8.7%, respectively). As in Bom Repouso, the most abundant Gamasina belonged to Ologamasidae, with *Caliphis* and *Neogamasellevans* as the predominant genera.

Table 2. Totals mites from 12 samples (400 cm^3 each) of coffee husk and pulp acquired in Ouro Fino, Minas Gerais and of soil + litter from a forest fragment located in Atibaia, São Paulo. All collected on February 13, 2020. continue

Taxa	Coffee husk and pulp	Forest Fragment
Sarcoptiformes (Oribatida Astigmatina: Acaridae**)**	**91**	**115**
Sarcoptiformes (other Oribatida)	**0**	**274**
Trombidiformes (Prostigmata)	**38**	**117**
Bdellidae	0	22
Cheyletidae	38	0
Eupodidae	0	33
Trombidiidae	0	25
Mesostigmata (Gamasina)	**183**	**344**
Ascidae		
Gamasellodes sp.	0	1
Protogamasellus sp.	0	1
Protogamasellus sp. Immature	0	1
Tropicoseius sp.	0	1
Blattisociidae		
Blattisocius dentriticus (Berlese)	123*	0
Cheiroseius sp.	0	2
Cheiroseius sp. Immature	0	2
Cheiroseius sp. Male	0	2
Lasioseius sp.	0	7
Male	0	2
Laelapidae		
Cosmolaelaps sp.	0	6
Pseudoparasitus sp.	0	1

Table 2. Totals mites from 12 samples (400 cm^3 each) of coffee husk and pulp acquired in Ouro Fino, Minas Gerais and of soil + litter from a forest fragment located in Atibaia, São Paulo. All collected on February 13, 2020. continuation

Taxa	**Coffee husk and pulp**	**Forest Fragment**
Mesostigmata (Gamasina)	**183**	**344**
Melicharidae		
Proctolaelaps bickleyi (Bram)	23*	1
Proctolaelaps diffissus Karg	0	1
Proctolaelaps pygmaeus (Müller)	37*	0
Proctolaelaps sp.	0	1
Immature	0	14
Ologamasidae		
Caliphis sp.	0	24*
Gamasiphis sp.	0	2
Geogamasus sp.	0	6
Neogamasellevans sp. 1	0	33
Neogamasellevans sp. 2	0	44*
New genre	0	5
Immature	0	17
Male	0	24
Parasitidae		
Amblygamasus sp.	0	1
Gamasodes sp.	0	1
Parasitus sp.	0	4
Immature	0	23
Phytoseiidae		
Immature	0	19
Podocinidae		
Podocinum sagax Berlese	0	1
Undefined Family		
Zygoseius sp.	0	7
Zygoseius sp. Immature	0	1
Zygoseius sp. Male	0	1
Mesostigmata (Uropodina: Uropodidae)	**0**	**81**
Total	**312**	**931**
Gamasina/ sample ± SE	**15.3 ± 7.4**	**28.7 ± 9.1**

(*) Predominant Gamasina species.

Mites at the surface of strawberry beds

In Bom Repouso, the total numbers of mites collected in treatments 1, 2 and 3 were 2,306, 2,024 and 169, respectively. Astigmatina were found in treatments 1 and 2, representing 17.3 and 32.1%, respectively, but not in treatment 3. Oribatida were found in treatments 1, 2 and 3, representing 4.1, 1.1 and 57.3%, respectively, while Prostigmata were found only in treatments 1 and 2, representing 8.8 and 11.6%, respectively (Table 3).

Gamasina were found in treatments 1, 2 and 3, representing 69.6, 55.0 and 42.6% of the total, but none belonged to Phytoseiidae (Table 3). The following Gamasina were classified as predominant (on at least one sampling date): treatment 1- *B. dentriticus*, *Lasioseius* sp., *Digamasellus* sp., *P. bickleyi* and *P. pygmaeus*; treatment 2 - *B. dentriticus, Lasioseius* sp. and *P. bickleyi*; treatment 3- *Lasioseius* sp. and *G. queenslandicus*.

In the three treatments, the highest number of Gamasina was determined on the second sampling date (January), although the difference between the numbers of the first and third samplings were not significantly different in treatments 1 and 3 ($p = 0.086$ and $p = 0.067$) (Table 3). Combining the Gamasina mites from the three sampling dates, the highest mean number was determined in treatment 1, followed by treatment 2 and then 3 (44.5 ± 19.1; 30.9 ± 13.5 and 2.0 ± 0.3 Gamasina mites/ sample) ($p<0.001$) (Table 3).

Table 3. Total number of mites collected in Bom Repouso, Minas Gerais at each sampling date (September 27, 2018, January 8 and April 10, 2019) from a sample (400 cm^3) of each of the 12 experimental units per treatment (1: coffee husk and pulp from the forest fragment; 2: dry coffee husk and pulp; and 3: polyethylene film). continue

	Treatment 1			**Treatment 2**			**Treatment 3**		
Taxa	**Sep**	**Jan**	**Apr**	**Sep**	**Jan**	**Apr**	**Sep**	**Jan**	**Apr**
Sarcoptiformes (Oribatida Astigmatina: Acaridae**)**	**132**	**216**	**53**	**98**	**342**	**211**	**0**	**0**	**0**
Sarcoptiformes (other Oribatida)	**67**	**12**	**16**	**0**	**17**	**7**	**23**	**41**	**33**
Trombidiformes (Prostigmata)	**71**	**90**	**44**	**75**	**110**	**50**	**0**	**0**	**0**
Cheyletidae	64	53	21	42	57	23	0	0	0
Cunaxidae	7	37	23	17	32	15	0	0	0
Pseudocheylidae	0	0	0	16	21	12	0	0	0
Mesostigmata (Gamasina)	**477**	**758**	**370**	**173**	**656**	**285**	**23**	**30**	**19**
Blattisociidae									
Blattisocius dentriticus (Berlese)	67*	87*	26*	51*	209*	59*	0	0	0
Lasioseius sp.	17	42*	15	21	47*	27	11*	9*	8*
Immature	25	12	7	11	3	1	3	9	0
Digamasellidae									
Digamasellus sp.	37*	45*	29	0	12	9	1	0	0
Immature	12	11	7	0	0	0	0	0	0
Laelapidae									
Stratiolaelaps sp.	22	29	17	0	0	0	0	0	0
Gaeolaelaps queenslandicus (Womersley)	0	18	32	0	0	12	7	9*	5
Macrochelidae									
Glyptholaspis sp.	13	6	8	0	0	0	0	0	0
Holostapella sp.	12	5	0	0	0	0	0	0	0
Macrocheles sp.	21	13	8	0	0	0	0	0	0
Male	3	0	0	0	0	0	0	0	0
Melicharidae									
Proctolaelaps bickleyi (Bram)	13	156*	36	59*	335*	132*	0	0	0
Proctolaelaps pygmaeus (Müller)	79*	172*	121*	0	0	0	0	0	0
Proctolaelaps sp. Immature	12	34	12	31	5	11	0	0	0
Proctolaelaps sp. Male	11	41	7	0	0	5	0	0	0

Table 3. Total number of mites collected in Bom Repouso, Minas Gerais at each sampling date (September 27, 2018, January 8 and April 10, 2019) from a sample (400 cm^3) of each of the 12 experimental units per treatment (1: coffee husk and pulp from the forest fragment; 2: dry coffee husk and pulp; and 3: polyethylene film). continuation

	Treatment 1			Treatment 2			Treatment 3		
Taxa	**Sep**	**Jan**	**Apr**	**Sep**	**Jan**	**Apr**	**Sep**	**Jan**	**Apr**
Mesostigmata (Gamasina)	**477**	**758**	**370**	**173**	**656**	**285**	**23**	**30**	**19**
Ologamasidae									
Neogamasellevans sp.	19	11	7	0	1	5	1	3	6
Male	9	1	4	0	5	9	0	0	0
Parasitidae									
Parasitus sp.	21	34	15	0	14	11	0	0	0
Male	12	7	8	0	0	0	0	0	0
Immature	68	31	1	0	25	4	0	0	0
Podocinidae									
Podocinela sp.	1	1	6	0	0	0	0	0	0
Podocinum sp.	3	2	4	0	0	0	0	0	0
Total	**747**	**1076**	**483**	**346**	**1125**	**553**	**46**	**71**	**52**
Gamasina/ sample ± SE**	**39.8 ± 14.6 b**	**63.2 ± 113.3 a**	**30.9 ± 11.2 b**	**14.4 ± 6.5 c**	**54.6 ± 22.8 a**	**23.7 ± 11.4 b**	**1.9 ± 0.2 b**	**2.5 ± 0.1 a**	**1.6 ± 0.7 b**

(*) Predominant Gamasina species. (**) Within each treatment, consecutive dates followed by the same letters do not differ by the *glht* function multicomp packet in R.

In Atibaia, the total numbers of mites collected in treatments 1, 2 and 3 were 1,766, 1,826 and 1,161, respectively. Of these totals, Astigmatina, Oribatida and Prostigmata were found in treatments 1, 2 and 3, representing 6.8, 36.1 and 9.5% (Astigmatina), 11.2, 3.4 and 21.8% (Oribatida), and 9.0, 18.8 and 17.0% (Prostigmata), respectively. In treatments 1 and 3, Uropodina represented 5.5 and 12.6%, respectively (Table 4).

Gamasina were the most abundant group in treatments 1, 2 and 3, representing 66.5, 41.7 and 39.5% of the total, but again, none was Phytoseiidae (Table 4). The following taxa were classified as predominant (on at least one sampling date): treatment 1- *B. dentriticus, Lasioseius* sp., *P. paulista, P. pygmaeus, Parasitus* sp. and *Pergamasus* sp.; treatment 2 - *B. dentriticus, Pseudoparasitus* sp., *P. paulista, Parasitus* sp. and *Zygoseius* sp.; treatment 3- *Protogamasellus* sp., *Cheiroseius* sp., *Cosmolaelaps* sp. and *Zygoseius* sp.

In treatment 1, the highest number of Gamasina was determined on the second sampling date (September), while the difference between the numbers of the second and third samplings were not significantly different in treatments 2 and 3 ($p = 0.14$ and p = 0, 21) (Table 4). Combining the Gamasina from the three sampling dates, the highest mean number was determined in treatment 1, followed by treatment 2 and then 3 ($p<0.001$) (32.9 ± 9.6; 21.1 ± 5.7 and 12.5 ± 5.7 Gamasina mites/ sample, respectively) (Table 4).

Table 4. Total number of mites collected in Atibaia, São Paulo at each sampling date (May 19, September 3 and December 1, 2020) from a sample (400 cm^3) of each of the 12 experimental units per treatment (1: coffee husk and pulp from the forest fragment; 2: dry coffee husk and pulp; and 3: polyethylene film). continue

Taxa	**Treatment 1**			**Treatment 2**			**Treatment 3**		
	May	**Sep**	**Dec**	**May**	**Sep**	**Dec**	**May**	**Sep**	**Dec**
Sarcoptiformes (Oribatida Astigmatina: Acaridae**)**	**72**	**37**	**12**	**231**	**256**	**173**	**18**	**71**	**21**
Tyrophagus sp.	72	37	12	231	256	173	18	71	21
Sarcoptiformes (other Oribatida)	**143**	**43**	**12**	**32**	**18**	**13**	**129**	**52**	**73**
Trombidiformes (Prostigmata)	**77**	**74**	**8**	**126**	**101**	**117**	**108**	**69**	**21**
Bdellidae	12	17	5	14	23	27	0	0	0
Cheyletidae	5	12	3	71	14	22	0	0	0
Cunaxidae	11	7	0	17	19	12	1	10	2
Ereynetidae	0	0	0	0	0	0	15	31	12
Eupodidae	0	0	0	0	0	0	32	28	7
Rhagidiidae	12	0	0	2	0	0	18	0	0
Trombidiidae	21	11	0	0	0	0	0	0	0
Tydeidae	16	27	0	22	45	56	42	0	0
Mesostigmata (Gamasina)	**285**	**579**	**321**	**177**	**304**	**278**	**143**	**168**	**142**
Ascidae									
Protogamasellus sp.	2	9	6	0	0	0	12	27*	13
Immature	0	6	0	1	0	0	1	12	5
Blattisociidae									
Blattisocius dentriticus (Berlese)	22*	7	0	53*	69*	34*	0	0	0
Blattisocius keegani Fox	0	8	0	3	14	26	0	0	0
Blattisocius tarsalis (Berlese)	0	7	0	1	19	32	0	0	0
Blattisocius sp. Male	0	3	0	1	21	27	0	0	0
Cheiroseius sp.	13	25	33	0	0	0	20*	0	0
Lasioseius sp.	33*	42*	54*	0	0	0	0	0	0
Immature	1	24	69	1	9	0	0	0	0
Laelapidae									
Cosmolaelaps sp.	12	16	19	0	0	0	13	21	24*
Gaeolaelaps sp.	0	0	0	0	0	0	2	11	8
*Holostaspis*sp.	0	0	0	0	0	0	1	5	0
Pseudoparasitus sp.	0	0	0	12	28*	32*	0	0	0
Immature	11	39	12	7	19	12	4	0	0
Melicharidae									
Proctolaelaps bickleyi (Bram)	9	19	12	14	3	11	12	0	0
Proctolaelaps diffissus Karg	22	17	32	0	0	0	0	0	0
Proctolaelaps paulista Mineiro et al.	21	38*	12	33*	41*	37*	11	8	17
Proctolaelaps pygmaeus (Müller)	28*	32	11	27	18	9	6	0	0
Proctolaelaps sp. Immature	9	2	0	12	1	0	14	1	0
Proctolaelaps sp. Male	2	4	0	11	6	5	6	2	0

Table 4. Total number of mites collected in Atibaia, São Paulo at each sampling date (May 19, September 3 and December 1, 2020) from a sample (400 cm^3) of each of the 12 experimental units per treatment (1: coffee husk and pulp from the forest fragment; 2: dry coffee husk and pulp; and 3: polyethylene film). continuation

Taxa	**Treatment 1**			**Treatment 2**			**Treatment 3**		
	May	**Sep**	**Dec**	**May**	**Sep**	**Dec**	**May**	**Sep**	**Dec**
Mesostigmata (Gamasina)	**285**	**579**	**321**	**177**	**304**	**278**	**143**	**168**	**142**
Macrochelidae									
Glyptolaspis sp.	8	14	0	0	0	0	0	0	0
Macrocheles sp.	10	15	0	0	0	0	0	0	0
Immature	1	1	0	0	0	0	0	0	0
Male	3	1	0	0	0	0	0	0	0
Ologamasidae									
Gamasiphis sp.	1	9	2	0	0	0	0	0	0
Neogamasellevans sp. 1	0	17	8	0	0	0	3	1	0
Neogamasellevans sp. 2	1	26	7	0	0	0	0	0	0
Immature	1	12	6	0	0	0	0	0	0
Male	3	2	0	0	0	0	1	1	0
Parasitidae									
Eugamasus sp.	0	0	0	0	0	0	1	0	0
Parasitus sp.	40*	56*	11	1	14	20*	0	0	0
Pergamasus sp.	2	37*	11	0	0	0	0	0	0
Immature	23	41	16	0	0	0	0	0	0
Phytoseiidae									
Neoseiulus sp.	2	0	0	0	0	0	3	0	0
Neoseiulus sp. Male	4	19	0	0	0	0	2	0	0
Proprioseiopsis sp.	0	0	0	0	0	0	1	1	0
Undefined Family									
Zygoseius sp.	0	8	0	0	26*	19*	25*	52*	41*
Zygoseius sp. Immature	0	6	0	0	7	0	2	7	9
Zygoseius sp. Male	1	17	0	0	9	14	3	19	25
Mesostigmata (Uropodina: Uropodidae)	**2**	**44**	**57**	**0**	**0**	**0**	**55**	**62**	**29**
Total	**579**	**777**	**410**	**566**	**679**	**581**	**453**	**422**	**286**
Gamasina/ sample ± SE**	**23.7± 7.2b**	**48.2± 12.4a**	**26.7± 9.4b**	**14.7± 5.5b**	**25.3± 7.6a**	**23.1± 4.2a**	**11.9± 5.8a**	**14.1± 6.2a**	**11.8± 5.1a**

(*) Predominant Gamasina species. (**) Within each treatment, consecutive dates followed by the same letters do not differ by the *glht* function multicomp packet in R.

Arthropods on plants at daytime

In Bom Repouso, the total numbers of essentially predatory mites collected on leaflets of treatments 1, 2 and 3 were 316, 251 and 58 respectively, of which 78.4, 80.4 and 100% were Gamasina (Table 5). The following Gamasina were classified as predominant (on at least one sampling date): treatment 1- *B. dentriticus, N. californicus* and *P. macropilis*; treatment 2 - *B. dentriticus, N. anonymus* and *P. macropilis*; treatment 3 - *N. anonymus* and *P. macropilis*.

Considering all evaluation dates, the highest number of *T. urticae* was found in treatment 3 (1.2 ± 0.2), followed by treatments 1 and 2 (0.6 ± 0.2 and 0.7 ± 0, 1 mite/leaflet, respectively) ($p < 0.01$). In treatment 3, the population of this mite increased from the second to the third month (November to December, 2018) (Fig. 1), followed by a reduction in the following month, which coincided with the increase in phytoseiid population and with the application of the acaricide Ortus®. Regarding phytophagous insects, the numbers were very low, but even so, it was possible to determine significant differences between treatments. The highest numbers of whiteflies and thrips occurred also in treatment 3 (0.3 ± 0.0 whitefly/ leaflet and 0.4 ± 0.1 thrips/flower), followed by treatments 1 and 2 (always 0.1 ± 0.0 / leaflet or 0.1 ± 0.0 / flower, respectively) ($p < 0.01$).

The low levels of relative humidity (0–45%) and dew point (-4–16°C) and higher amplitude of temperature (17–33°C) inside the tunnel of the treatment 3 were notorious (Fig. 2). This was quite different from what happened in the tunnels of treatments 1 and 2 (36–93%, -0.7–25°C and 17–22°C, respectively), for which these parameters were similar to the climatic data recorded at the vicinity of the strawberry field, where relative humidity ranged from 42 to 94% RH and temperature from 14 to 33°C.

Table 5. Total arthropods collected in six monthly evaluations (September 2018 to April 2019) from 18 leaflets of each of 12 experimental units (total 1512 leaflets), in a strawberry field in Bom Repouso, Minas Gerais (treatment 1: coffee husk and pulp from the forest fragment; treatment 2: dry coffee husk and pulp and treatment 3: polyethylene plastic).

Taxa	**Treatments**		
	1	**2**	**3**
Essentially predatory mites	**316**	**251**	**58**
Trombidiformes (Prostigmata)	**68**	**49**	**0**
Cunaxidae	12	27	0
Cheytletidae	56	22	0
Mesostigmata (Gamasina)	**248**	**202**	**58**
Blattisociidae			
Blattisocius dentriticus (Berlese)	29*	44*	0
Melicharidae			
Proctolaelaps bickleyi (Bram)	25	11	0
Proctolaelaps pygmaeus (Müller)	21	17	0
Parasitidae			
Parasitus sp.	1	2	0
Phytoseiidae			
Neoseiulus californicus ((McGregor)	43*	11	6
Neoseiulus anonymus (Chant & Baker)	17	22*	11*
Phytoseiulus macropilis (Banks)	28*	21*	12*
Male	18	17	5
Immature	66	57	24
Essentially phytophagous mites	**336**	**579**	**790**
Trombidiformes (Prostigmata: Tetranychidae**)**			
Tetranychus urticae Koch	336	579	790
Others mites	**22**	**27**	**0**
Sarcoptiformes (Oribatida Astigmatina: Acaridae**)**			
Tyrophagus sp.	22	27	0
Insects	**288**	**231**	**767**
Hemiptera: Aleyrodidae			
Trialeurodes vaporariorum (Westwood)	177	159	378
Thysanoptera: Thripidae			
Frankliniella occidentalis (Pergande)	111	72	389

(*) Predominant Gamasina species.

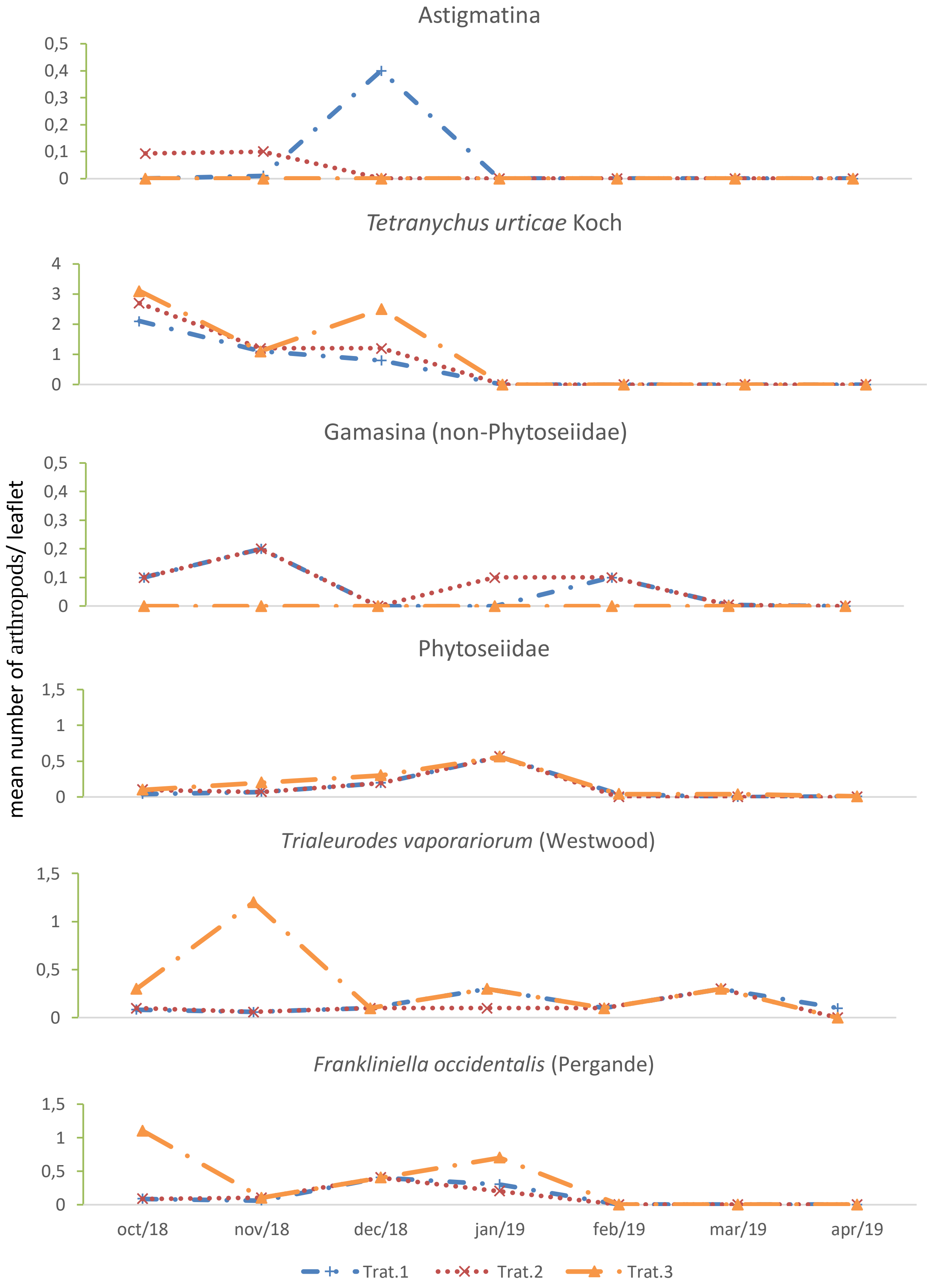

Figure 1. Number of Astigmatine mites, *Tetranychus urticae*, Gamasina (non-Phytoseiidae), Phytoseiidae, *Trialeurodes vaporariorum* and *Frankliniella occidentalis*/ strawberry leaflet (N=18 leaflets/repeat) in Treatments 1, 2 and 3, from October 2018 to April 2019, in Bom Repouso, MG (treatment 1: Coffee husk and pulp from the forest fragment; treatment 2: Dry coffee husk and pulp and treatment 3: Polyethylene plastic).

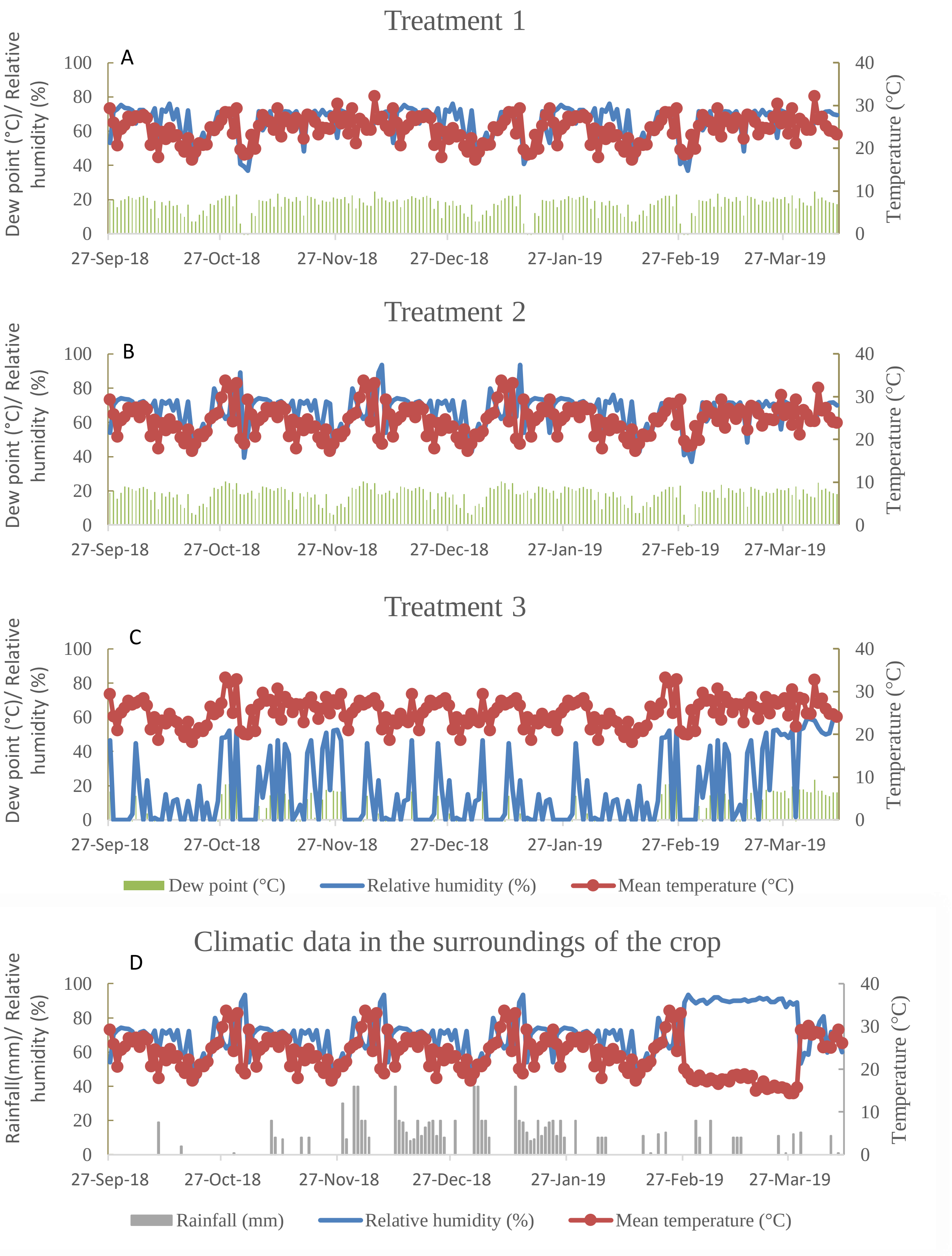

Figure 2. Dew point (°C), relative humidity (%) and mean temperature (°C) collected from 3 thermo-hygrometers installed 10 cm above the mulching surface of treatments 1, 2 and 3 (A, B, and C, respectively). Rainfall (mm), relative humidity (%) and mean temperature (°C) collected from a pluviometer e and thermo-hygrometer installed 10 m from the strawberry crop (D), from September 27, 2018 to April 10, 2019, in Bom Repouso, Minas Gerais. (treatment 1: coffee husk and pulp from the forest fragment; treatment 2: dry coffee husk and pulp and treatment 3: polyethylene film).

In Atibaia, the total numbers of essentially predatory mites collected on the leaflets of treatments 1, 2 and 3 were respectively 210, 218 and 67, of which 100, 88.5 and 100% were Gamasina (Table 6). The following Gamasina were classified as predominant (on at least one sampling date) in the three treatments: *N. californicus*, *N. anonymus* and *P. macropilis*.

Considering all evaluation dates, the highest number of *T. urticae* was found in treatment 3 (1.3 ± 0.3), followed by treatments 1 and 2 (0.7 ± 0.2 and 0.4 ± 0, 1 mite/leaflet, respectively) ($p < 0.03$). In treatment 3, the population remained a little higher between the second to the fifth month, followed by a reduction in the following months (Fig. 3). Regarding phytophagous insects, the numbers were even lower than in Bom Repouso, but even so, it was possible to determine significant differences between treatments. The highest numbers of aphids and thrips occurred in treatment 3 (0.08 ± 0.01 aphid/ leaflet and 0.03 ± 0.02 thrips/flower), followed by treatments 1 and 2 (0.02 ± 0.01 aphid/ leaflet and 0.02 ± 0.01 thrips/flower and 0.02 ± 0.01 aphid/leaf and 0.01 ± 0.00 thrips/flower, respectively) ($p < 0.04$).

In the vicinity of the plants of treatment 3, the levels of relative humidity (20–62%) and dew point (-8–17°C) were much lower, while the temperature range was much larger (4–40°C) than in treatments 1 and 2 (35–92% RH, 5–25°C and 9–29°C), and these data were even higher than the climatic data of the surroundings of the crop (28–67% and 4–25°C).

Table 6. Total arthropods collected in six monthly evaluations (June to December, 2020) from 18 leaflets of each of 12 experimental units (total 1296 leaflets), in a strawberry field in Atibaia, São Paulo (treatment 1: coffee husk and pulp from the forest fragment; treatment 2: dry coffee husk and pulp and treatment 3: polyethylene film).

Taxa	**Treatments**		
	1	**2**	**3**
Essentially predatory mites	**210**	**218**	**67**
Trombidiformes (Prostigmata)	**0**	**25**	**0**
Cunaxidae	0	16	0
Cheytletidae	0	9	0
Mesostigmata (Gamasina)	**210**	**193**	**67**
Blattisociidae	0	0	0
Cheiroseius sp.	3	2	0
Blattisocius dentriticus (Berlese)	1	12	0
Parasitidae			
Parasitus sp.	0	2	0
Phytoseiidae			
Amblydromalus limonicus (Garman & McGregor)	9	11	3
Neoseiulus californicus (McGregor)	39*	27*	12*
Neoseiulus anonymus (Chant & Baker)	63*	51*	18*
Neoseiulus idaeus Denmark & Muma	0	5	0
Phytoseiulus macropilis (Banks)	21*	12*	12*
Proprioseiopsis sp.	11	4	4
Male	18	17	5
Immature	45	50	13
Essentially phytophagous mites	**1161**	**635**	**2059**
Trombidiformes (Prostigmata: Tetranychidae**)**			
Tetranychus urticae Koch	1161	635	2059
Others mites	**2**	**47**	**1**
Sarcoptiformes (Oribatida, Astigmatina: Acaridae**)**			
Tyrophagus sp.	2	47	1
Insects	**41**	**4**	**81**
Hemiptera			
Aphididae			
Chaetosiphon fragaefolii (Cockerell)	10	0	21
Thysanoptera			
Thripidae			
Frankliniella occidentalis (Pergande)	31	4	60

(*) Predominant Gamasina species.

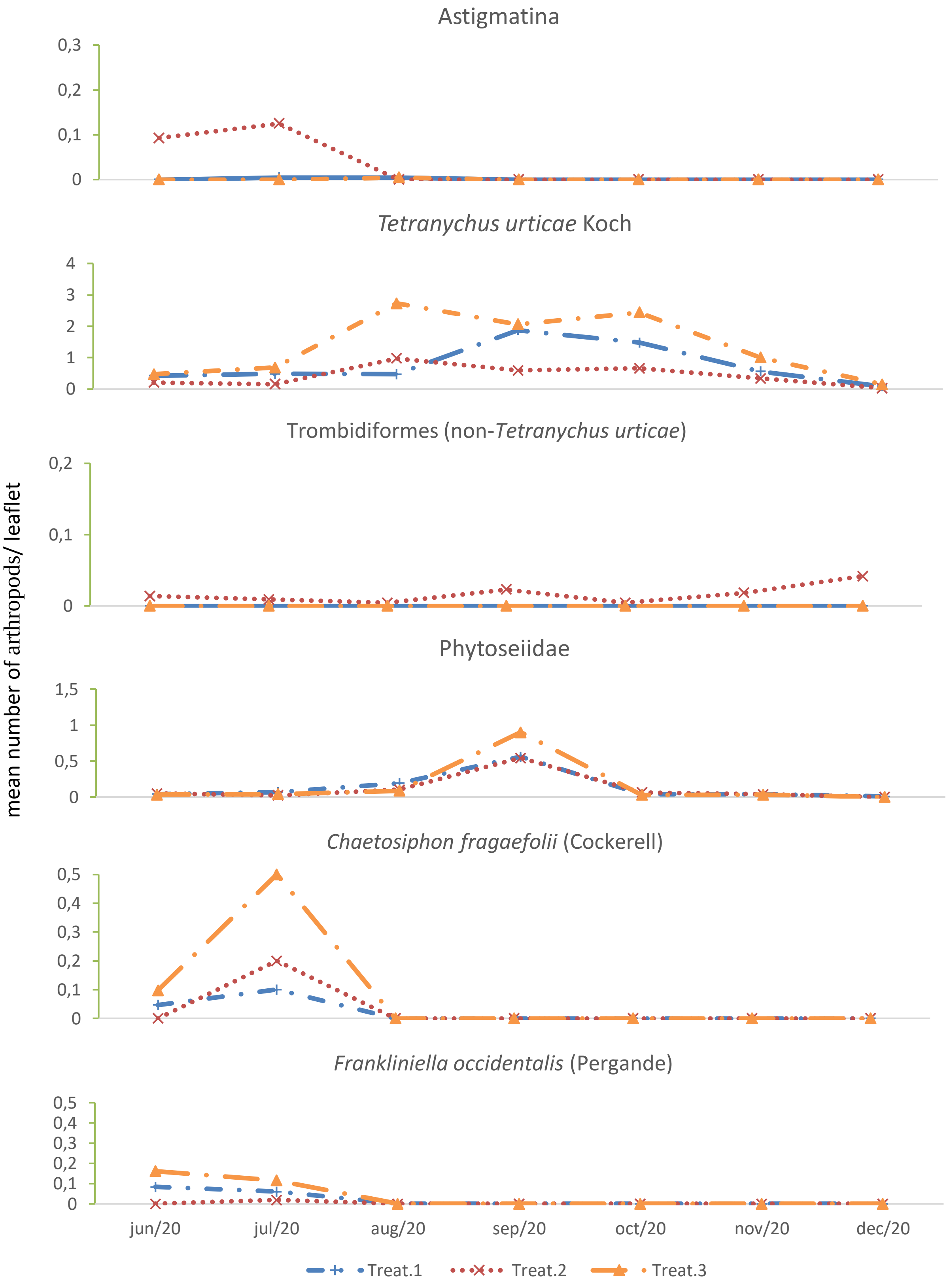

Figure 3. Number of Astigmatina mites, *Tetranychus urticae*, Trombidiformes (non-*Tetranychus urticae*), Phytoseiidae, *Chaetosiphon fragaefolii* and *Frankliniella occidentalis*)/ strawberry leaflet (N=18 leaflets/repeat) in Treatments 1, 2 e 3, from June to December 2020, in Atibaia, São Paulo (Treatment 1: Coffee husk and pulp from the forest fragment; Treatment 2: Dry coffee husk and pulp and Treatment 3: Polyethylene plastic).

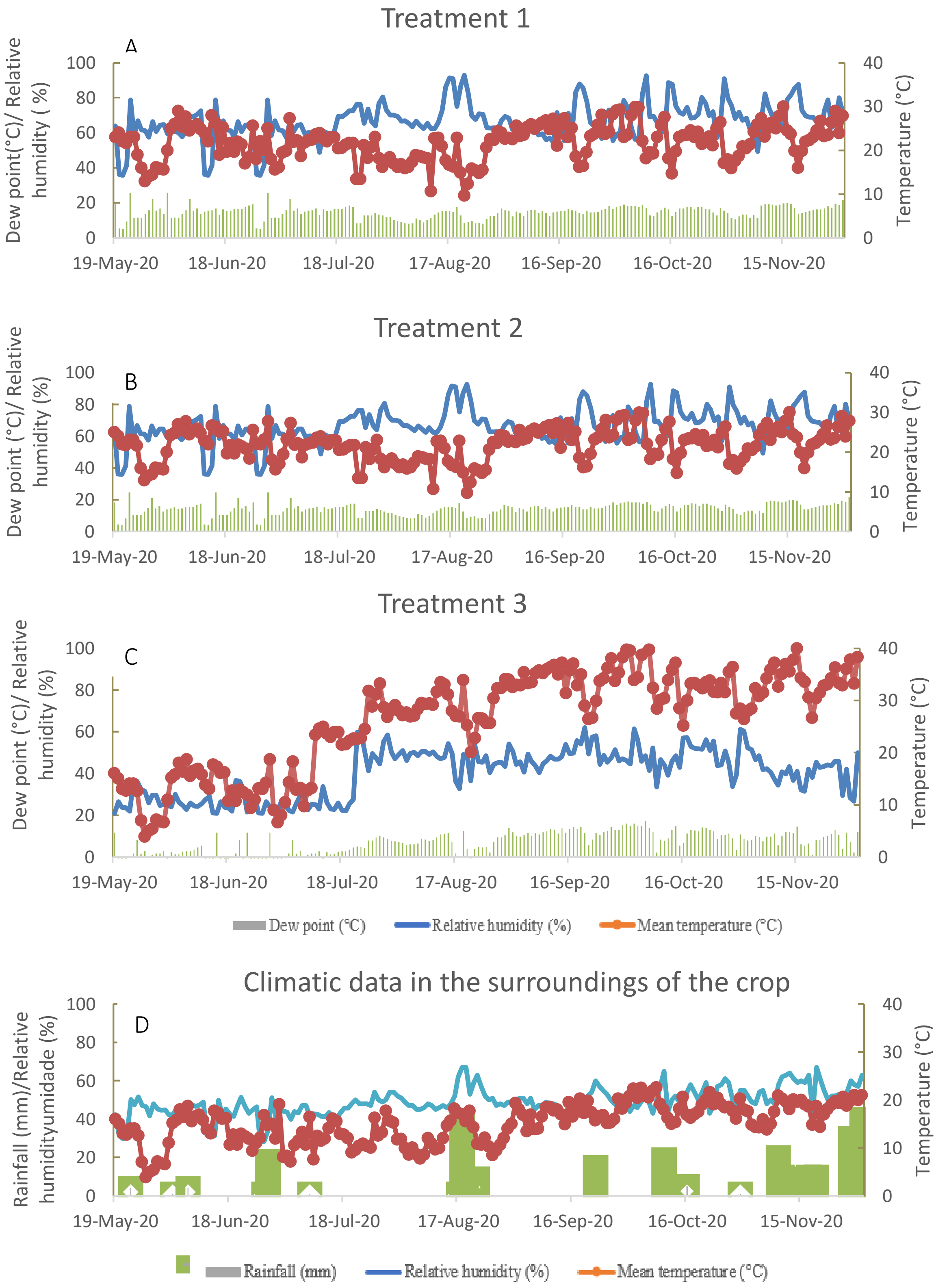

Figura 4. Dew point (°C), relative humidity (%) and mean temperature (°C) collected from 3 thermo-hygrometers installed 10 cm above the mulching surface of treatments 1, 2 and 3 (A, B, and C, respectively). Rainfall (mm), relative humidity (%) and mean temperature (°C) collected from a pluviometer e and thermo-hygrometer installed 10 m from the strawberry crop (D), from May 19 to December 1, 2020, at Atibaia, São Paulo. (treatment 1: coffee husk and pulp from the forest fragment; treatment 2: dry coffee husk and pulp and treatment 3: polyethylene film).

Edaphic mites on whole strawberry plants

By pooling the data of both sampling times, totals of 371, 267 and 359 mites were collected from the plants of treatments 1, 2 and 3, respectively (Table 7). Despite the similarity in those numbers, the highest number of *T. urticae* was found in treatment 3 (4.4 ± 2.7 two-spotted spider mite/ plant; p < 0.01), with no significant difference between treatments 1 and 2 (Table 7) (2.9 ± 1.6 and 2.2 ± 0.8 two-spotted spider mite/ plant, respectively; p = 0.6), as also observed when the mites were evaluated only at daytime (previous item). The number of phytoseiids was higher in treatment 1 (1.9 ± 1.1 phytoseiids/ plant) (p=0.05), while the difference between treatments 2 and 3 was not significant (1.0 ± 0.3 and 1.2 ± 0.5 phytoseiids/plant, respectively; p=0.43). Other Gamasina were significantly more numerous in treatment 2 (1.4 ± 0.1 other Gamasina/ plant; p=0.05), while the difference between treatments 1 and 3 was not significant (1.2 ± 0.1 and 0.9 ± 0.4 other Gamasina/plant, respectively; p= 2.4).

Of all mites collected, the total numbers of Gamasina in treatments 1, 2 and 3 were respectively 85, 84 and 86 for samples collected at nighttime and 63, 39 and 21 for samples collected at daytime. The predominant Gamasina were: treatment 1- *Lasioseius* sp. and *P. bickleyi* (nighttime), *N. californicus* and *Proprioseiopsis* sp. (at both times); treatment 2 - *Lasioseius* sp. (nighttime), *N. anonymus* (at both times) and *Proprioseiopsis* sp. (nighttime); treatment 3 - *Lasioseius* sp. (nighttime), *N. anonymus* (diurnal), *N. californicus* and *Proprioseiopsis* sp. (nighttime).

Comparing the number of mites between sampling times, no significant difference was observed for *T. urticae* and for the phytoseiids in any of the three treatments (p = 0.75 and p = 1.2, respectively) (Table 7). For other Gamasina, significant differences were observed for all treatments, with the numbers at nighttime being always higher (p<0.001).

In summary, the most important results of this part of the experiment were that there was: more *T. urticae* in treatment 3; more Gamasina in the plants at night, and that the number of Gamasina was the same in the three treatments, including more *Lasioseius* in the treatment 3 than in others.

Table 7. Totals of mites in four whole strawberry plants from beds covered with Treatment 1, 2 and 3, in day (10 h) and night (22 h) collection, on October 27, 2020, Atibaia, São Paulo (treatment 1: coffee husk and pulp from the forest fragment; treatment 2: dry coffee husk and pulp and treatment 3: polyethylene film). continue

Taxa	**Treatment 1**		**Treatment 2**		**Treatment 3**	
	10:00 AM	**10:00 PM**	**10:00 AM**	**10:00 PM**	**10:00 AM**	**10:00 PM**
Essentially predatory mites	**69**	**100**	**52**	**101**	**25**	**97**
Trombidiformes (Prostigmata)	**6**	**15**	**19**	**17**	**4**	**11**
Bdellidae	1	1	2	3	0	0
Cheyletidae	2	2	8	6	0	1
Cunaxidae	3	12	9	8	4	10
Mesostigmata (Gamasina)	**63**	**85**	**35**	**84**	**21**	**86**
Ascidae						
Protogamasellus sp.	0	2	0	0	0	0
Immature	0	0	0	1	0	1
Blattisociidae						
Cheiroseius sp.	0	0	3	6	0	1
Lasioseius sp.	4	11*	3	22*	1	31*
Immature	0	2	0	1	0	3
Laelapidae						
Cosmolaelaps sp.	3	1	3	5	0	1
Gaeolaelaps queenslandicus (Womersley)	0	0	1	0	0	0
Stratiolaelaps sp.	1	0	0	1	0	0
Pseudoparasitus sp.	1	0	0	0	1	0
Immature	0	8	0	4	0	3
Melicharidae						
Proctolaelaps bickleyi (Bram)	5	7*	0	3	0	1
Proctolaelaps pygmaeus (Müller)	0	0	0	1	0	1
Macrochelidae						
Macrocheles sp.	0	0	2	1	0	1
Ologamasidae						
Caliphis sp.	0	3	0	0	0	2
Neogamasellevans sp.	0	0	0	1	0	0
Parasitidae						
Immature	0	9	1	6	0	0
Phytoseiidae						
Amblydromalus sp.	5	1	1	2	1	1
Neoseiulus anonymus (Chant & Baker)	6	2	5*	5*	4*	3
Neoseiulus californicus ((McGregor)	14*	12	4	4	2	10*
Neoseiulus idaeus Denmark & Muma	0	0	0	0	0	1
Propioseiopsis sp.	13*	21*	3	10*	3	18*
Immature	11	5	6	1	7	6
Male	0	1	3	4	2	2
Undefined Family						
Zygoseius sp.	0	0	0	5	0	0
Zygoseius male	0	0	0	1	0	0

Table 7. Totals of mites in four whole strawberry plants from beds covered with Treatment 1, 2 and 3, in day (10 h) and night (22 h) collection, on October 27, 2020, Atibaia, São Paulo (treatment 1: coffee husk and pulp from the forest fragment; treatment 2: dry coffee husk and pulp and treatment 3: polyethylene film). continuation

Taxa	**Treatment 1**		**Treatment 2**		**Treatment 3**	
	10:00 AM	**10:00 PM**	**10:00 AM**	**10:00 PM**	**10:00 AM**	**10:00 PM**
Essentially phytophagous mites	**70**	**72**	**57**	**50**	**122**	**103**
Trombidiformes (Prostigmata: Tetranychidae**)**						
Tetranychus urticae (Koch)	67	72	56	49	117	99
Paraplonobia (Anaplonobia) sp.	3	0	1	1	5	4
Others mites	**2**	**4**	**2**	**5**	**0**	**12**
Sarcoptiformes (Oribatida Astigmatina: Acaridae**)**	1	2	1	4	0	7
Tyrophagus sp.	1	2	1	4	0	7
Trombidiformes (Prostigmata)						
Eupodidae	0	0	0	1	0	3
Tydeidae	1	2	1	0	0	2
Total	**141**	**176**	**111**	**156**	**147**	**212**
Tetranychus urticae* /plant ± SE)*	**2.8 ± 1.2 a**	**3.0 ± 2.1 a**	**2.3 ± 1.1 a**	**2.1 ± 0.7 a**	**4.8 ± 2.6 a**	**4.1 ± 2.9 a**
Phytoseiidae/plant ± SE)**	**2.1 ± 1.4 a**	**1.7 ± 0.9 a**	**0.9 ± 0.3 a**	**1.1 ± 0.4 a**	**0.8 ± 0.4 a**	**1.7 ± 0.7 a**
Other Gamasina/plant ± SE)**	**0.6 ± 0.3 a**	**1.8 ± 0.5 b**	**0.5 ± 0.2 a**	**2.4 ± 0.7 b**	**0.1 ± 0.0 a**	**1.8 ± 0.9 b**

(*) Predominant Gamasina species. (**) Within each treatment, consecutive dates followed by the same letters do not differ by the *glht* function multicomp packet in R.

Strawberry quality

In Bom Repouso (Fig. 5A), the average weight (g) of 18 strawberries was higher in treatments 1 and 2, which did not differ from each other (p= 0.03). In Atibaia (Fig. 5B), the mean weight of 18 strawberries was significantly higher in treatment 1 than in treatment 3 (p=0.04), with the mean weight in treatment 2 in an intermediate position between other two treatments, although the differences between treatments 1 and 2 (p=1.2) and 2 and 3 (p=0.75) were not significant.

The °Brix values of strawberries from Atibaia were also higher in treatments 1 and 2 (which did not differ from each other: p=2.1), than in treatment 3 (p=0.04) (respectively 9.3±1.5, 9.5±1.3 and 7.9±0.8).

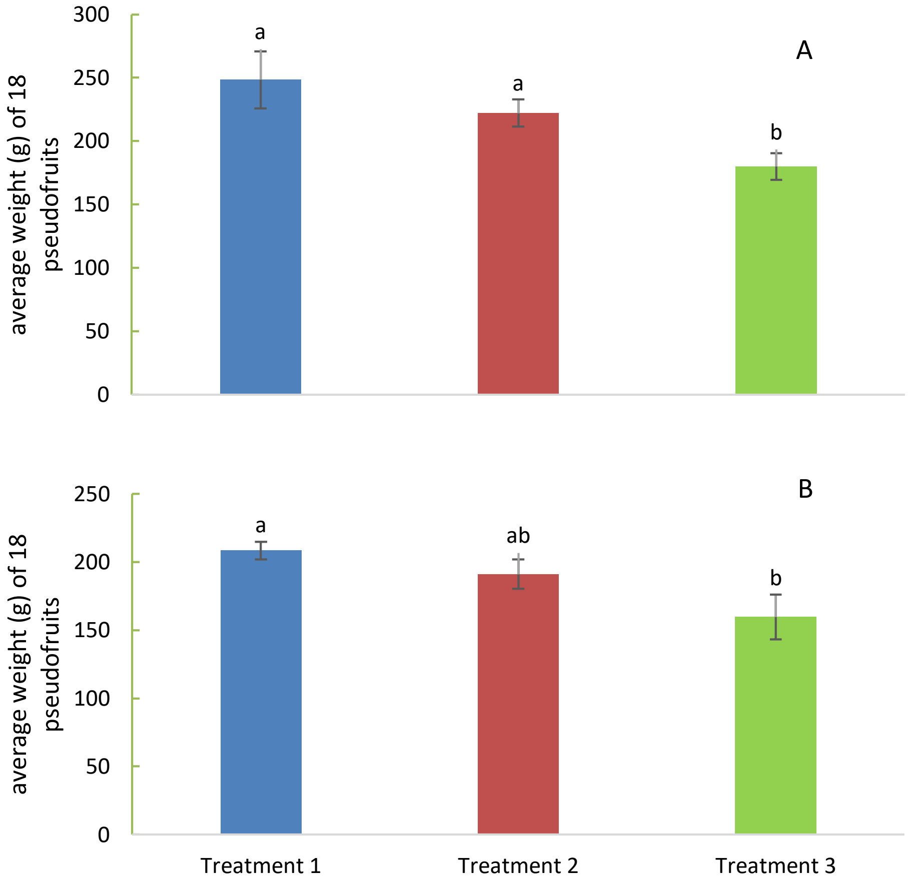

Figure 5. Average weight (g) of 18 strawberry pseudofruits/plot in the three treatments (treatment 1: coffee husk and pulp from the forest fragment; treatment 2: dry coffee husk and pulp and treatment 3: polyethylene film) in Bom Repouso, Minas Gerais (Figure 5A) and in Atibaia, São Paulo (Figure 5B). *Bars topped by different letters are significantly different. Means were compared using the *glht* function multicomp packet in R.

Leaf content of macro and micronutrients

Determined contents of K, Zn, Mn and B were higher in treatments 1 and 2 than in treatment 3 (Table 8). The content of K was higher in treatment 1, followed by treatment 2 and 3, which did not differ between themselves (p=0.0007). The contents of the Zn, Mn and B were higher in the treatment 1 and 2, which did not differ between themselves, than in treatment 3 (p=0.0003, 0.0008 and 0.0004, respectively).

Table 8. Average contents of macro and micronutrients in strawberry leaflets (n= 6 samples/treatment) cultivated under treatments 1–3, in Atibaia, São Paulo (treatment 1: coffee husk and pulp from the forest fragment; treatment 2: dry coffee husk and pulp and treatment 3: polyethylene film).

Treatments	N	P	K	Ca	Mg	S	Cu	Fe	Zn	Mn	B
	---------------- g/kg					-----------------------	---------------- mg/kg				--------------------
1	26.7 ± 0.1 a	3.4 ± 1.3 a	32.3± 0.3 a	10.8 ± 1.2 a	4.5 ± 2.1 a	1.4 ± 1.2 a	4.7 ± 3.0 a	292.0 ± 7.9 a	24.6 ± 0.1 a	22.9 ± 0.4 a	40.0 ± 2.9 a
2	24.8 ± 0.2 a	3.0 ± 1.0 a	28.9 ± 1.1 b	10.6 ± 2.2 a	4.1 ± 1.9 a	1.4 ± 0.6 a	4.0 ± 2.5 a	299.0 ± 9.0 a	24.3 ± 0.1 a	22.8 ± 0.7 a	37.0 ± 3.3 a
3	23.7 ± 0.3 a	2.9 ± 0.9 a	19.6 ± 0.6 c	8.9 ± 2.9 a	4.4 ± 2.1 a	1.3 ± 0.9 a	4.9 ± 1.8 a	330.0 ± 12.2 a	19.6 ± 0.3 b	14.0 ± 0.9 b	32.0 ± 2.2 b

Within each column, consecutive dates followed by the same letters do not differ by the *glht* function multicomp packet in R.

Predator attack rate and oviposition on Tetranychus urticae

For all predator species, daily predation rate was relatively low, the maximum reaching 2.0 eggs for *Lasioseius* sp. (Table 9). For other predators, rates were significantly lower (Df = 4; $\chi^2 = 87.5$; p <0.001), at most 1.0 egg per day. Daily oviposition rates were also low, although significantly higher for *Lasioseius* sp., *P. bickleyi* and *P. pygmaeus* (0.5 to 0.8 egg) (Df = 4; $\chi^2 = 95.1$; p <0.001) than for other predators. Survival rates were generally high (at least 9.8 days during the maximum period considered in the experiment, 10 days), not differing statistically between the evaluated mites (Df = 4; $\chi^2 = 28.2$; p = 0.69).

Table 9. Daily predation (average number of eggs fed/ female/ day ± SE), oviposition (average number of eggs laid/ female/ day ± SE) and mean survivorship (in days) of different predators for a maximum of 10 days when *Tetranychus urticae* eggs were offered as prey at 25 ± 2 °C, 70 ± 10% RH, in the dark.

	Predation	Oviposition	Survivorship
Blattisociidae			
Blattisocius dentriticus (Berlese)	0.1 ± 0.0 c	0.1 ± 0.01 b	10.0 ± 0.0 a
Lasioseius sp.	2.0 ± 0.16 a	0.8 ± 0.10 a	10.0 ± 0.0 a
Melicharidae			
Proctolaelaps bickleyi (Bram)	0.9 ± 0.06 b	0.5 ± 0.08 a	9.25 ± 0.37 a
Proctolaelaps pygmaeus (Müller)	1.0 ± 0.06 b	0.7 ± 0.07 a	9.85 ± 0.47 a
Laelapidae			
Gaeolaelaps queenslandicus (Womersley)	0.0 ± 0.0 c	0.1 ± 0.02 b	10.0 ± 0.0 a

In the same column, treatments followed by the same letters do not differ by the $\chi 2$ test (p <0.05).

6.4. Discussion

Preliminary mite evaluations

The similar mite composition in the recently acquired samples of coffee husk and pulp destined for use in Bom Repouso and Atibaia was expected, given that the material was purchased from the same source. In both cases, Blattisociidae and Melicharidae were predominant, represented by *B. dentriticus*, *Lasioseius* sp., *P. bickleyi* and *P. pygmaeus*. Moraes et al. (2015) presented a review of the feeding habits of mites of these families, highlighting that they develop and reproduce on fungi, other mites, small insects and nematodes. In the present study, it seems that those mites could be feeding mostly on acarid mites.

In both Bom Repouso and Atibaia forest fragments, Ologamasidae was the predominant family. Castilho et al. (2015) summarized the predatory habits of mites of this family, concluding that they feed on other mites, small insects, nematodes and Collembola. Also in both sites, mites of several other families not found in the recently acquired coffee husk and pulp were present in the samples of soil + litter (including predatory groups as Laelapidae and Parasitidae), indicating that they could move to the husk and pulp in the period that this material was exposed on the fragment floor.

The much higher total number of mites collected in the evaluation of the soil + litter samples of the fragment in Bom Repouso was due to the high density of non-Astimatina Oribatida in that fragment, about five times as that in Atibaia. In contrast, the density of Gamasina, group of greater interest in this study for the predominantly predatory behavior of its constitutive species, was actually higher in Atibaia.

The results of this part of the study showed the absence of potentially harmful mite species to strawberry plants in the samples of coffee husk and pulp and of soil + litter. On the contrary, beneficial species with the potential to contribute to pest control in the crop were found.

Mites at the surface of strawberry beds

The detection of some mite groups only in treatments 1 and 2, and not in treatment 3, suggests that they were introduced to the strawberry beds for being present in the acquired coffee husk and pulp material (as Cheyletidae, Tydeidae, Cunaxidae, some *Blattisocius* and some *Proctolaelaps* spp.) or because that material was effectively "enriched" with mites (as *Stratiolaelaps* sp., Macrochelidae, Ologamasidae and Parasitidae) when exposed onto the floor of the forest fragment. As determined in the preliminary evaluation of mites, the diversity in treatment 2, especially in the first of the three samplings was expected to be about the same in both, as the samples came from the same source (recently acquired from Ouro Fino), although at different dates. And this is about what happened, with totals of 2,014 in nine families in Bom Repouso and 1,897 in 11 families in Atibaia, in both cases, without considering the Oribatida species. However, while the number of mites obtained from treatment 1 was higher in Bom

Repouso (2,306 in ten families) than in Atibaia (1,695 in 15 families), the number of mites obtained from treatment 3 was much lower in Bom Repouso (169 in four families) than in Atibaia (1,161 in 14 families.

These results suggest that in Atibaia the environmental conditions were more adequate for the mites to survive in the soil of the strawberry field than in Bom Repouso. This could be related to the difference in the employed cultivation system (low tunnel in Bom Repouso and open field in Atibaia) or to natural ecological conditions. During this experiment, determined rainfall, temperature and relative humidity levels were: Bom Repouso (1442 m above sea level) 1091 mm, 20°C and 75%, and Atibaia (766 m above sea level) 263 mm, 25°C and 78%.

Blattisociidae and Melicharidae were predominant in treatments 1 and 2, in both experiments, being represented by *B. dentriticus* and *Lasioseius* sp. (Blattisociidae) and *P. pygmaeus* (Melicharidae), as also determined in the study of Esteca et al. (2018), carried out in a field about 10 km from the Bom Repouso and 130 km from the Atibaia fields in which the present study was conducted. Moraes et al. (2015) presented a review of the feeding habits of mites of these families, highlighting that they develop and reproduce on fungi, other mites, small insects and nematodes.

The results of this part of the study allowed to conclude that the maintenance of coffee husk and pulp on the forest fragment floor before using as mulching promoted the incorporation of local edaphic predatory mites (relatively more numerous in the forest fragment) in the strawberry field.

Arthropods on plants at daytime

In the three treatments, *T. urticae* population levels were always much lower than what is reported in the literature as the economic damage level for several cultivars (Bernardi et al. 2015). The low incidence of that species coincided with the results obtained by Esteca et al. (2018) and by Castilho et al. (2015). However, the levels might have been affected the control measures adopted by producers.

Regardless of that, it was possible to determine the higher *T. urticae* densities in treatment 3, which could be related to the presence of predators, as these were more numerous on plants of treatments 1 and 2 than on plants of treatment 3. And these predators included species that were also present on the organic mulching with coffee husk and pulp (treatments 1 and 2), but not in strawberry bed soil (treatment 3), namely Cunaxidae, Cheyletidae, *B. dentriticus*, *Proctolaelaps* spp., or they were present at seemingly lower levels on plants of treatment 3, as some phytoseiid species. But the higher *T. urticae* densities in treatment 3 could also be related to the different environmental conditions in this treatment, compared to the others, due to the use of the polyethylene film. Kivijärvi et al. (2002) also reported higher *T. urticae* levels in beds covered with polyethylene than in beds covered with dry grass, barley straw, wheat straw or pine bark, but the underlying cause was not discussed.

In the present study, the polyethylene film reducing air relative humidity (Duso et al. 2004; Castilho et al. 2015) and interfered with temperature. Temperature measured 10 cm above the mulching surface was considerably more stable in treatments 1 and 2 than in treatment 3. In the coldest period of the day (11:00 p.m. to 6:00 a.m.), the average was higher (12 to 15°C) than in treatment 3 (4 to 10°C), while at the hottest times of the day (11:00 p.m. to 2:00 p.m.), the average was lower (20 to 22°C) in treatments 1 and 2 than in treatment 3 (30 to 40°C). The dew point might also have played a role. In treatment 3, the dew point was always lower than in treatments 1 and 2, being even negative in some days. The dew point data is related to air humidity; the lower the relative humidity, the lower the dew point and the higher the suitability to spider mite performance. In both experiments, even soon after a period of rainy days, humidity was higher in treatments 1 and 2 than in treatment 3. The more stable temperature and humidity might have favored the performance of the predators (Ramachandran et al. 2021) and/ or disfavored the performance of *T. urticae*.

The reduced relative humidity levels in beds covered with polyethylene film coincided with the results of Cadavid et al. (1998) and Costa et al. (2007). Esteca et al. (2018) had speculated about this possible effect.

Variation in temperature, relative humidity and dew point in treatment 3 was higher in Bom Repouso than in Atibaia, suggesting that microclimate under low tunnel is less stable than in the open field, and this could have different effects on different organisms affecting the strawberry plants. However, a comparison of the levels of the organisms in those two places should take into account that several other factors were also different.

The results of this part of the study suggested that mulching with coffee husk and pulp maintained a microclimate close to the strawberry plants favorable to the development of predatory mites and harmful to the development of the two-spotted spider mites.

Edaphic mites on whole strawberry plants

The predominance of the edaphic Gamasina *Lasioseius* sp. (all treatments) and *P. bickleyi* (treatment 1) on leaflets at nighttime evaluations in this work was similar to the results obtained by Esteca et al. (2018, 2020). Those authors concluded that mites of these same genera are found during the day mainly in the soil, climbing to plants at night. Other studies have also reported the difference between the mite fauna on the leaves of certain plants in daytime and nighttime observations (Novotny et al. 1999; Saigusa et al. 2000; Onzo et al. 2003; Parecis-Silva et al. 2016).

It is conceived that some mites climb the strawberry plants at night in search of food, favored by lower diversity and abundance of predatory mites in the aerial part of the plants than in the soil, thus avoiding competition and possibly reducing intra-guild predation. Associated with this, the increased environmental relative humidity at night, probably allows the movement of edaphic predators to the aerial plant parts, given their high sensitivity to low humidity levels. Our previous experience has shown that representatives of edaphic Gamasina (including *Lasioseius floridensis* Berlese (Blattisociidae), *P. bickleyi* will perish in a few hours when subjected to relative humidity levels below 70%.

Within the phytophagous organisms, some (such as ants and Orthoptera) found on *Ficus wassa* Roxb. (Maraceae) forage the plants at night in order to escape their main predators, the ants *Tapinoma melanocephalum* Fabricius (Formicidae), which have the diurnal predation habit (Novotny et al. 1999).

The results of this part of the study supported previous findings that suggested edaphic predatory mites, especially *Lasioseius* sp. and *P. blickleyi*, may move to strawberry plants at nighttime. Hence, their preservation in the soil might be helpful in pest control.

Strawberry quality

The observed higher average weight and concentration of soluble solids (°Brix) in treatments 1 and 2 proved one of the initial hypotheses of this work. This finding may be related to the fact that during the hottest period of the day (11:00 a.m. to 2:00 p.m.), temperature close to coffee husk and pulp mulching remained mild (20-22°C) in comparison with what was observed when mulching consisted of the use of polyethylene film (30 and 40°C), which may have favored the visit of pollinators and/ or the physiology of the plants. In a study conducted in Spain, Medina (2003) concluded that the optimum temperature for pollinators to visit strawberry flowers is between 20 and 25ºC, which coincided in the present work with the predominant range in the plots of treatments 1 and 2.

Increased bee visits may have led to higher average strawberry weight (Castle et al. 2019). Size is one of the most relevant characteristics considered in the valuation of strawberries for fresh market (Calvete et al. 2003; Godoy, 1998; MacInnis and Forrest 2019).

Regarding the total soluble solids content, the use of coffee husk and pulp as mulching was found to improve the quality of strawberries, enabling values (at the extreme) of 11° Brix, while in polyethylene mulching, the maximum was 8.2° Brix. Similar results were obtained by Oliveira Neto et al. (2011) who determined significant increase in the soluble solids content in sugar beet when using Cameroon grass as ground cover. This could be related to the higher levels of some elements determined in leaf analysis, especially potassium, in plants of treatments 1 and 2. Among the various functions of potassium in plant physiology, its role in photosynthetic activity and in the biosynthesis of sugars, organic acids, vitamin C and total soluble solids stands out (Lopes 1995; Taiz and Zeiger 2004).

The results of this part of the study allowed us to conclude that in addition to being advantageous in reducing pest problems, the use of coffee husk and pulp as might result in the production of heavier and sweeter strawberries.

***Predator attack rate and oviposition on* Tetranychus urticae**

The hypothesis concerning the predation ability of the predators on *T. urticae* was partially proven. At least three of the evaluated predator species (*Lasioseius* sp., *P. bickleyi* and *P. pygmaeus*) were able to attack and oviposit when offered *T. urticae* eggs as prey. Despite the low predation and oviposition rates, the results suggested that when climbing strawberry plants at night, these edaphic predators may complement the role of other predators, especially phytoseiids, present on strawberry leaflets at day and nighttime, to reduce *T. urticae* population levels. The high survivorship of all evaluated predator on this prey does not necessarily indicate that all evaluated predator species fed on it, given that certain Gamasina species are known to survive relatively long periods in absence of food, provided water is available. This seems to have been the case with at least two of the predators evaluated in this study, *B. dentriticus* and *G. queenslandicus*, which seem to have attacked very few or no prey.

Hence, *Lasioseius* sp., *P. bickleyi* and *P. pygmaeus* may have been partially responsible for the lower *T. urticae* population on plants of treatments 1 and 2, coinciding with the higher number of edaphic predators on plants of these treatments, mainly at night.

Momen et al. (2011) reported that *Lasioseius lindquisti* Nasr & Abou-Awad neither fed nor developed on *T. urticae* nymphs, while Abou-Awad et al. (2001) concluded that *Lasioseius athiasae* Nawar & Nasr was able to complete its life cycle and sustain oviposition when fed with *T. urticae* nymphs. Mathys & Tencalla (1959) reported that although *P. pygmaeus* was not considered a good spider mite predator, it managed to feed and reproduce on *T. urticae*, as seemingly happened in the present work. *Proctolaelaps bickleyi* and *Proctolaelaps bulbosus* Reis et al. had been reported to consume *T. urticae*, which however was considered unsuitable for their development to adult and for oviposition (Lawson-Balagbo et al. 2008; Galvão et al. 2011).

Blattisocius dentriticus has never been reported to consume spider mites, despite feeding on Astigmatina and fungi (Mohamed 2013). Likewise, *Gaeolaelaps* are known to be aggressive predators of various arthropods and nematodes, but not of phytophagous mites (Moreira and Moraes 2015).

In addition to *T. urticae*, other small arthropods present on the strawberry plants, especially whiteflies and thrips, may have been attacked by the edaphic predatory mite climbing the plants, both found in numbers two to five times lower on plants of treatments 1 and 2 than on plants of treatment 3 in Bom Repouso, and several times lower on plants of treatments 1 and 2 in Atibaia. This possibility should be investigated.

Future studies should include the evaluation of cost/benefit ratio of using coffee husk and pulp compared to polyethylene film as strawberry mulching, in addition to evaluating the effect on crop yield.

REFERENCES

Abbatiello, M.J. (1965) A culture chamber for rearing soil mites. Turtox News, 43: 162-163.

Abou-Awad BA, Korayem AM, Hassan MF, Abou-Elela MA (2001) Life history of the predatory mite *Lasioseius athiasae* (Acari: Ascidae) on various kinds of food substances: a polypeptide analysis of prey consideration. Journal of Applied Entomology, 125: 125-130.

Antunes. L.E.C.; Bonow. S.; Reisser Júnior. C. (2020) Morango: Crescimento constante em área e produção. Campo e Negócios, 1, 88-92.

Bernardi, D.; Botton, M.; Nava, D.E.; Zawadneak, M.A.C. (2015) Guia para a identificação e monitoramento de pragas e seus inimigos naturais em morangueiro. Embrapa Uva e Vinho-Livro científico, Brasília. (ALICE).

Cadavid, L.F.; El-Sharkawy, M.A.; Acosta, A.; Sánchez, T. (1998) Long-term effects of mulch, fertilization and tillage on cassava grown in sandy soils in northern Colombia. Field Crops Research, 57(1): 45-56.

Calvete, E.O.; Rocha, H.C; Cechetti, D.; Maran, R.E; Carli, W.D.E. (2003) Polinização entomófila de morangueiro cultivado em ambiente protegido. Horticultura Brasileira, 21: 281 (suplemento I).

Carrillo, D.; Moraes, G.J.; Peña, J.E. (2015) Prospects for biological control of plant feeding mites and other harmful organisms. Springer International Publishing: Cham, Switzerland, p. 337.

Castilho, R.C.; Duarte, V.S.; Moraes, G.J.; Westrum, K.; Trandem, N.; Rocha, L.C.D.; Delalibera, I. Jr.; Klingen, I. (2015) Two-spotted spider mite and its natural enemies on strawberry grown as protected and unprotected crops in Norway and Brazil. Experimental and Applied Acarology, 66(4): 509-528.

Castle, D.; Grass, I.; Westphal, C. (2019) Fruit quantity and quality of strawberries benefit from enhanced pollinator abundance at hedgerows in agricultural landscapes. Agriculture, Ecosystems & Environment, 275: 14-22.

Costa, D.M.A.; Melo, H.N.D.S.; Ferreira, S.R. (2007) Eficiência da cobertura morta na retenção de umidade no solo. Holos, Rio Grande do Norte, 1, 59-69.

Cruz. P.C. (1999) A situação da cultura do morangueiro no estado de São Paulo. In: Duarte Filho. J.; Cançado. G.M.A.; Regina. M.A.; Antunes. L.E.C.; Fadini. M.A.M. Eds. Morango: tecnologia de produção e processamento. Caldas: EPAMIG. p. 129-130.

Dara. S.K. (2016) Managing strawberry pests with chemical pesticides and non-chemical alternatives. International Journal of Fruit Science, 16(sup1): 129-141.

Duso, C.; Chiarini, F.; Conte, L.; Bonora, V.; Dalla Montà, L.; Otto, S. (2004) Fogging can control *Tetranychus urticae* on greenhouse cucumbers. Journal of Pest Science, 77: 105-111.

Esteca, F.C.N.; Rodrigues, L.R.; Moraes, G.J.; Delalibera Jr., I.; Klingen, I. (2018) Mulching with coffee husk and pulp in strawberry affects edaphic predatory mite and spider mite densities. Experimental and Applied Acarology, 76(2): 161-183.

Esteca, F.C.N.; Trandem, N., Klingen, I., Cruz, J. S.; Delalibera Jr., I.; Moraes, G.J. (2020) Cereal straw mulching in strawberry—A facilitator of plant visits by edaphic predatory mites at night? Diversity, 12(6): 242.

Filgueira, F.A.R. (2000) Novo Manual de Olericultura: Agrotecnologia moderna na produção e comercialização de hortaliças. Editora UFV, Viçosa, p 402.

Galvão, A.S.; Gondim, M.G.; Moraes, G. J. (2011) Life history of *Proctolaelaps bulbosus* feeding on the coconut mite *Aceria guerreronis* and other possible food types occurring on coconut fruits. Experimental and Applied Acarology, 53(3): 245-252.

Godoy, W.I. (1998) Polinização entomófila em duas cultivares de morangueiro (*Fragaria* x *ananassa* Duch.) sob diferentes coberturas do solo. Porto Alegre: (Dissertação de Mestrado em Fitotecnia) – Programa de Pós-Graduação em Agronomia, Universidade Federal do Rio Grande do Sul.162p (Dissertação de Mestrado em Fitotecnia).

Hoddle, M.S.; Morse, J.G.; Phillips, P.A.; Faber, B.A.; Jetter, K.M. (2002) Avocado thrips: A new challenge for growers. California Agriculture, 56: 103-107.

Jamieson, L.E.; Stevens, P.S. (2006) The effect of mulching on adult emergence of Kelly's citrus thrips (*Pezothrips kellyanus*). New Zealand Native Plant Protection, 59: 42-46.

Jensen, L.; Simko, B.; Shock, C.; Saunders, L. (2002) Alternative methods for controlling onion thrips (*Thrips tabaci*) in Spanish onions. Malheur Experiment Station Annual Report 2001; Oregon State University Agricultural Experiment Station: Ontario, OR, Canada, 1038: 104-111.

Johnson, J.M.; Hough-Goldstein, J.A.; Vangessel, M.J. (2004) Effects of straw mulch on pest insects, predators, and weeds in watermelons and potatoes. Environmental Entomology, 33: 1632-1643.

Kivijärvi, P.; Parikka, P.; Tuovinen, T. (2002) The effect of different mulches on yield, fruit quality and strawberry mite in organically grown strawberry. Organic production of fruit and berries. The Danish Institute of Agricultural Sciences, Department of Horticulture, Årslev, Denmark. Accessed on July 16, 2021.

Krantz, G.W.; Walter, D.E. (2009) A Manual of Acarology, 3rd ed.; Texas Tech University Press: Lubbock, TX, USA, p. 411.

Lasmini, S. A.; Nasir, B.; Hayati, N.; Edy, N. (2018) Improvement of soil quality using bokashi composting and NPK fertilizer to increase shallot yield on dry land. Australian Journal of Crop Science, 12(11): 1743-1749.

Lawson-Balagbo, L.M.; Gondim, M.G.C.; Moraes, G.J.; Hanna, R.; Schausberger, P. (2008) Exploration of the acarine fauna on coconut palm in Brazil with emphasis on *Aceria guerreronis* (Acari: Eriophyidae) and its natural enemies. Bulletin of Entomological Research, 98(1): 83-96.

Lopes, A.S. (1995) Manual internacional de fertilidade do solo. Associação brasileira para pesquisa da potassa e do fósforo. Piracicaba: ESALQ, 177p.

MacInnis, G.; Forrest, J. R. (2019) Pollination by wild bees yields larger strawberries than pollination by honey bees. Journal of Applied Ecology, 56(4): 824-832.

Mathys, G.; Tencalla, Y. (1959) Note préliminaire sur la biologie et la valeur prédatice de *Proctolaelaps hypudaei* Oudms (Acarien: Mesostigmata: Aceosejidae). Stations Fédérales D'Essais Agricoles, 600: 645-654.

Medina, J. (2003) Análisis y evolución agronômica de lãs deformaciones de fruto em fresa. Posibles soluciones. Jornadas Agrícolas y Comerciales de El Monte, 20: 101-115.

Mohamed, A. E. (2013). Biological aspects and life table parameters of predator gamasid ascid mite, *Blattisocius dentriticus* (Berlese) (Acari: Gamasida: Ascidae). Egyptian Academic Journal of Biological Sciences, 6(2): 97-105.

Momen, F.M.; Abou-Elela, A.M.; Metwally, A.M.; Naser, A.K.; Saleh, K.H.M. (2011) Biology and feeding habits of the predacious mite, *Lasioseius lindquisti* (Acari: Ascidae) from Egypt. Acta Phytopathologica Entomologica Hungarica, 46: 151-163.

Montagnana, J.M.; Costa, A.L.; Pereira, J.M.; Tonelli, M.; Leite, L.G. (2017) Eficiência de Steinernema Brazilense no controle de *Colaspis* sp. em morangueiro. In: 9ª Jornada Científica e Tecnológica e 6º Simpósio da Pós-graduação do IFSULDEMINAS, Machado.

Moraes, G.J.; Venancio, R.; Santos, V.L.V.; Paschoal, A.D. (2015) Potential of Ascidae, Blattisociidae and Melicharidae (Acari: Mesostigmata) as biological control agents of pest organisms. In: *Prospects for biological control of plant feeding mites and other harmful organisms*; Carillo, D.; Moraes, G.J.; Peña, J.E. (Eds.). Springer International Publishing: Cham, Switzerland, chap. 2, pp. 33–76.

Moreira, G.F.; Moraes, G.J. (2015) The potential of free-living laelapid mites (Mesostigmata: Laelapidae) as biological control agents. In: *Prospects for biological control of plant feeding mites and other harmful organisms*; Carillo, D.; Moraes, G.J.; Peña, J.E. (Eds.). Springer International Publishing: Cham, Switzerland, chap. 3, pp. 77-102.

Mori, H.; Chant, D.A. (1966) The influence of humidity on the activity of Phytoseiulus persimilis Athias-Henriot and its prey, *Tetranychus urticae* (Koch) (Acarina: Phytoseiidae, Tetranychidae). Canadian Journal of Zoology, 44(5): 863-871.

Morra, L.; Bilotto, M.; Cerrato, D.; Coppola, R.; Leone, V.; Mignoli, E.; Pasquariello, M.S.; Petriccione, M.; Cozzolino, E. (2016) The Mater-Bi® biodegradable film for strawberry (*Fragaria* x *ananassa* Duch.) mulching: effects on fruit yield and quality. Italian Journal of Agronomy, 11: 731.

Novotny, V.; Basset, Y.; Auga, J.; Boen, W.; Dal, C.; Drozd, P.; Kasbal, M.; Isua, B.; Kutil, R.; Manumbor, M. (1999) Predation risk for herbivorous insects on tropical vegetation: A search for enemy-free space and time. Australian Journal of Ecology, 24: 477-483.

Oliveira Neto, D.H.; Carvalho, D.F.; Silva, L.D.; Guerra, J.G.M.; Ceddia, M.B. (2011) Evapotranspiração e coeficientes de cultivo da beterraba orgânica sob cobertura morta de leguminosa e gramínea. Horticultura Brasileira, 29: 330-334.

Oliveira, A.D.; Moraes, G.D.; Demétrio, C.D.; Nardo, E.A.B. (2001) Efeito do vírus de poliedrose nuclear de *Anticarsia gemmatalis* sobre Oribatida edáficos (Arachnida: Acari) em um campo de soja. Boletim de Pesquisa, 13: 31-37.

Onzo, A.; Hanna, R.; Zannou, I.; Sabelis, M.W.; Yaninek, J.S. (2003) Dynamics of refuge use: Diurnal, vertical migration by predatory and herbivorous mites within cassava plants. Oikos, 101: 59-69.

Parecis-Silva, P.V.; Nuvoloni, F.M.; Feres, R.J. (2016) Day vs. night: The importance of the circadian cycle over metacommunities and predator–prey densities. International Journal of Acarology, 42: 141-148.

Pinzón, J.; Spence, J.R. (2010) Bark-dwelling spider assemblages (Araneae) in the boreal forest: dominance, diversity, composition and life-histories. Journal of Insect Conservation, 14(5): 439-458.

R Core Team, (2018). R: A Language and Environment for Statistical Computing R Foundation for Statistical Computing, Vienna, Austria (2018) Available online: https://www.R-project.org/ (accessed on May 12, 2019).

Ramachandran, D.; Lindo, Z.; Meehan, M. L. (2021) Feeding rate and efficiency in an apex soil predator exposed to short-term temperature changes. Basic and Applied Ecology, 50: 87-96.

Sánchez-Moreno, S.; Ferris, H. (2007) Suppressive service of the soil food web: Effects of environmental management. Agriculture, Ecosystems & Environment, 119: 75-87.

Solomon, M.G.; Jay, C.N.; Innocenzi, P.J.; Fitzgerald, J.D.; Crook, D.; Crook, A.M.; Easterbrook, M.A.; Cross, J.V. (2001) Review: natural enemies and biocontrol of pests of strawberry in Northern and Central Europe. Biocontrol Science and Technology, 11: 165-216.

Strassburger. A.S.; Martins. D.D.S.; Reisser Junior. C.; Schwengber. J.E.; Peil. R.M.N.; Philipsen. L.C. (2009) Sistema de produção do morangueiro: fatores que influenciam o manejo da irrigação. In: Timm. L.C.; Tavares. V.E.Q.; Reisser Junior. C.; Estrela. C.C. (Ed.). Morangueiro irrigado: aspectos técnicos e ambientais do cultivo. Pelotas: Universidade Federal de Pelotas. p. 30-50.

Taiz, L.; Zeiger, E. (2004) Fisiologia Vegetal. Porto Alegre: Artmed. 719 p.

Raij, B.; Canatrella, H.; Quaggio, J. A.; Furlano, A. M. C. (1996) Recomendações de adubação e calagem para o estado de São Paulo. Campinas: Instituto Agronômico - FUNDAG, 285 p. (IAC. Boletim técnico, 100).

7. CEREAL STRAW MULCHING IN STRAWBERRY - A FACILITATOR OF PLANT VISITS BY EDAPHIC PREDATORY MITES AT NIGHT?

Fernanda de Cássia Neves Esteca[1]*, Nina Trandem[2,3], Ingeborg Klingen[3], Jandir Cruz Santos[4], Italo Delalibera Júnior[1] and Gilberto José de Moraes[1]

[1] Department of Entomology and Acarology, “Luiz de Queiroz” College of Agriculture (ESALQ), University of São Paulo (USP), Av. Pádua Dias, 11, Piracicaba SP 13418-900, Brazil; delalibera@usp.br (I.D.J.); moraesg@usp.br (G.J.d.M.)

[2] Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences (NMBU), P.O. Box 5003, 1432 Ås, Norway; nina.trandem@nibio.no

[3] Biotechnology and Plant Health Division, Norwegian Institute of Bioeconomy Research (NIBIO), P.O. Box 115, 1431 Ås, Norway; ingeborg.klingen@nibio.no

[4] Centre for Biodiversity Genomics, 50 Stone Road East, University of Guelph, Guelph, ON N1G2W1, Canada; jandir_jc@hotmail.com

***** Correspondence: fernanda.esteca@usp.br

Manuscript submitted to the Journal Diversity on 3 May 2020, accepted: 27 May 2020 and published: 13 June 2020. (Diversity 2020, 12, 242; doi:10.3390/d12060242)

Abstract

In Norway, strawberry producers use cereal straw mulching to prevent berries from contacting the soil and to control weeds. We hypothesized that organic matter such as straw mulch also favors the maintenance of predatory mites which visit strawberry plants at nighttime. We compared mite diversity in cereal straw exposed for different periods in strawberry fields and evaluated their possible migration to plants in two experiments with potted plants in 2019. An ‘Early season’ experiment compared no mulching (T1), oat straw mulch exposed in field since 2018 (T2), or 2017 (T3), while a ‘Mid-season’ experiment compared no mulching (T1), barley straw mulch from 2018 (T2), or a mix from 2017 and 2018 (T3). To provide edaphic predatory mites with a potential source of food, all plants were infested with two-spotted spider mite (*Tetranychus urticae* Koch). Results suggested that straw mulch facilitates the prevalence of predatory mites in strawberry fields. Most predatory mite visits were at night, confirming our initial hypothesis. Predominant nocturnal mites on leaves belonged to Melicharidae (*Proctolaelaps* sp.) ('Early season', T2), Blattisociidae (*Lasioseius* sp.) ('Early and Mid-season', T3) and Phytoseiidae ('Mid-season', T2). *Parasitus consanguineus* Oudemans & Voigts was the predominant species ('Early season', T3) at the base of plants. Anystidae were diurnal visitors only ('Mid-season', T2). Future studies should evaluate the predation potential of *Proctolaelaps* sp. and *Lasioseius* sp. on two-spotted spider mite and other strawberry pests.

Keywords: 1. Oat straw mulch 2. Barley straw mulch 3. Biological control 4. Two-spotted spider mite 5. Edaphic mites

7.1. Introduction

The use of mulching (a plastic film or an organic material) is an important technique in strawberry (*Fragaria* x *ananassa* Duch.) cultivation, to regulate soil temperature, suppress weeds, prevent erosion, and prevent berries from contact with the soil [1].

In Norwegian strawberry production, cereal straw is commonly used as a mulching material. In roughly half of the strawberry production area, cereal straw is the only mulching material, whilst in another 25%, straw is combined with a black plastic film beneath the straw [2]. Strawberry is an important fruit crop in Norway, accounting for 70% of the yield of soft fruit production in the country [3]. In 2018, about 8000 tons of strawberries were produced by Norwegian farmers, with revenue of about 432 million NOK [4]. Most of the strawberry production is done in open fields, where the plants are harvested for 2-3 years. Among the most common pests in strawberry cultivation in Norway are two phytophagous mites (two-spotted spider mite, *Tetranychus urticae* Koch [5], and the strawberry tarsonemid mite, *Phytonemus pallidus* (Banks), as well as two weevils, *Anthonomus rubi* Herbst and *Otiorhynchus sulcatus* F., and a capsid bug, *Lygus rugulipennis* L. [6-8].

According to Kader et al. [9], there are many advantages in using organic material instead of plastic as mulching, as for example the fact that it can improve soil structure throughout the degradation process, in addition to a more efficient balancing of soil temperature. Another advantage is the maintenance of higher densities of certain pest predators, such as beetles of the families Carabidae and Staphylinidae, and spiders of the families Linyphiidae and Lycosidae, providing them with a more suitable environment [10]. Further, Larentzaki et al. [11] reported reduction in thrips populations in onion crops whose soil was covered with oat straw.

Beneficial mites can also benefit from organic mulching. An increase in the abundance and diversity of Gamasina mites and/or decreasing pest populations in different crops have been reported by several authors [12-15]. Gamasina is a cohort of the order Mesostigmata, which comprises a large group of mites found in different habitats, but especially in the soil. Although many of these can feed on fungi, they are well known as predators of small invertebrates, including mites, nematodes and insects. For this reason, the members of this group have been studied as predators of edaphic pests [16].

Mites, as well as Collembola and some other Hexapoda are part of the soil mesofauna (length 200 μm - 2 mm), many of which fragmentize the organic matter available on the soil surface [17]. The macrofauna (> 2 mm), in particular the earthworms, also participate in the process of organic matter fragmentation [18]. The soil microbiota, which includes the microfauna and other small organisms (<100 μm), including bacteria, fungi and nematodes, is also a key element in the cycling of organic matter [19]. Thus, it is expected that throughout the process of decomposition of organic matter, such as straw mulch, the characteristics of the soil will vary, influencing the fauna of edaphic predatory mites.

Hence, soil with better coverage, naturally represented by dead plant structures on its surface or resulting from the purposeful introduction by growers, has a higher density of predatory mites, as extensively reported in the literature [20-23]. Consequently, in agricultural crops, the use of organic cover should benefit pest control, by acting as a reservoir of edaphic predatory mites.

An aspect that has been little mentioned in the literature on predatory mites is the movement of mites from protected to exposed habitats, like plants, at night, [24-26]. In Brazil, Esteca et al. [20] found that *Proctolaelaps pygmaeus* (Muller) (Melicharidae) and *Blattisocius dentriticus* (Berlese) (Blattisociidae) were present on strawberry leaflets mainly at night, indicating their possible daily migration from the edaphic environment to the plants. The reason for this behavior has not been properly studied. It could be casual, or linked to the direct effect of light or other abiotic factors (as temperature and humidity), or to biotic factors. In an unpublished work, we found that representatives of edaphic Gamasina (*Lasioseius floridensis*, *Cosmolaelaps* sp., *Proctolaelaps bickleyi*, *Protogamasellopsis posnaniensis* and *Stratiolaelaps scimitus*) will perish in a few hours when subjected to relative humidity levels below 70% [27]. Due to the increase in relative humidity at night, this period is probably the most suitable for predatory Gamasina mites to temporarily migrate onto plants in search of prey. The possible migration of the Gamasina predator *Lasioseius floridensis* Berlese (Blattisociidae) from the soil to plants and vice versa was conceived by Britto et al. [28]. But it is expected that the movement of edaphic Gamasina to plants will vary from place to place, according to the faunistic composition and the intrinsic preference of the local mite fauna.

The hypotheses of the work we will present were: a) The diversity and abundance of predatory mites (especially Gamasina) in organic matter increases with the time the matter has been exposed on the soil surface in the strawberry field. b) Mites present in the organic mulching at daytime climb to strawberry plants at nighttime. These hypotheses were recently proposed by Castilho et al. [5] for strawberry cultivations.

The objective of this study was therefore to compare the diversity and prevalence of mites in cereal straw subjected to different periods of exposure in the strawberry field, evaluating their possible temporary movement from the edaphic habitat to strawberry plant leaves at nighttime.

7.2. Materials and Methods

Two consecutive experiments, one ‘Early season’ and one ‘Mid-season’, were carried out during May-July 2019, in a climatic room at the Department of Biotechnology and Plant Health of NIBIO (Norwegian Institute of Bioeconomy Research, Ås, Norway). The room was maintained at 18 ± 2 °C, 70 ± 5% relative humidity and a daily photoperiod of 15 h, turning the lights on at 6:00 AM (500 µmol [quanta] $m^{-2}.s^{-1}$) and off at 9:00 PM. These parameters simulated the average light conditions of the region during the study period.

Experimental set-up

In both experiments, potted strawberry plants received one of three treatments (T): In T1 no mulch was added, in T2 cereal straw mulch present in a strawberry crop since last autumn (standard practice) was added, and in T3 cereal straw one year older than in T2 was added. Each treatment had 13 replicates per experiment, and each experiment lasted 5 weeks.

Preparing experimental pots and plants

Pots (5 L) were filled to 80% of the capacity with a commercial organic substrate (Kompostert Plantejord®; composition: 50% peat, 50% garden waste, macro- and micronutrients). One strawberry plant of cv 'Korona', approximately 10 cm high and with ca five trifoliate leaves, free of pests or diseases, was transplanted into each pot. Pots were placed 15 cm apart on four shelves in the climatic room. To avoid movement of mites from one pot to another, the edge of each pot was covered with entomological glue (Tangle trap, Biocontrol®).

To provide edaphic predatory mites with a potential source of food on the plants, two adult females of the two-spotted spider mite were transferred to each of six random leaflets per plant two days after transplanting. Leaflets with *T. urticae* were marked with a pen (3 mm in diameter), so that they could be examined for mite presence along the experiment. One week after the transplant, plants were fertilized with 8 g of Plantagen® fertilizer (2 N: 3P: 1K) per pot. The plants were irrigated manually to field capacity (about 10 mL per pot) once every two days.

Sampling straw mulch and soil in the field

For both experiments, cereal straw mulch of two ages, but otherwise as similar as possible, was collected on a strawberry farm near NIBIO (details given in Table 1). A frame of 55 x 25 cm was used to standardize sampling, each subsample taken about 4 m apart, always next to bed margin, maximum 15 cm from a strawberry plant. Subsamples of ca 2L were collected and pooled into 40L plastic bags and taken to the laboratory. The straw originated from cereal production at the same farm as the strawberry field, where no insecticides were used.

Soil samples from the same fields were also collected by the use of a standard probe (6 cm deep, 10 cm in diameter), transferring each sample to a plastic bag that was brought to the laboratory.

Table 1. Details about the different types of straw used in the study and the fields they were collected. All fields were at the same grower (County of Viken) in SE Norway. RH= Relative humidity.

Experiment Treatment(T)*	Date collected	Temperature, RH, Precipitation**	Location	Type of straw	Length of exposure in the field
'Early season' T2	May 6, 2019	3.3–4.0°C; 77–82% RH; 6 mm	59°39'38"N; 10°40'37"E, Altitude 90 masl, Loam soil	Oat	Since autumn 2018
'Early season' T3	May 6, 2019	3.3–4.0°C; 77–82% RH; 6 mm	59°39'30"N, 10°41'13"E, Altitude 100 masl, Silty loam soil	Oat	Since autumn 2017
'Mid-season' T2	June 23, 2019	11.4–13.0°C; 75-83% RH; 29 mm	59°39'51"N; 10°41'4"E, Altitude 100 masl, Silty loam soil	Barley	Since autumn 2018
'Mid-season' T3	June 23, 2019	11.4–13.0°C; 75-83% RH; 29 mm	59°39'51"N; 10°41'4"E, Altitude 100 masl, Silty loam soil	Barley	Mixture of autumn 2017 (lower layer) and 2018 (upper layer)

*Treatment 1 was without straw in both experiments. **During last 7 days

At the time of the ‘Early season’ sampling, the strawberry plants in the two fields sampled were approximately 30 cm high and had 15 leaves each, BBCH 55. They had been covered with Agryl fleece a few weeks before, to avoid frost damage to flowers, a common practice in the region. The fleece was removed to sample straw and soil. The cultivation practices of both fields were approximately the same, belonging to the same grower, and no pesticides had been used that year.

At the ‘Mid-season’ sampling, plants in the sampled field were approximately 30 cm high and had 30 leaves each; with no flowers open BBCH 57. The straw and soil collection process in the field was the same as in the ‘Early season’.

Berlese extraction of mites

To get data on prevalence of predatory mite in the different straw treatments at the time of collection, straw samples were processed in laboratory by placing a part of the samples (not the same to be used as mulching in the pots in the experiment of the climatic room) in Berlese funnels at the beginning of each experiment. For each straw treatment, 13 Berlese funnels were employed. The corresponding soil sampled in the strawberry fields was also subjected to Berlese funnels (13 samples) to get data on the predatory mite prevalence. The sample volume in each Berlese funnel was 1L. Mites dropping from each funnel were caught in a container with 70% ethanol. The extraction process lasted 7 days.

The potting commercial organic substrate used in experimental pots was investigated in the same way to reveal whether the potting commercial organic substrate contained mites that would affect the study.

At the end of each experiment, similar Berlese extractions were performed with the material from experimental pots (straw or potting substrate): For T1 (potting substrate only), a sample (1L per pot) of the upper part of the potting substrate was taken from each pot. For T2 and T3, all the straw (1L) covering the substrate of each pot was analyzed.

The material from each Berlese funnel extraction was screened in a stereo microscope, and all mites were mounted in Hoyer's medium for identification.

Observation and sampling of mites on marked leaflets during experiments

Once a week for four consecutive weeks, the number of mites was counted on the six leaflets of each plant previously infested with two-spotted spider mites. Each mite was categorized as Mesostigmata, Prostigmata or Oribatida. At each date, evaluations were carried out at 3, 7, 11 AM, and 3, 7 and 11 PM. Evaluations were conducted with a hand held lens

(40x) on both leaflet surfaces. During the dark phase (3 AM and 11 PM), evaluations were conducted by the use of a hand held lens (40x) illuminated with a headlight. All non-*T. urticae* mites found on the first evaluated leaflet of each plant on the second and fourth evaluations were collected and mounted on slides using Hoyer's medium for later identification.

Extraction of mites from the whole plant (end of experiments)

Each experiment was ended after 5 weeks, by a destructive sampling in which 6 of the plants of each treatment were cut at the plant base at 11 AM, and the remaining 7 plants were cut at 11 PM. Each plant was divided into three parts: a) basal region (first two centimeters from the plant base); b) young leaves, still folded; c) remainder of the plant (mature leaves and petioles). Each part was immediately placed in a plastic bag containing 70% ethanol and shaken vigorously. The liquid was subsequently poured through a 160 µm sieve. To extricate any remaining mites, the plant material was further rinsed by the use of jets of 70% ethanol. The mites retained in the sieve were mounted for identification and quantification under a stereomicroscope (100 x).

To investigate the possible occurrence of the fungus *Neozygites floridana* (Weiser & Muma) Remaudière & Keller (Entomophthorales: Neozygitaceae), a pathogen of the two-spotted spider mite, a sample of *T. urticae* were mounted in Hoyer's medium (maximum 10 mites / plant part/ plant) for examination under a microscope (100x).

Statistical analysis

Numbers of Mesostigmata and two-spotted spider mites in the different treatments were analyzed statistically as follows: for Mesostigmata, the means were contrasted (just between T2 and T3 in both experiments, given that Mesostigmata were not found in T1) by the F test, from the generalized linear model of the quasi-Poisson distribution (Anova, model.QuasiPoisson, test = “F”), in R [29]. For the two-spotted spider mites, the untransformed data were fitted to a quasi-Poisson distribution. Means were compared using the *glht* multicomp package, in R [29]. The predominant species of Mesostigmata were calculated as proposed by Pinzón and Spence [30].

7.3. Results

Mites extracted from commercial potting substrate, field soil and straw mulch

No mites were found in the commercial potting substrate, neither at the start nor at the end of the experiments. This strongly indicates that all mites eventually found on the strawberry plants of T2 and T3 originated from the straw used as mulch. As to the soil sampled in the strawberry fields, the average number of mites per soil sample of the 'Early season' were low in both T2 and T3 (1.2 ± 0.4 and 1.7 ± 0.5 mites, respectively), none of them Gamasina. In the 'Mid-season', the averages were also low (0.5 ± 0.2 and 0.7 ± 0.3 mites, respectively), and the only Gamasina found were *Amblygamasus* sp. and *Pergamasus longicornis* (Berlese) (both Parasitidae) and *Rhodacarellus epigynalis* Shels, 1956 (Rhodacaridae).

In contrast, the total numbers of mites found in the samples taken from straw to be used in T2 and T3 in the 'Early season', were relatively high, with the proportion of Gamasina higher in T3 than in T2 (18 and 6.0%, respectively) (Table 2). Consequently, the mean number of Gamasina per sample was significantly higher in T3 (7.8 ± 0.6) than in T2 (3.5 ± 0.7) (Df = 1; $F = 16.5$; $P < 0.001$). In the 'Mid-season', the total numbers of mites in T2 and T3 were lower at the start, of which 15.8 and 38.6% were Gamasina. As in the 'Early season', the mean number of Gamasina per sample was higher in the straw of T3 (11.9 ± 1.9) than in the straw of T2 (2.7 ± 0.7) (Df = 1; $F = 26.9$; $P < 0.001$). These results suggested that it would be expected to find a higher number of Gamasina on plants of T3 in both experiments.

The predominant gamasine families in 'Early season' T2 were Blattisociidae (33.3%), Melicharidae (31.1%) and Eviphididae (22.2%), each of the others representing a maximum of 4.4% of the total Gamasina. In T3, the predominant gamasine families were Parasitidae (49.5%) and Phytoseiidae (38.6%), each of the others representing a maximum of 4.9%. At the species level in the 'Early season', the predominant ones were *Alliphis halleri* (G. & R. Canestrini) (Eviphididae) and *Proctolaelaps* sp. (Melicharidae) in T2, and *Pergamasus* sp. (Parasitidae) in T3. In the 'Mid-season', the predominant family was Parasitidae in both treatments with straw (T2= 76.1% and T3= 71.4%), other families representing a maximum of 4.5 and 14.3%, respectively. At the species level in this experiment, *P. longicornis* (Berlese) (Parasitidae) and *Lasioseius* sp. (Blattisociidae) were predominant in T2 and T3, and *Veigaia nemorensis* (Koch) (Veigaiidae), in T3. These results suggest that most Gamasina to be found on strawberry plants should belong to the families Ascidae, Melicharidae and Eviphididae in T2 of the 'Early season' and to Parasitidae in the other cases.

At the end of the 'Early season', the total numbers of mites found in the straw of T2 and T3 were 231 and 105, respectively, Gamasina representing 52.8 and 16.1% of these (Table 2). Consequently, the mean number of Gamasina per sample was significantly higher in T2 than in T3 (9.4 ± 1.3 and 1.3 ± 0.4, respectively) (Df = 1; $F = 47.7$; $P < 0.001$). At the end of the 'Mid-season', the total numbers of mites in T2 and T3 were similar (166 and 152, respectively), and the proportions of Gamasina were also similar, 24.1 and 23.6% respectively (Table 4), resulting in no statistical difference between their mean numbers per sample (3.1 ± 0.7 and 2.8 ± 0.7, respectively) (Df = 1; *F* = 0.1; *P* = 0.75).

At the end of experiments, the predominant gamasine families in 'Early season' were Melicharidae (83.6%) and Blattisociidae (9.8%) in T2, each of the other families representing a maximum of 4.1% of the total Gamasina. In T3, the predominant gamasine families were Parasitidae (84.2%) and Phytoseiidae (10.5%), each of the other representing 5.2%. In this experiment, the predominant species were *Proctolaelaps* sp. (Melicharidae) in T2, and *Pergamasus* sp. (Parasitidae) in T3. In the 'Mid-season', the predominant families in both treatments were Parasitidae (T2= 52.5%; T3= 30.5%) and Blattisociidae (T2= 37.5%; T3= 38.9%), other families representing a maximum of 10 and 8.3%, respectively. In this experiment, *Lasioseius* sp. (Blattisociidae) was the predominant species at the end of both T2 and T3 and *Porrhostaspis lunulata* Müller, 1859 (Parasitidae), in T2.

Table 2. Mites extracted in Berlese funnels from samples of four types of cereal straw mulching used in two climates chamber experiments in 2019. The mulching had been present in strawberry fields for different periods of time, as indicated in column headings. B = Samples taken at the start of experiment; E=taken at the end of the experiment. N=13 samples of 1L per straw type and sampling occasion. continue

	'Early season' experiment				**'Mid-season' experiment**			
Taxa	**T2 (oat straw 2018)**		**T3 (oat straw 2017)**		**T2 (barley straw 2018)**		**T3 (barley straw 2017-2018)**	
	B	**E**	**B**	**E**	**B**	**E**	**B**	**E**
Sarcoptiformes, Oribatida, Astigmatina								
Acaridae								
Tyrophagus putrescentiae	48	92	130	35	17	22	15	7
Winterschimidtiidae	0	0	8	0	0	0	0	0
Sarcoptiformes, other Oribatida								
-	119	11	109	7	23	5	149	16
Trombidiformes, Prostigmata								
Anystidae								
Anystis sp.	0	0	0	0	32	27	2	0
Cunaxidae								
Cunaxoides croceus	0	0	3	0	0	0	0	0
Ereynetidae								
Ereynetes sp.	135	0	17	0	1	2	0	6
Eupodidae								
Eupodes sp.	239	0	46	0	64	50	31	66
Pygmephoridae								
Siteroptes sp.	1	0	2	0	0	0	1	0
Tydeidae								
Tydeus sp.	167	6	129	46	22	20	33	21
Parasitiformes, Mesostigmata, Gamasina								
Ascidae								
Gamasellodes bicolor	0	0	1	0	0	0	0	0
Neojordensia sinuata ♂	1	0	0	0	0	0	0	0
Blattisociidae								
Lasioseius sp.	7	10	3	1	5	15*	1	14*
Lasioseius sp. ♂	7	2	0	0	0	0	0	0
Eviphididae								
Alliphis halleri	9*	5	5	0	0	0	7	3
Alliphis sp. immature	1	0	0	0	0	0	0	0
Melicharidae								
Proctolaelaps sp.	14*	102*	0	0	0	0	0	0
Parasitidae								
Amblygamasus sp.	0	0	0	0	4	3	20	0
Amblygamasus sp. (immature)	0	0	0	0	0	1	16	0
Parasitus consanguineus	0	0	1	7	0	0	0	0
Parasitus sp. (deutonymph)	1	0	7	0	0	0	0	0
Pergamasus longicornis	0	0	3	2	14*	4	42*	2
Pergamasus septentrionalis	0	0	10	0	0	0	5	2
Pergamasus sp. (deutonymph)	0	0	26*	2	1	4	17	0
Pergamasus sp. ♂	0	0	0	2	0	0	0	0
Porrhostaspis lunulata	0	0	0	0	0	8	4	7*
Porrhostaspis sp. (deutonymph)	0	0	1	2	0	0	1	0
Immature	1	0	2	1	2	1	3	0

Table 2. Mites extracted in Berlese funnels from samples of four types of cereal straw mulching used in two climates chamber experiments in 2019. The mulching had been present in strawberry fields for different periods of time, as indicated in column headings. B = Samples taken at the start of experiment; E = taken at the end of the experiment. N=13 samples of 1L per straw type and sampling occasion. continuation

	'Early season' experiment				**'Mid-season' experiment**			
Taxa	**T2 (oat straw 2018)**		**T3 (oat straw 2017)**		**T2 (barley straw 2018)**		**T3 (barley straw 2017-2018)**	
	B	**E**	**B**	**E**	**B**	**E**	**B**	**E**
Parasitiformes, Mesostigmata, Gamasina								
Phytoseiidae								
Neoseiulus alpinus	1	2	3	1	0	0	0	0
Neoseiulus cucumeris	0	0	0	0	2	2	0	1
Neoseiulus sp. immature	0	1	3	1	0	0	0	0
Proprioseiopsis okanagensis	0	0	3	0	1	2	0	0
Typhlodromips masseei	0	0	8	0	0	0	5	2
Typhlodromips sp. ♂	1	0	5	0	0	0	0	0
Immature	0	0	17	0	0	0	0	0
Rhodacaridae								
Rhodacarellus epigynalis	0	0	0	0	1	0	2	3
Rhodacarellus kreuzi	0	0	1	0	0	0	0	0
Rhodacarellus sp. immature	1	0	0	0	0	0	0	0
Veigaiidae								
Veigaia nemorensis	1	0	0	0	0	0	22*	2
Veigaia sp. immature	0	0	2	0	0	0	0	0
Total	**754**	**231**	**545**	**105**	**189**	**166**	**376**	**152**

(*) Predominant Gamasina species; (-) Not identified until family.

Mites observed on marked leaflets

Tetranychus urticae was the only mite species found on the T1 marked leaflets of both experiments, which was expected, given that no mites were observed in the commercial substrate used for all treatments and that this was the only material present in the pots of T1 (Table 3). On the other hand, the totals of Gamasina on T2 and T3 leaflets were low throughout both experiments, with a maximum of 4 mites in T2 (in the third week of evaluation of the 'Early season') and of 5 mites in T3 (in the fourth week of evaluation of the 'Mid-season'). This makes it difficult to compare treatments statistically. Nevertheless, gamasine mites could be seen moving on the straw surface at night.

In the 'Early season', the predominant Gamasina in T2 were Melicharidae, of which *Proctolaelaps* sp. was the most abundant species. In T3, the predominant family was Blattisociidae, of which *Lasioseius* sp. as the most abundant species. In the 'Mid-season', the predominant Gamasina in T2 were Phytoseiidae, with *Typhlodromips masseei* (Nesbitt) as the most abundant species, whereas the predominant family in T3 was Blattisociidae, with *Lasioseius* sp. as the most abundant species.

Table 3. Total mites (except *Tetranychus urticae*) found on the second and fou rth evaluations at 3, 7, 11 AM and 3, 7, 11 PM on the 6 marked leaflets with *T. urticae* / plant (N = 13 plants/ treatment) in two lab experiments for different periods of time: 'Early season' experiment (oat straw: T2, since 2018; T3, since 2017); 'Mid-season' experiment (barley straw: T2, since 2018; T3, lower half layer since 2017 and top half layer since 2018). Light was on from 6 AM to 9 PM

(*) Predominant species

Taxa	**'Early season' experiment**		**'Mid-season' experiment**	
	T2 (oat straw 2018)	**T3 (oat straw 2017)**	**T2 (barley straw 2018)**	**T3 (barley straw 2017-2018)**
Trombidiformes, Prostigmata				
Anystidae				
Anystis sp.	0	0	22	0
Parasitiformes, Mesostigmata, Gamasina				
Ascidae				
Neojordensia sinuata	1	0	0	0
Blattisociidae				
Lasioseius sp.	0	3	1	11*
Melicharidae				
Proctolaelaps sp.	6*	0	0	0
Parasitidae				
Parasitus sp. (deutonymph)	0	0	1	0
Pergamasus sp. (deutonymph)	0	0	0	1
Phytoseiidae				
Neoseiulus cucumeris	0	0	1	0
Typhlodromips masseei	1	0	3*	0
Proprioseiopsis okanagensis	0	0	1	1
Total	**8**	**3**	**29**	**13**

To sum up, in the 'Early season', the total numbers of non-*T. urticae* mites found on the marked leaflets of T2 and T3 were 8 and 3, of which only one mite was Phytoseiidae (in T2, *T. masseei*). In the 'Mid-season', the total number of non-*T. urticae* mites found on marked leaflets of T2 and T3 were 29 and 13, of which 22 on T2 plants were *Anystis* sp. (Anystidae); of Phytoseiidae there were five mites in T2 (1 *Neoseiulus cucumeris* (Oudemans), 3 *T. masseei* and 1 *Proprioseiopsis okanagensis* (Chant)) and one in T3 (*P. okanagensis*). Despite small the numbers of edaphic Gamasina, they were seen on the plants only at night (Figures 1 A, B, C, D), except for Phytoseiidae and Anystidae, seen only at daytime (Figure 1 C).

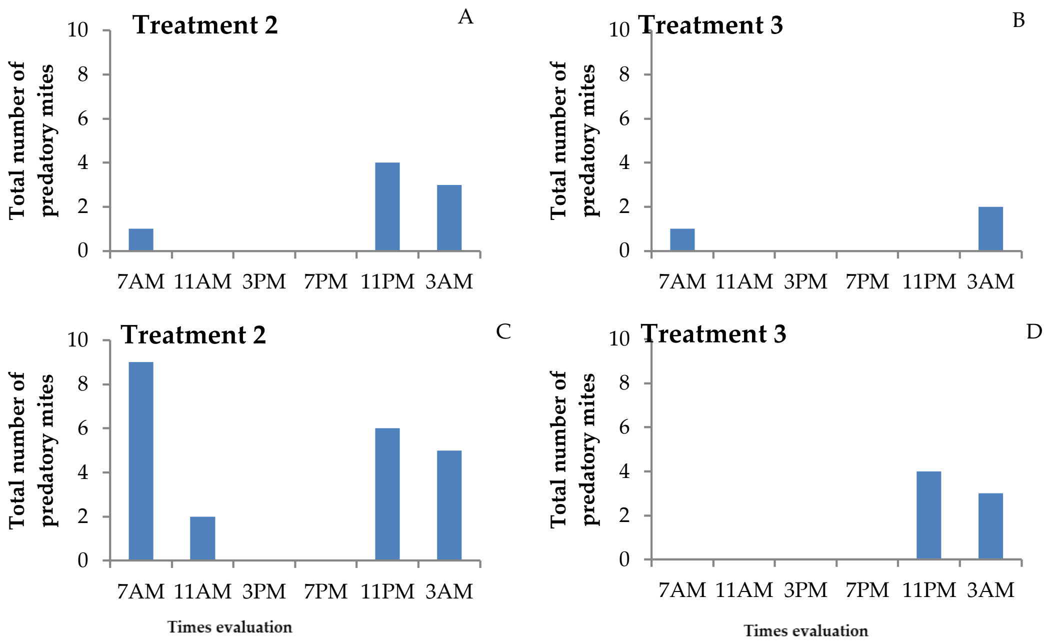

Figure 1. Weekly total numbers of Gamasina mites observed on 6 marked leaflets with *Tetranychus urticae* per plant (N= 13 plants per treatment). A and B: 'Early season' experiment (T2, oat straw since 2018; T3, oat straw since 2017); C and D: 'Mid-season' experiment (T2, barley straw since 2018; T3, barley straw lower half layer since 2017 and top half layer since 2018. Light was on from 6 AM to 9 PM.

For *T. urticae*, the mean number observed per leaflet in the fourth week of the experiment were: 'Early season': T1= 15.4 ± 2.3, T2= 9.2 ± 3.8 and T3= 10.4 ± 3.7; 'Mid-season': T1= 20.4 ± 4.1, T2=14.3 ± 4.0 and T3=14.5 ± 4.4. Significant differences were observed in the 'Early season' between T1 and T2 (Df = 2; Z = 2.55; P = 0.02), and in the 'Mid-season' between T1 and T2 (Df = 2; Z = 2.4; P = 0.03) and T1 and T3 (Df = 2; Z = 2.3; P = 0.03) (Figures 2 A, B). In addition, the number of *T. urticae* did not vary according to evaluation times, as expected, leading us to consider each evaluation as a replicate in the statistical analysis.

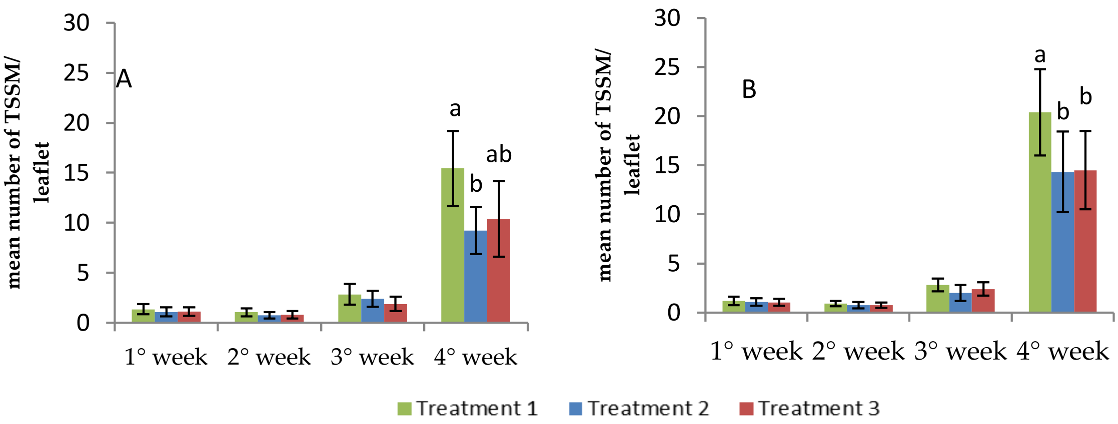

Figure 2. Weekly mean number of *Tetranychus urticae* observed on 6 marked leaflets per plant (N= 13 plants per treatment). A: 'Early season' experiment (T2, oat straw since 2018; T3, oat straw since 2017); B: 'Mid-season' experiment (T2, barley straw since 2018; T3, barley straw lower half layer since 2017 and top half layer since 2018. Light was on from 6 AM to 9 PM.

Mites extracted from whole plants at the end of experiments

No Gamasina was found on T1 plants. Considering T2 and T3 together and the two evaluation times, the number of Gamasina found in the 'Early season' (107 mites) was higher than in the 'Mid- season' (76 mites) (Table 4), coinciding with the higher number of mites in the straw at the end in the 'Early season'. The largest number of Gamasina was found on plants of T2 in both experiments. Also considering T2 and T3 together, Gamasina were rarely found at 11 AM (total of five mites), with a much larger number found at 11 PM (total of 102 mites); this resulted in significantly higher density of Gamasina at 11 PM than at 11 AM in both treatments and in both experiments (Table 4).

Table 4. Total number of mites (except *Tetranychus urticae*) of different groups extracted with 70% alcohol from whole strawberry plants. Six plants were put in alcohol at 11 AM and another seven at 11 PM and 11 in two lab experiments for different periods of time: Early season experiment (oat straw: T2, since 2018; T3, since 2017); Mid-season experiment (barley straw: T2, since 2018; T3, lower half layer since 2017 and top half layer since 2018).

	T2				**T3**			
Experiment	**'Early season'**		**'Midseason'**		**'Early season'**		**'Midseason'**	
Taxa	**11AM**	**11PM**	**11AM**	**11PM**	**11AM**	**11PM**	**11AM**	**11PM**
Trombidiformes, Prostigmata: Anystidae								
Anystis sp.	0	0	11	0	0	0	0	0
Sarcoptiformes, Oribatida, Astigmatina: Acaridae								
Tyrophagus sp.	0	14	0	11	0	16	0	24
Parasitiformes, Mesostigmata, Gamasina								
Blattisociidae								
Lasioseius sp. 1	0	3	0	11*	0	2	0	0
Lasioseius sp. immature	0	1	0	0	0	0	0	0
Lasioseius sp. 1 ♂	0	2	0	0	0	0	0	0
Eviphididae								
Alliphis halleri	0	11	0	0	0	0	0	1
Melicharidae								
Proctolaelaps sp.	0	33*	0	0	0	0	0	0
Parasitidae								
Amblygamasus sp.	0	0	0	1	0	0	0	0
Amblygamasus (deutonymph)	0	0	0	1	0	0	0	0
Pergamasus sp. (deutonymph)	0	0	0	0	0	0	1	0
Porrhostaspis lunulata	0	0	2	0	0	0	0	0
Parasitus consanguineus	0	0	0	0	0	16*	0	0
Pergamasus longicornis	0	0	0	0	0	1	0	4
Pergamasus septentrionalis	0	0	0	0	0	0	0	1
Pergamasus sp. (deutonymph)	0	0	0	0	0	4	0	0
Porrhostaspis lunulata	0	0	0	1	0	0	0	0
Immature	0	2	1	0	0	0	0	0
Phytoseiidae								
Neoseiulus cucumeris	0	1	0	2	0	0	0	0
Proprioseiopsis okanagensis	0	0	1	1	0	0	0	0
Typhlodromips masseei	0	0	0	1	0	1	0	0
Rhodacaridae								
Rhodacarellus sp. (immature)	0	0	0	1	0	0	0	0
Total	**0**	**67**	**15**	**30**	**0**	**40**	**1**	**30**

In the'Early season', at 11 AM no Gamasina was found on T2 or T3 plants, in contrast to the considerable number found at 11 PM (Table 5). The average numbers of Gamasina per plant was significantly different between night and day (between 11AM and 11PM in T2: Df=1; F= 73.4; P< 0.001; between 11AM and 11PM in T3: Df= 1; F= 33.3; P< 0.001). In the 'Mid-season', the number of Gamasina on T2 plants at 11 PM was higher than at 11 AM (Df=1, F= 10.6, P< 0.01), whilst in T3 there was no statistical difference between 11AM and 11PM (Df= 1, F= 2.1, P= 0.15) .

As to the faunistic composition, in the 'Early season' at 11 PM, the predominant families were Melicharidae, in T2, with *Proctolaelaps*. sp. as the most abundant species, only 18.2% of which were located at the base of the plant or on the young leaflets. In T3, the predominant family was Parasitidae, with *Parasitus consanguineus* Oudemans & Voigts as the most abundant species; about 87.5% of these were found at the base of the plants. In the 'Mid-season', in T2 the predominant family was Blattisociidae, with *Lasioseius* sp. as the most abundant species. In T3, Gamasina always occurred at very low numbers. These results were similar to those of the evaluation of mites on leaflets only, that is, in both experiments, the largest number of predatory mites occurred in T2, both in the evaluations of only leaflets and on the whole plant.

The mean number of *T. urticae* per plant on leaves in T1, T2 and T3 were 176.3 ± 10.2, 143.6 ± 14.3 and 137.5 ± 11.2, respectively, in the 'Early season', and 223.8 ± 2.5, 138.4 ± 18.1 and 94.4 ± 18.5 in the 'Mid-season' experiment. Significant differences were observed in the 'Early season' between T1 and T3 (Df = 2; Z = -2.2; P = 0.0) and in the 'Mid-season' between T1 and T2 (Df = 2; Z = - 3.2; P = 0.003) and T1 and T3 (Df = 2; Z = -5.1; P <0.001). Infection of the two-spotted spider mite by the fungus *N. floridana* was not observed.

Table 5. Mean number (± SE) of Gamasina mites per plant put in alcohol at 11 AM and 11 PM in two lab experiments (N=6 plants) with straw mulching: Early season experiment (oat straw: T2, since 2018; T3, since 2017); Mid-season experiment (barley straw: T2, since 2018; T3, lower layer since 2017 and top layer since 2018).

	T2		**T3**	
	11AM	**11PM**	**11AM**	**11PM**
'Early season' experiment	0.0 ± 0.0 a	4.1 ± 0.5 b	0.0 ± 0.0 a	1.8 ± 0.3 b
'Mid-season' experiment	0.3 ± 0.1 a	1.5 ± 0.4 b	0.2 ± 0.1 a	0.5 ± 0.2 a

In each row, different lower case letters indicate significant statistical difference between sampling at 11AM and at 11PM within each treatment (Means contrasted by F test, generalized linear model of the quasi-Poisson type in R).

7.4. Discussion

The results of both experiments confirmed our suspicion, showing that Gamasina are present in cereal straw used as mulch in strawberry and that straw maintained longer in the field has the highest numbers and diversity of Gamasina, suggesting that organic mulch could serve as a reservoir for those organisms. This is in accordance with what Esteca et al. [20] found for coffee husk maintained on the floor of a forest patch and then used as mulch in a Brazilian strawberry field. It should be expected, however, that there should be a limit to this effect over time, beyond which abundance and diversity would be reduced, due to the natural process of decomposition of the organic matter and changes in ecological conditions, as described by some authors [31-33].

The results also show that typically edaphic predators (except Anystidae and Phytoseiidae) move from cereal straw and are present on strawberry plants at night. This is in accordance with what was conceived by Britto et al. [28] and show that this is also the case for our system. Further it is in accordance with Esteca et al. [20] who found that Gamasina that *Proctolaelaps pygmaeus* (Muller) (Melicharidae) and *Blattisocius dentriticus* (Berlese) (Blattisociidae) were present on strawberry leaflets mainly during night, indicating their possible daily migration from the edaphic environment to the plants. Our results also show a higher prevalence of *T. urticae* in pots without than with straw, suggesting that the Gamasina moving from the straw to the plant during night might have preyed on *T. urticae.* But other factors associated with the absence of straw should not be ruled out, and this would be an aspect for complementary investigation.

Mites from the straw, underlying soil and commercial potting substrate

Although in this study our main interest centered on the Gamasina and the two-spotted spider mite, mites of other groups were also found in the straw. The diversity of mites found in the soil taken from the field was apparently low, with the occurrence of representatives of about 3–5 families (non-Astigmatina Oribatida were not identified to family) in each field. However, at least 13–14 families (also without identifying families of non-Astigmatina Oribatida families) were represented in the straw used in the experiments. Among the mites collected in this study, some Prostigmata (especially the Tydeidae) and some Astigmatina (especially Acaridae) have been considered important as alternative food for several predatory mites. Some species of Astigmatina are extensively used as factitious food in the mass production of Gamasina for use as biological control agents [34]. In the present study, acarids were quite numerous in both experiments, and might have served as prey for some of the Gamasina collected. In fact the acarid *Tyrophagus* sp. was quite abundant on the strawberry plants at night, in both treatments and in both experiments. These were not present on the plants at daytime. The presence of these mites onto the plants could somehow be related to the concurrent presence of the Gamasina on the plants.

There is a lot of information about the groups of mites effectively or potentially useful for biological pest control [15]. In a study conducted by Castilho et al. [5] in Norwegian strawberry fields, three species of some of the same genera reported in this study were collected from litter samples, namely *Porrhostaspis* (Parasitidae), *Proprioseiopsis* (Phytoseiidae) and

Lasioseius (Blattisociidae). Among the Gamasina collected, some are members of the Phytoseiidae, a family that contains several species extensively used for biological pest control [35]. Of the four phytoseiid species found in this study, *N. cucumeris* has been widely commercialized for the control of the two-spotted spider mite and thrips on plants in different countries, including Norway. Of the other families collected, Parasitidae is also commonly found in studies conducted worldwide, but these have not been reported as plant mites. Instead, they are soil inhabitants that feed mostly on nematodes and immature flies [36-38]. Based on the evaluation of the mites present on both types of straw, it was expected that parasitid species could be some of the predominant mites on strawberry leaves. This was not the case, most certainly because despite of their dominance in the straw, strawberry leaves in the climatic room had nothing attractive to them. Parasitid species have been evaluated for the biological control of pest organisms, especially of thrips [38]. The parasitid *P. longicornis* was previously reported from several coastal habitats in Norway [39]. Mites of the same genus have also been found in soil of strawberry fields in Brazil [20].

Ascidae, Blattisociidae and Melicharidae, Gamasina groups found in this study, have not been used commercially, but some studies have demonstrated their potential as biological control agents, especially in humid habitats [40]. Mites of these groups seem to be generalists, being able to feed on fungi and to prey on mites and small insects. Melicharids have been reported to feed on immatures of drosophila flies, an insect group important in several countries, including Norway, as a direct pest of strawberry [40]. The melicharid *P. pygmaeus* was found in Norway in a coniferous forest [41]. In a study carried out in Brazil, this mite was found in large numbers in coffee husk used as mulching in strawberry fields [20]. It has been reported to consume drosophila eggs [40] and *T. urticae* [42].

The Rhodacaridae have been inadequately studied for use as a biocontrol agent, but an important characteristic of species of this group is their small size, facilitating their movement below the soil surface, where they can encounter plant feeding nematodes, which they have been shown to be able to consume [37]. Eviphidids and veigaiids have also received little attention as biocontrol agents. This is apparently the first report of the eviphidid *A. halleri* in Norway, but this mite has already been reported from neighboring Sweden [43]. Eviphidids are commonly found in agricultural soils, phoretically on insects, especially beetles, and in manure. Interest in this group stems in part from the fact that it contains species known to prey on plant feeding nematodes [44]. The veigaiid *V. nemorensis* was previously reported from Norway, both in a natural and in a modified environment [37]. Mites of this family are common in litter, feeding on other mites and other small arthropods [45].

Some of the Prostigmata collected (especially Anystidae and Cunaxidae) are also known to be predators [34]. But they have not been developed as commercially available biocontrol agents probably because of the difficulties in mass production.

Mites on strawberry plants

What would cause mites to move from the ground surface to plants and vice-versa? In general, the movement could occur while escaping from stressing factors of relatively long duration. An example is the excess of humidity, as evidenced by the movement of Oribatida from the soil to the trees in the Amazon forest, in the rainy season of each year [46]. Other stressors may lead to the occurrence of diapause, common in phytoseiids [35, 47] and tetranychids [48], stimulating the mites to move away from their usual habitat while active.

But factors of shorter duration might also be involved, and related to daily movements. They could move from plants to soil, during the day to escape the stressful conditions of low levels of relative humidity. But mites could move to plants at night, among other reasons, in search of food and / or while searching for places with lower competition (due to the diversity and abundance of predators, usually lower on plants than in the soil) and where they are usually less subjected to intra-guild predation. In doing so, it could promote the biological control of potential pest organisms.

The diversity of mites on plants was quite high in this study compared to other study conducted in strawberry fields [5]. About eight species were found when only leaflets were examined, but the number of species detected was about the double when the whole plant was examined, although, with few exceptions, the number of specimens was low. In relation to the most common groups on plants (the blattisociid *Lasioseius* sp., the melicharid *Proctolaelaps* and the anystids), the same response was observed in the evaluation of mites only on leaflets and on the whole plants. But there was a difference, and that referred to the parasitid *P. consanguineus*, not collected from leaflets, but collected in relatively large total number on whole plants. The main reason for this difference refers to the occurrence of this species onto the crown of the strawberry plants, not on leaves.

In the present study, anystids were only found in the ‘Mid-season’, in which they were observed in significant numbers on plants with young cereal straw, and only at daytime. The anystid *Anystis agilis* (Banks) has been cited as a predator of the mites *Panonychus citri* (McGregor), two-spotted spider mite and the thrips *Scirtothrips citri* Moultan [49]. These pests are found in the aerial part of the plants, although *S. citri* is also found in the soil or litter in the stages of "pre-pupa" and "pupa". The anystids found in this study could have helped to reduce the number of *T. urticae* on the strawberry leaflets on plants with young cereal straw of the 'Midseason' experiment, but reports of predation of anystids on *T. urticae* are not satisfactory in the literature, and this should be further investigated.

The phytoseiids *N. cucumeris*, *P. okanagensis* and *T. masseei* have previously been collected on strawberry in Norway [5, 50, 51], but this is the first time *N. cucumeris* has been found in a Norwegian strawberry crop where it has never been released as a biocontrol agent of mites (including *T. urticae* [52]) or thrips. Only one specimen of this species has ever been recorded as naturally occurring in Norway (on *Corylus avellana* L., Denmark and Edland 2002). So, *N. cucumeris* is either naturally occurring in the area or it has followed the plant material. Regarding *P. okanagensis,* this species is definitely native in Norway. According to Meshkov [53], it does not feed on two-spotted spider mite to a significant extent.

Final remarks

Our study suggests that the use of cereal mulching by strawberry producers in Norway allows the maintenance of a larger number of predatory mites in the environment than would be possible without mulching. Further, it suggests that cereal straw maintained in the field for a longer time (2 years) hosts more predatory mites than cereal straw maintained for a shorter period (1 year). This is suggested by the striking differences in numbers of Gamasina in the straw and in the underlying soil, although a precise numerical comparison cannot be made, given the different sample sizes in this study. Organic soil covering facilitates the maintenance of predators in the field, as extensively reported in the literature [10-15]. The difference between abundance of Gamasina mites in the soil and in the straw was to be expected, as these mites are not only predators (usually unspecific), but also feed on other organisms found in the mulching, including fungi [42, 54, 55].

The movement of certain Gamasina onto strawberry plants at night and the lower *T. urticae* numbers in straw treatments indicates that edaphic Gamasina prey on *T. urticae* and that using straw with Gamasina may be an interesting conservational biocontrol strategy and effectiveness of these organisms as biological control agents. Different studies reported the difference between the mite fauna on the leaves of certain plants in daytime and nighttime observations [20, 25, 56, 57].

However, not all groups of Gamasina may be inclined to climb plants at night. The results of this study suggested that while some Gamasina apparently ventured to visit the regions of more extensive surface in the plant, that is, the leaflets (Blattisociidae, Melicharidae), others visit only the parts closest to the soil, that is, the region of the crown of the plants, as observed for the parasitid *P. consanguineous*. Yet, in certain plant species, this region is attacked by pests, especially fly larvae of the family Sciaridae, which could theoretically be attacked by predators of this group. The results also suggested that some Gamasina, such as Eviphididae, even when relatively abundant in the substrate, do not appear to climb the plants too often. This suggests that their role as biological control agents on strawberry plants may not be significant.

Although the main focus of the present study was the nocturnal movement of the Gamasina from mulching to plants, an opposite characteristic was also determined. Unlike Gamasina, anystids were observed on plants only during the day, suggesting an inverse movement of the Gamasina, perhaps this being a way to reduce intra-guild predation.

The results of this study may subsidize subsequent research to evaluate the performance of strawberry crops that incorporate the use of cereal straw used in previous years as soil cover, favoring the maintenance of edaphic predatory mites in the cultivation. In subsequent research, it seems recommendable to evaluate the potential predation and oviposition of Gamasina mites found in the aerial part of the strawberry plants when offered common Norwegian strawberry herbivore pests in the aerial part of the strawberry plants. Moreover, in future studies, when sampling predatory mites on strawberry plants, the time of the day should be taken into consideration, and sampling should also be done at night.

Results could have been different, had we inoculated the plants with other organisms, onto which the edaphic mite could feed. It might be worthwhile to conduct a similar study under field conditions, where environmental factors alter more slowly and in concert, what was not possible to incorporate in the present study.

Author Contributions: Conceptualization, Fernanda de Cássia Neves Esteca, Nina Trandem, Ingeborg Klingen and Gilberto José de Moraes; Data curation, Fernanda de Cássia Neves Esteca, Nina Trandem, Ingeborg Klingen, Jandir Cruz Santos, Italo Delalibera Junior and Gilberto José de Moraes; Formal analysis, Fernanda de Cássia Neves Esteca, Jandir Cruz Santos and Gilberto José de Moraes; Funding acquisition, Nina Trandem, Ingeborg Klingen, Italo Delalibera Junior and Gilberto José de Moraes; Investigation, Fernanda de Cássia Neves Esteca, Nina Trandem, Ingeborg Klingen and Gilberto José de Moraes; Methodology, Fernanda de Cássia Neves Esteca, Nina Trandem, Ingeborg Klingen and Gilberto José de Moraes; Project administration, Fernanda de Cássia Neves Esteca, Nina Trandem, Ingeborg Klingen and Gilberto José de Moraes; Resources, Nina Trandem, Ingeborg Klingen, Italo Delalibera Junior and Gilberto José de Moraes; Supervision, Nina Trandem, Ingeborg Klingen and Gilberto José

de Moraes; Writing – original draft, Fernanda de Cássia Neves Esteca, Nina Trandem, Ingeborg Klingen, Italo Delalibera Junior and Gilberto José de Moraes.

Funding: This research was funded by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and The Research Council of Norway through the SMARTCROP project (Project Number 244526).

Acknowledgments: We are grateful to Marta Bosque Fajardo and Marit Helgheim at NIBIO for their practical assistance in conducting this work; to the José Bruno Malaquias at ESALQ-USP for help with statistics; to the Saxebøl family for answering lots of questions and letting sample to us straw mulching on their farm and to the Marina Ferraz de Barbosa Camargo for help in identifying Astigmatina mites.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Chakraborty, D.; Nagarajan, S.; Aggarwal, P.; Gupta, V.K.; Tomar, R.K.; Garg, R.N.; Sahoo, R.N.; Sarkar, A.; Chopra, U.K.; Sarma, K.S.S.; Kalra, N. Effect of mulching on soil and plant water status: and the growth and yield of wheat (*Triticum aestivum* L.) in a semi-arid environment. *Agric. Water Manag.* **2008**, *95*, 1323-1334.
2. Haslestad, J. (Norsk Landbruksrådgivning, Ribadu, Hedmark, Norway). Personal communication, 2020.
3. Vi spiser mer utenlandske enn norske jordbær. https://www.ssb.no/jord-skog-jakt-og-fiskeri/artikler-og-publikasjoner/vi-spiser-mer-utenlandske-enn-norske-jordbaer (Accessed on 22, Jan, 2020).
4. Statistics Norway. https://www.ssb.no/en (Accessed on 22, Jan, 2020).
5. Castilho, R.C.; Duarte, V.S.; Moraes, G.J.; Westrum, K.; Trandem, N.; Rocha, L.C.D.; Delalibera, I. Jr.; Klingen, I. Two-spotted spider mite and its natural enemies on strawberry grown as protected and unprotected crops in Norway and Brazil. *Exp. Appl. Acarol.* **2015**, *66*, 509–528.

6. Trandem, N. Greenhouse production of strawberries and blackberries in Norway–arthropod pests and biological control. IOBC/ WPRS Working group “Integrated Plant Protection in Orchards” Subgroup “Soft Fruits”, Proceedings of the meeting at Dundee, Scottish Crop Research Institute, Scotland, 18-21 September 2001; Gordon, S.C.; Cross J. V.; Dundee, Scotland, 2003; *26*, 45-50.
7. Klingen, I.; Westrum, K.; Meyling, N.V. Effect of Norwegian entomopathogenic fungal isolates against *Otiorhynchus sulcatus* larvae at low temperatures and persistence in strawberry rhizospheres. *Biol. Control.* **2015**, *81*, 1-7.
8. Aasen, S.S.; Trandem, N. Strawberry blossom weevil *Anthonomus rubi* Herbst (Col.: Curculionidae): relationships between bud damage, weevil density, insecticide use, and yield. *J. Pest. Sci*. **2006**, *79*, 169-174.
9. Kader, M.A.; Senge, M.; Mojid, M.A.; Ito, K. Recent advances in mulching materials and methods for modifying soil environment. *Soil Till. Res*. **2017**, *168*, 155-166.
10. Johnson, J.M.; Hough-Goldstein, J.A.; Vangessel, M.J. Effects of straw mulch on pest insects, predators, and weeds in watermelons and potatoes. *Environ. Entomol*. **2004**, *33*, 1632–1643.
11. Larentzaki, E.; Plate, J.; Nault, B.; Shelton, A. Impact of straw mulch on populations of onion thrips (Thysanoptera: Thripidae) in onion. *J. Econ. Entomol*. **2008**, *101*, 1317-1324.
12. Hoddle, MS, Morse, JG, Phillips, PA, Faber, BA, Jetter, KM Avocado thrips: a new challenge for growers. *Cali. Agr.* **2002**, *56*,103-107.
13. Jensen, L.; Simko, B.; Shock, C.; Saunders, L. Alternative methods for controlling onion thrips (*Thrips tabaci*) in Spanish onions. Malheur Experiment Station Annual Report 2001, Oregon State University Agricultural Experiment Station, Ontario, Canada, 2002; *1038*, 104-111
14. Jamieson, L.E.; Stevens, P.S. The effect of mulching on adult emergence of Kelly’s citrus thrips (*Pezothrips kellyanus*). *N. Z. Plant Prot*. **2006**, *59*, 42–46.
15. Sánchez-Moreno, S.; Ferris, H. Suppressive service of the soil food web: effects of environmental management. *Agric. Ecosyst. Environ.* 2007, *119*, 75–87.
16. Carrillo, D.; Moraes, G. J.; Peña, J. E. *Prospects for biological control of plant feeding mites and other harmful organisms*, Springer International Publishing: Switzerland, 2015; pp. 337.

17. Walter, D.E.; Proctor, H.C. *Mites: Ecology, Evolution and Behaviour Life at a Microscale* (second edition), Springer Science and Business Media: Dordrecht, Netherlands, 2013, pp. 509.
18. Bottinelli, N.; Jouquet, P.; Capowiez, Y.; Podwojewski, P.; Grimaldi, M.; Peng X. Why is the influence of soil macrofauna on soil structure only considered by soil ecologists? *Soil Till. Res.* **2015**, *146*, 118-124.
19. Raven, P.H.; Evert, R.F.; Eichorn, S.E. *Biologia vegetal*. Guanabara-Koogan: Rio de Janeiro, Brasil, 2001, pp. 906.
20. Esteca, F.C.N.; Rodrigues, L.R.; Moraes, G.J.; Júnior, I.D.; Klingen, I. Mulching with coffee husk and pulp in strawberry affects edaphic predatory mite and spider mite densities. *Exp. Appl. Acarol.*, **2018**, *76*, 161-183.
21. Karg, W. Syno¨kologische Untersuchungen von Bodenmilben aus forstwirtschaftlich und landwirtschaftlich genutzten Böden. *Pedobiologia*. **1967**, 7, 198–214
22. van de Bund C.F. Gamasides as predators of phytophagous nematodes. In: Problems of acarology, symposium; Bozek J, Suski ZW, Jakubowska J (Eds.); Publisher: Polska Akademia Nauk, Polska, 1970, pp. 280.
23. Walter, D.E.; Behan-Pelletier, V. Mites in forest canopies: Filling the size distribution shortfall? *Annu. Rev. Entomol*. **1999**, *44*, 1– 19.
24. Onzo, A.; Hanna, R.; Zannou, I.; Sabelis, M.W.; Yaninek, J.S. Dynamics of refuge use: diurnal, vertical migration by predatory and herbivorous mites within cassava plants. *Oikos.* **2003**, *101*, 59–69.
25. Parecis-Silva, P.V.; Nuvoloni, F.M.; Feres, R.J. Day vs. night: the importance of the circadian cycle over metacommunities and predator–prey densities. *Int. J. Acarol.* **2016**, *42*, 141–148.
26. Fagan, L.L.; Didham, R.K.; Winchester, N.N.; Behan-Pelletier, V.; Clayton, M.; Lindquist, E.E.; Ring, R. A. An experimental assessment of biodiversity and species turnover in terrestrial versus canopy leaf litter. Oecologia, **2006**, *147*, 335– 347.
27. Esteca, F.C.N.; Moraes, G.J. Effect on relative humidity in the hatching of larvae and in adults of different mites (Acari). (in preparing)
28. Britto, E.P.J.; Gago, E.; Moraes, G.J. How promising is *Lasioseius floridensis* as a control agent of *Polyphagotarsonemus latus*? *Exp. Appl. Acarol.*, **2012**, *56*, 221–231.
29. R Development Core Team R: a language and environment for statistical computing, v.3.0.1. R foundation for Statistical Computing, Vienna, Austria. 2013

30. Pinzón, J.; Spence, J.R. Bark-dwelling spider assemblages (Araneae) in the boreal forest: dominance, diversity, composition and life-histories. *J. Insect. Conserv*. **2010**, 14, 439-458.
31. Sjursen, H.; Michelsen, A.; Jonasson, S. Effects of long term soil warming and fertilization on microarthropod abundances in three sub-artic ecosystems. *Appl. Soil Ecol*. **2005**, *30*, 148-161.
32. Blankinship, J.C.; Niklaus, P.A.; Hungate, B.A. A meta-analysis of responses of soil biota to global change. *Oecologia.* **2011,** *165*, 553-565.
33. Bedano, J.C.; Ruf, A. Sensitivity of different taxonomic levels of soil Gamasina to land use and anthropogenic disturbances. *Agr. Forest Entomol.* **2010**, *12*, 203-212.
34. Hernandes, F.A.; Castro, T.M.M.; Venancio, R. Prostigmata (Acari: Trombidiformes) as biological control agents. In *Prospects for biological control of plant feeding mites and other harmful organisms*; Carillo D., Moraes G.J., Peña J.E. (Eds.); Springer International Publishing: Switzerland, 2015, pp. 151–184.
35. McMurtry, J.A.; Sourassou, N.F.; Demite, P.R. The Phytoseiidae (Acari: Mesostigmata) as biological control agents. In *Prospects for Biological Control of plant feeding mites and other harmful organisms*; Carillo D., Moraes G.J., Peña J.E. (Eds.); Springer International Publishing: Switzerland, 2015, pp. 133-149.
36. Szlendak, E.; Lewandowski, M. Development and reproductive capacity of the predatory mite *Parasitus consanguineus* (Acari: Parasitidae) reared on the larval stages of *Megaselia halterata* and *Lycoriella ingenua*. *Exp. Appl. Acarol.* **2009**, *47*, 285-292.
37. Castilho, R.C.; Venancio, R.; Narita, J.P.Z. Mesostigmata as biological control agents, with emphasis on Rhodacaroidea and Parasitoidea. In *Prospects for Biological Control of plant feeding mites and other harmful organisms;* Carillo D., Moraes G.J., Peña J.E. (Eds.); Springer International Publishing: Switzerland, 2015, pp. 1–31.
38. Rueda-Ramírez, D.; Rios-Malaver, D.; Varela-Ramírez, A.; Moraes, G. J. Biology and predation capacity of *Parasitus bituberosus* (Acari: Mesostigmata: Parasitidae) on *Frankliniella occidentalis* (Thysanoptera: Thripidae), and free-living nematodes as its complementary prey. Pest Manag. Sci., **2019**, *75*, 1819-1830.
39. Bolger, T.; M; Devlin; Seniczak, A. First records of ten species of Mesostigmata (Acari, Mesostigmata) added to the published Norwegian species list. *Nor. J. Entomol*. **2018,** *65*, 94–100.

40. Moraes, G.J.; Venancio, R, dos Santos, V.L.V.; Paschoal, A.D. Potential of Ascidae, Blattisociidae and Melicharidae (Acari: Mesostigmata) as biological control agents of pest organisms. In *Prospects for Biological Control of plant feeding mites and other harmful organisms*; Carillo D., Moraes G.J., Peña J.E. (Eds.); Springer International Publishing: Switzerland, 2015, pp. 33–75.
41. Slomian, S.; Gulvik, M.E.; Madej, G.; Austad, I. Gamasina and Microgyniina (Acari, Gamasida) from soil and tree hollows at two traditional farms in Sogn og Fjordane, Norway. *Norw. J. Entomol.* **2005,** *52*, 39-48.
42. Lindquist, E.E.; Krantz, G.W.; Walter, D.E. Order Mesostigmata. In *A Manual of Acarology*; 3rd ed.; Krantz, G.W.; Walter, D.E. (Eds.); Texas Tech University Press: Lubbock, Texas, 2009, pp. 124–232.
43. Halliday, R.B. *Alliphis siculus* (Oudemans 1905) is not a synonym of *Alliphis halleri* (G. & R. Canestrini 1881) (Acari: Eviphididae). *Syst. Appl. Acarol.* **2008**, *13*, 51-64.
44. Karg, W.; Grosse, E. Raubmilben als Antagonisten von Nematoden. *Nachrichtenblatt für den Pflanzenschutz in der DDR*. **1983**, 37,208–212.
45. Mathys, G.; Tencalla, Y. Note préliminaire sur la biologie et la valeur prédatice de *Proctolaelaps hypudaei* Oudms (Acarien: Mesostigmata: Aceosejidae). *Stations Fédérales D'Essais Agricoles.* **1959**, *600*, 645–654
46. Moraes, J.; Franklin, E.; Morais, J.W.; Souza, J.L.P. Species of edaphic mites (Acari: Oribatida) and effects of topography, soil properties and litter gradients on their qualitative and quantitative composition in 64 km^2 of forest in Amazonia. *Exp. Appl. Acarol*. **2011**, *55*, 39–63.
47. Overmeer, W.P.J. Diapause. In *Spider mites; their biology, natural enemies and control*; IB ed.; Helle, W.; Sabelis, M.W. (Eds.); Elsevier: Amsterdam, Netherlands, 1985, pp. 95–102.
48. Veerman, A. Diapause. In *Spider mites: their biology, natural enemies and control;* 1st ed.; Helle, W.; Sabelis, M.W.(Eds.). Elsevier: Amsterdam, Netherlands, 1985, pp. 270-316.
49. Laing, J.E.; Knop, N.F. Potential use of predaceous mites other than Phytoseiidae for biological control of orchard pests. In *Biological control of pests by mites, Special Publications;* Hoy, M.A.; Cunningham, G.L.; Knutson, L. (Eds). University of California: Berkeley, 1983, pp. 12–20.
50. Karg, W.; Edland, T. Neue Raubmilbenarten der Phytoseiidae Berlese, 1916. *Dtsch. Entomol. Z.* **1987,** *34*, 387–395.

51. Trandem, N.; Klingen, I.; Haukeland, S.; Moraes, G.J. The occurrence of two pest mites and three groups of biocontrol agents in organic and conventional strawberry fields (abstract only). *IOBC/wprs Bull*. **2011**, *70*, 96.
52. Knapp, M.; van Houten, Y.; van Baal, E.; Groot, T. Use of predatory mites in commercial biocontrol: current status and future prospects. *Acarologia.* **2018**, *58*, 72-82.
53. Meshkov, Y.I. Phytoseiid mites (Parasitiformes, Phytoseiidae) on main berry plantings. PhD. Thesis, Bolshije Vjazemi, in Russian, 1996.
54. Zaitsev, A.S.; Chauvat, M.; Pflug, A.; Wolters, V. Oribatid mite diversity and community dynamics in a spruce chronosequence. *Soil Biol. Biochem.* **2002,** *34*, 1919–1927.
55. Walter, D.E.; Hunt, H.W.; Elliott, E.T. Guilds or functional groups? An analysis of predatory arthropods from a shortgrass steppe soil. *Pedobiologia.* **1988**, *31*, 247–260.
56. Novotny, V.; Basset, Y.; Auga, J.; Boen, W.; Dal, C.; Drozd, P.; Kasbal, M.; Isua, B.; Kutil, R.; Manumbor, M. Predation risk for herbivorous insects on tropical vegetation: a search for enemy-free space and time. *Aust. J. Ecol.* **1999**, *24*, 477–483.
57. Saigusa, M.; Oishi, K.; Ikumoto, A.; Iwasaki, H.; Terajima, M. Emergence patterns of small subtidal arthropods in relation to day/night, tidal, and surface/bottom factors: investigations in the Boreal Sea, Japan (Akkeshi, Hokkaido). *J. Oceanogr.* **2000**, *56*, 295–310.

8. FINAL CONCLUSIONS

The main aspects of this work refer to the growing problems that strawberry crop has been facing in the main producing region in Brazil (southern Minas Gerais and neighboring region in São Paulo state), concerning the excessive use of pesticides and the use of non-biodegradable material (polyethylene film) for mulching. Within the scope of Integrated Pest Management, the objective of this work was to help to obtain the solution to those problems, focusing on the use of biological control of pest organisms with predatory mites, use of beneficial fungi that contribute to direct pest control of promote plant growth, and adequate crop management, including the use of organic cover (coffee husk and pulp).

The results of the study reported in Chapter 2 suggest that although *Drosophila suzukii* (Matsumura) has been found in the three evaluated fields in southern Minas Gerais (two in Bom Repouso and one in Senador Amaral), the levels of occurrence seem low, especially in cultivations done under low tunnels.

In Chapter 3, it was shown that some Gamasina mites have the ability to attack and oviposit when offered *D. suzukii* as prey. Predators as *Macrocheles embersoni* Azevedo et al. (Macrochelidae) and *Macrocheles robustulus* (Berlese) (both Macrochelidae), *Protogamasellopsis zaheri* Abo-Shnaf et al. (Rhodacaridae) and *Stratiolaelaps scimitus* (Womersley) (Laelapidae) showed promising results, and, therefore, should be further investigated as potential candidates for periodic releases.

In the Chapter 4, the monthly inoculation of the fungus *Metarhizium humberi* Luz et al. (Clavicipitaceae) by dripping the conidia suspension into strawberry growing substrate (through the irrigation system) was sufficiently effective to positively affect the plants. The introduced fungus was recovered from samples of planting substrate, root, petiole and leaflet of the inoculated plants. The inoculation resulted in reduced levels of the two-spotted spider mite, thrips and anthracnose and increased levels of predatory mite populations. Future work needs to investigate whether the results obtained here were only possible due to the monthly inoculation of beneficial fungi or whether the results would also be promising at a lower frequency of applications. In addition, it seems warranted to repeat this study under conditions of higher levels of pest incidence, under the hypothesis that greater productivity can be obtained from plants inoculated with the fungus.

Based in the results of Chapter 5, it was concluded that the highest number of predatory mites can be found in coffee husk and pulp at the beginning of the year, coincidentally the period of greatest strawberry planting in southern Minas Gerais. This is most probably due to the fact that the storage site is not hermetic, allowing access of mites from the surroundings remaining environmentally more adequate to the mites in that period. The density and composition of mites varied among the forest fragments studied. Therefore, further studies should be conducted to better understand the variation of Gamasina composition and densities in different fragments along the year, to determine the most appropriate combinations of sites and periods for eventual enrichment of coffee husk and pulp acquired commercially for use as mulching in strawberry fields.

The results obtained in Chapter 6 were very relevant and can contribute to a more sustainable cultural management of the strawberry crop. They suggested that: a) maintenance of coffee husk and pulp on forest fragment floor before using as mulching can promote the incorporation of local edaphic predatory mites (relatively more numerous in the forest floor) in the strawberry field; b) mulching with coffee husk and pulp promote the maintenance of a microclimate close to the strawberry plants more favorable for the development of predatory mites and less favorable for the development of the two-spotted spider mites; c) edaphic predatory mites, especially *Lasioseius* sp. (Blattisociidae) and *Proctolaelaps blickleyi* (Bram) (Melicharidae), may move to strawberry plants at nighttime. Hence, their preservation in the soil might be helpful in pest control; d) in addition to being advantageous by reducing pest problems, the use of coffee husk and pulp might result in the production of heavier and sweeter strawberries; e) at least three of the evaluated predator species (*Lasioseius* sp., *P. bickleyi* and *Proctolaelaps pygmaeus* (Müller) (Melicharidae) were able to attack and oviposit when offered two-spotted spider mite eggs as prey.

Finally, in the Chapter 7, our study suggested that the use of cereal mulching for strawberry productionnin Norway allows the maintenance of a larger number of predatory mites in the environment than would be possible without mulching. Further, it suggested that cereal straw maintained in the field longer (2 years) may host more predatory mites than cereal straw maintained for a shorter period (1 year). This wass suggested by the striking differences in numbers of Gamasina in the straw and in the underlying soil, although a precise numerical comparison cannot be made, given the different sample sizes in that study. The movement of certain Gamasina onto strawberry plants at night and the lower *T. urticae* numbers in straw treatments indicated that edaphic Gamasina prey on *T. urticae* and that the use of straw with Gamasina may be an interesting conservational biocontrol strategy, promoting the effectiveness of these organisms as biological control agents.

It is expected that the conduction of this work may contribute to strawberry production in Brazil, reducing pest problems through the use of alternative techniques involving biological control and environmental management.