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Sciarídeos em morangueiro semi-hidropônico no sul do Rio Grande do Sul: identificação e estratégias de manejo biológico e químico

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A tarefa não é tanto ver aquilo que ninguém viu, mas pensar o que ninguém ainda pensou sobre aquilo que todo mundo vê. (Arthur Schopenhauer)

Resumo

DUARTE, Adriane da Fonseca. **Sciarídeos em morangueiro semi-hidropônico no sul do Rio Grande do Sul: identificação e estratégias de manejo biológico e químico.** Orientador: Uemerson Silva da Cunha. 2020. 92f. Tese (Doutorado em Fitossanidade) – Programa de Pós-Graduação em Fitossanidade, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas, 2020.

A cultura do morangueiro tem se destacado, principalmente pela grande expressão econômica, uma vez que, geralmente, emprega mão-de-obra familiar. Considerando que grande parte do cultivo era realizado em túnel baixo, onde tem-se diversos problemas fitossanitários e ergonométricos, muitos produtores estão migrando para o cultivo fora do solo, também chamado de semi-hidropônico. Este sistema está em plena expansão no sul do país, porém, novos problemas podem surgir e precisam ser identificados. Insetos do grupo black fungus gnats, por exemplo, são comumente encontrados em cultivos de cogumelo, viveiros e ambiente protegido, onde tem-se condições ideais para seu desenvolvimento. Os danos ocasionados podem ser diretos, através da alimentação das larvas, e indiretos através da abertura de entradas para fungos patogênicos, além de que, larvas e adultos podem auxiliar na disseminação de patógenos. O objetivo principal deste trabalho foi identificar as espécies de black fungus gnats que ocorrem no cultivo de morangueiro em sistema semi-hidropônico no sul do Rio Grande do Sul, bem como avaliar o potencial de uso de duas espécies de ácaros predadores (*Cosmolaelaps brevistilis* e *Stratiolaelaps scimitus*) para o manejo de uma das espécies, além de avaliar a seletividade de alguns agrotóxicos utilizados na cultura do morangueiro sobre estes ácaros predadores. Ademais, visando mais alternativas para o manejo destes insetos, onze agrotóxicos registrados para o manejo de pragas na cultura do morangueiro ou culturas próximas foram avaliados quanto à eficiência de controle. Inicialmente quatro propriedades foram visitadas durante o ano de 2017, para coleta e identificação dos insetos. Na sequência, criações de laboratório foram estabelecidas para a condução dos demais experimentos. Como resultados, duas espécies de black fungus gnats foram encontradas, *Bradysia impatiens* (Johannsen, 1912) e *Bradysia ocellaris* (Comstock, 1882), correspondendo a 90,79 e 9,21%, respectivamente. Com relação à eficiência dos ácaros predadores, *S. scimitus* foi mais eficiente para o manejo de *B. ocellaris*, consumindo em média 8,25 ± 0,33 larvas de segundo e terceiro ínstar por fêmea/dia, enquanto *C. brevistilis* consumiu apenas 4,45 ± 0,13. Além disso, *S. scimitus* também foi menos afetado pelos principais agrotóxicos utilizados na cultura do morangueiro, uma vez que dentre os avaliados apenas lambda-cialotrina foi moderadamente perigoso (classe III) e os demais inofensivos (classe I), enquanto para *C. brevistilis* lambda-cialotrina foi classe III, spinetoram levemente perigoso (classe II) e os demais inofensivos (classe I). O controle de *B. ocellaris* com inseticidas, foi considerado eficiente apenas com acetamiprido e novalurom, sendo a Cl_{50} de 19,18 e 1,24 mg de ingrediente ativo.L^{-1}, respectivamente. Embora outros produtos tenham proporcionado uma mortalidade maior (86 e 96% para malatiom e tiametoxam respectivamente), acetamiprido e novalurom são os menos prejudiciais para organismos benéficos. No entanto, mais estudos são necessários, visando o uso de *S. scimitus* que não contamina o meio ambiente, o aplicador e não deixa resíduo no produto final, que geralmente é consumido *in natura,* mas também para avaliar a CL_{90} de acetamiprido e novalurom.

Palavras-chave: Black fungus gnats. *Bradysia* sp.. *Stratiolaelaps scimitus*. Controle biológico.Controle químico.

Abstract

DUARTE, Adriane da Fonseca. **Sciarids in semi-hydroponic strawberry in southern Rio Grande do Sul: identification, biological and chemical management strategies**. Advisor: Uemerson Silva da Cunha. 2020. 92f. Thesis (Doctor in Crop Protection) – Graduate Program in Crop Protection, Faculty of Agronomy Eliseu Maciel, Federal University of Pelotas, 2020.

The strawberry culture has highlighted, mainly due to its economic importance, since it generally, employs a familiar labor. Considering that most of the cultivation is carried out in low tunnels, where there are several phytosanitary and ergonomic problems, many producers are migrating to the cultivation outside the soil, also called semi-hydroponic system. This system is in full expansion in the south of the country, however new problems may arise and need to be identified. Insects of the black fungus gnats group are commonly found in mushroom crops, nurseries and protected environments, where ideal conditions for larval development are found. The damage caused can be direct, through the feeding of the larvae, and indirect through the opening of entrances for pathogenic fungi, besides that, larvae and adults can help in the spread of pathogens. The main objective of the work was to identify the species of black fungus gnats that occur in strawberry cultivation in a semi-hydroponic system in the south of Rio Grande do Sul, as well as to evaluate the potential use of two species of predatory mites (*Cosmolaelaps brevistilis* and *Stratiolaelaps scimitus*) for the management of one of the species, besides evaluating the selectivity of some pesticides used in strawberry cultivation over these predatory mites. Furthermore, aiming at more alternatives for the management of these insects, eleven pesticides registered for pest management in strawberry or similar crops were evaluated for control efficiency. Initially, four farms were visited during 2017, to collect and identify the insects. Following, laboratory colony were established to conduct the other experiments. As a result, two species of black fungus gnats were found *Bradysia impatiens* (Johannsen, 1912) and *Bradysia ocellaris* (Comstock, 1882), corresponding to 90.79 and 9.21%, respectively. Regarding the efficiency of predatory mites, *S. scimitus* was more efficient for the management of *B. ocellaris*, consuming an average of 8.25 ± 0.33 second and third instar larvae per female/day, while *C. brevistilis* consumed only 4.45 ± 0.13 larvae. Furthermore, *S. scimitus* was also less affected by the main pesticides used in strawberry cultivation, since among those evaluated, only lambda-cyhalothrin was moderately dangerous (class III) and the others harmless (class I), while for *C. brevistilis* lambda-cyhalothrin it was class III, slightly dangerous spinetoram (class II) and the other were harmless (class I). The control of *B. ocellaris* with insecticides was considered efficient only with acetamiprid and novaluron, with LC_{50} from 19.18 and 2.14 mg of active ingredient.L^{-1}, respectively. Although other products have provided highest mortality (86 and 96% for malathion and tiamethoxam respectively). acetamiprid and novaluron are the least harmful to beneficial organisms. However, further studies are needed, aiming at the use of *S. scimitus* that does not contaminate the environment, the applicator and leaves no residue in the final product, which is usually consumed *in natura*, but also to evaluate the LC_{90} of acetamiprid and novaluron.

Keywords: Fungus gnats. *Bradysia* sp.. *Stratiolaelaps scimitus*. Biological control. Chemical control.

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Sumário

1 Introdução Geral

O Brasil, apesar de possuir apenas 13,5% de sua população em áreas rurais, dispõe uma área agrícola de produção muito grande (FAOSTAT, 2019), a qual responde pela alimentação de inúmeras pessoas. Com uma das maiores taxas de produtividade do mundo, o país apresenta potencial para expandir sua área agrícola em 69 milhões de hectares até 2024 (OECD-FAO, 2015). Com essa área é possível incrementar em 136% a atual produção de grãos e fibras, por exemplo, que hoje é de cerca de 210 milhões de toneladas (MAPA, 2016).

Dentre a produção de pequenas frutas, o morangueiro (*Fragaria* x *ananassa*) tem se destacado por ser a espécie de maior expressão econômica, sendo produzido e apreciado nas mais variadas regiões do mundo (PIROVANI et al., 2015). A cultura enquadra-se perfeitamente no sistema de agricultura familiar, podendo ser cultivado em pequenas áreas e proporcionar rápido retorno do capital investido (BERNARDI et al., 2015) além de empregar mão-de-obra familiar (CECATTO et al., 2013).

No Brasil, a área plantada é de aproximadamente 4.000 hectares, com uma produção anual ultrapassando 105 mil toneladas (RESENDE, 2018), onde as maiores áreas de cultivos estão localizadas nos estados de Minas Gerais, São Paulo, Rio Grande do Sul e Paraná, sendo Minas Gerais o principal estado produtor, com cerca de 1.500 ha (CORRÊA ANTUNES; JUNIOR, 2007). Já o estado do Rio Grande do Sul é responsável por 29% da produtividade nacional (REISSER JUNIOR et al., 2014).

O consumo de morango tem aumentando dentre as pequenas frutas (berry), principalmente devido a composição nutricional, sendo uma excelente fonte de antioxidantes e, principalmente, devido ao seu alto teor de vitamina C e fenólicos (PINELI et al., 2011), necessitando assim, um aumento da produção para atender o mercado consumidor (RESENDE, 2018).

Embora grande parte do cultivo seja realizado em sistema de túneis baixos, onde problemas fitossanitários na parte aérea e no sistema radicular acabam se agravando com o decorrer do tempo, pois o cultivo sucessivo, na mesma área, favorece a incidência de pragas (ANDRIOLO; BONINI; BOEMO, 2002).

O cultivo fora do solo, também chamado de semi-hidropônico, tem seus primeiros registros no Rio Grande do Sul na região da serra gaúcha por volta do final da década de 1990 (GONÇALVES et al., 2016) e esta em plena expansão no sul do país, sendo uma das alternativas para reduzir problemas principalmente relacionados com organismos que se mantém no solo (RADIN et al., 2009), diminuindo a utilização de agrotóxicos aplicados na cultura (CECATTO et al., 2013).

Este sistema também permite a produção por um período maior, além de melhorar questões ergométricas de trabalho durante a manutenção e colheita (GONÇALVES et al., 2016), sendo sustentado pelas questões sócio-ambientais, porém não deixa o sistema totalmente imune, e problemas fitossanitários na cultura sempre podem surgir, exigindo constante acompanhamento (BERNARDI et al., 2012).

Dentre os principais problemas fitossanitários da cultura em sistema semi-hidropônico, destacam-se doenças causadas por *Xanthomonas fragariae, Verticillium* spp., *Phythophtora* spp., *Fusarium* spp., *Pythium* spp. e *Rhizoctonia* spp. (SANHUEZA; CALEGARIO, 2006). Dentre os artrópodes, a principal praga é o *Tetranychus urticae* (Koch, 1836) (ácaro-rajado) (MORAES; FLECHTMANN, 2008), seguido por brocas, besouros, lagartas, afídeos (KOVALESKI et al., 2006) e mais recentemente fungus gnats, as quais foram relatadas neste sistema (ALVES et al., 2016; DUARTE et al., 2018; RADIN et al., 2009) causando danos de grande importância na cultura, uma vez que, além de ocasionar danos diretos, larvas e adultos são importantes agentes disseminadores de doenças como *Phytium, Verticilium, Fusarium* e *Botrytis* (CLOYD, 2008; GARDINER; JARVIS; SHIPP, 1990).

A origem do termo fungus gnats é em função de que, larvas de dípteros deste grupo possuem como principal hábito alimentar a fungívoria, ou seja, alimentam-se de fungos (LEITE et al., 2007). Neste grupo, fungus gnats, diversas famílias são representadas como, Mycetophilidae, Keroplatidae, Sciaridae e algumas outras famílias de menor importância (SCHÜHLI et al., 2014), as quais possuem como característica em comum, o desenvolvimento em ambiente com presença de matéria orgânica (húmus) no solo, sendo um grupo onipresente em cultivo protegido (CLOYD, 2015).

Na superfamília Sciaroidea, as larvas ocorrem associadas com corpos de frutificação de fungos ou com micélios, (MOHRIG; MENZEL, 2009; SCHÜHLI, 2014), porém há também famílias que não são inteiramente fungívoras, ao exemplo de Sciaridae, também chamada de black fungus gnats, em que as larvas vivem no solo alimentando-se fungos e também raízes de plantas (HUNGERFORD, 1916), podendo minar as hastes e folhas de plantas herbáceas, principalmente em cultivos em ambiente protegido (CLOYD, 2008; LEITE et al., 2007; MOHRIG; MENZEL, 2009).

A família é constituída por 92 gêneros e 2455 espécies (PAPE; BLAGODEROV; MOSTOVSKI, 2011), as quais são encontradas nos mais variados ambientes e regiões do mundo, com membros adaptados a diversas condições climáticas (COLLESS; MCALPINE, 1991).

Dentro de Sciaridae, adultos de *Bradysia* Winnertz é separada morfologicamente dos demais gêneros pela presença de pente na tíbia I, ausência de macrosetas nas veias M e CuA, palpo com três segmentos e com fosseta sensorial (MOHRIG; MENZEL, 2009). Contudo a identificação de espécies dentro do gênero é difícil, visto que em alguns casos tem-se a possibilidade da existência de espécies crípticas, as quais são separadas apenas com análises moleculares (SHIN et al., 2015).

No gênero *Bradysia*, por exemplo, larvas foram encontradas alimentando-se de *Eucalyptus* (eucalipto) (AMORIM, 1992). Em cultivos de *Agaricus* sp. e *Lentinula* sp. (cogumelos) sete espécies diferentes foram encontradas, com predominância para duas espécies, *Lycoriella ingenua* (Dufour, 1839) *e Bradysia difformis* Frey 1948 (SHIN; LEE; LEE, 2012), as quais são relatadas também em cultivos de *Pinus montezumae* (pinus) e viveiros florestais, sendo que no México os danos ocasionados em plântulas de coníferas chegam a 30% (MARÍN-CRUZ et al., 2015).

Em viveiros (produção de mudas florestais, ornamentais, tabaco) assim como em cultivos protegidos, principalmente com substrato rico em matéria orgânica, larvas de black fungus gnats são importantes pragas (CLOYD, 2015; MOHRIG et al., 2012). No Japão, por exemplo, em cultivo de morangueiro sem solo, produzido em casa-de-vegetação, *Bradysia impatiens* (Johannsen, 1912) é considerada a principal praga de cultura (ARIMOTO et al., 2018). Na China *Bradysia odoriphaga* Yang & Zhang 1985, é considerada a principal praga da cebolinha (ZHANG et al., 2014).

Contudo no Brasil, a carência de especialistas em taxonomia aliada ao desconhecimento da bioecologia e ocorrência de insetos deste grupo (SCHÜHLI, 2014) indicam que mais estudos precisam ser realizados, principalmente para estabelecer um sistema de manejo deste grupo.

O ciclo biológico de *Bradysia* compreende as fases de ovo, larva, pupa e adulto (Fig. 1), o qual é completado num intervalo entre 17-43 dias (dependendo das condições ambientais e da espécie). O ciclo de *B. impatiens*, por exemplo, de acordo com Wilkinson & Daugherty (1970) em temperatura de 23,89ºC compreende a fase de ovo (4-5 dias), larva (8-24 dias), pupa (3-5 dias) e durante a fase adulta a longevidade varia de 2-9 dias (Fig. 1). Na fase adulta, as moscas deste gênero são pequenas, com cerca de 2 mm de comprimento, enquanto que as larvas são esbranquiçadas, alongadas e com a cabeça preta, a pupa é do tipo obtecta (TRIPLEHORN; JOHNSON, 2015) diferentemente da grande maioria dos dípteros que possuem pupas coarctadas.

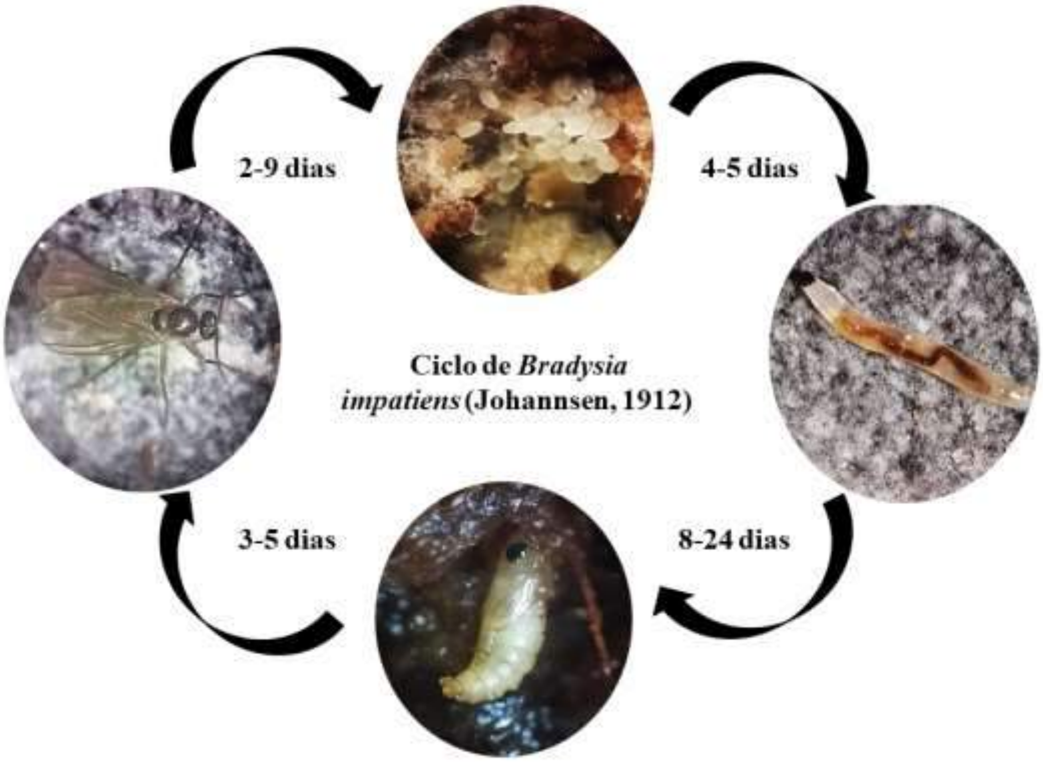

Figura 1. Ciclo biológico de *Bradysia impatiens* (Johannsen, 1912) com período aproximado de duração de cada fase de desenvolvimento, sob temperatura de aproximadamente 24ºC, adaptado de Wilkinson & Daugherty (1970). Fotos: Arquivo pessoal.

Os adultos preferem ambientes mais escuros, onde são observados realizando vôos baixos/rasteiros, uma vez que as fêmeas buscam por substratos ricos em umidade, atividade microbiana e matéria orgânica para realização da postura (CLOYD, 2008). A oviposição de algumas espécies é influenciada pela presença de determinadas espécies de fungo (CLOYD, 2015), assim como algumas espécies alimentam-se basicamente de fungos, enquanto outras utilizam os fungos do substrato como fonte primária de alimento e depois migram para as raízes de plantas (KENNEDY, 1974) ocasionando enormes perdas exigindo assim medidas de contenção das populações.

No Brasil, as espécies relatadas até o momento importunando os ambientes de produção são poucas, e convém destacar a ocorrência de *B. matogrossensis* (Lane), *B. ocellaris* (Comstock) e *B. difformis* Frey=*B. impatiens* (Johannsen), todas encontradas em cultivos de cogumelo (CASTILHO et al., 2009; MENZEL; SMITH; COLAUTO, 2003).

A ocorrência de *Bradysia* em plantas, já foi relatada em produção de mudas de *Coffea* (cafeeiro) (SOUZA et al., 2011) em cultivos de *Fragariae x* *ananassa* (morangueiro) (ALVES et al., 2016; DUARTE et al., 2018; RADIN et al., 2009) bem como em plântulas de *Citrus* (citros), *Nicotiana* (fumo), e ornamentais mantidas em viveiros (LEITE et al., 2007; TAVARES et al., 2012).

Na cultura do morangueiro no Brasil, como a ocorrência destes insetos é recente (RADIN et al., 2009), são necessários, primeiramente, estudos para identificar as espécies que ocorrem nos cultivos de importância econômica, devido à capacidade de ambas as fases (larval e adultos) transmitirem doenças, o nível de tolerância para a presença desta praga é muito baixo (CLOYD, 2015).

O controle destas espécies na grande maioria é realizado com a utilização de produtos químicos registrados nos países. Na Austrália, por exemplo, o inseticida fipronil é incorporado no substrato de produção de cogumelo desde 1996 para o controle de *B. ocellaris,* porém sua eficiência já é reduzida devido a problemas de resistência (SHAMSHAD, 2010).

No entanto, alguns inseticidas reguladores de crescimento como ciromazina demonstram-se bastante eficientes para o controle desta espécie (SHAMSHAD; CLIFT; MANSFIELD, 2009), assim como piriproxifeno afetou negativamente larvas de segundo e terceiro instar de *B.* sp nr. *coprophila* (CLOYD; DICKINSON, 2006). No grupo das benzoiluréias, triflumuron demostrou um bom controle de *B. ocellaris* em cultivos de cogumelo (SHAMSHAD; CLIFT; MANSFIELD, 2009), assim como novaluron foi eficiente para o controle de populações de *L. ingenua* via irrigação, em substrato com cogumelo (ERLER et al., 2011).

Dentre os microbiológicos, o uso de biopesticidas a base de bactérias entomopatogênicas principalmente *Bacillus thuringiensis* Berliner é estudado há muitos anos, sendo que a subespécie *israelensis* (Bti) foi eficiente no controle de *B. coprophila* (OSBORNE; BOUCIAS; LINDQUIST, 1985), porém em outro trabalho realizado por Cloyd & Dickinson (2006), ou autores concluíram que *Bti* não foi efetivo para o controle de larvas *B. coprophila* nas doses de $0,29 \times 10^6$ mg.L^{-1} e $1,15 \times 10^6$ mg.L^{-1} para segundo e terceiro instar respectivamente, necessitando assim de mais estudos.

Com relação aos macrobiológicos, espécies de ácaros predadores Laelapidae são amplamente utilizados para o controle de larvas em diversos países (FREIRE et al., 2007; SHAMSHAD, 2010). *Stratiolaelaps miles*

(Mesostigmata: Laelapidae), mencionado pelos autores como *Hypoaspis miles* (Berlese) por exemplo, reduziu significativamente a população de *L. ingenua* em cultivos de cogumelo (JESS; SCHWEIZER, 2009), enquanto *Stratiolaelaps scimitus* é amplamente utilizado para o controle de espécies de *Bradysia,* sendo efetivo no controle de *B. matogrossensis* em cogumelo no Brasil (CASTILHO et al., 2009). Contudo para *B. ocellaris* e *B. impatiens*, estudos com inseticidas químicos e principalmente com micro (Bts) e macrobiológicos (ácaros predadores) são escassos.

No Brasil tem-se uma carência muito grande de opções de produtos para o manejo de espécies deste grupo, o que pode também favorecer o surgimento de populações resistentes (CHEN et al., 2017; SHAMSHAD, 2010).

De acordo com o Ministério da Agricultura, Pecuária e Abastecimento, para o controle de *B. ocellaris* não há produto registrado, enquanto para *B. impatiens* há apenas um produto químico disponível, pertencente ao grupo dos tetranortriterpenoides, e para *B. matrogrossensis* existe um produto a base de ácaros predadores da espécie *Stratiolaelaps scimitus* (BRASIL, 2019), o qual pode ser utilizado com eficiência em todos os cultivos em que *B. matogrossensis* for encontrada, inclusive em cultivos orgânicos (BRASIL, 2015).

Este produto a base de *S. scimitus,* assim como alguns inseticidas, principalmente os reguladores de crescimento, podem ser ferramentas úteis para o manejo das espécies de *Bradysia* que forem encontradas em morangueiro semi-hidropônico, porém são necessários estudos iniciais para conhecer as espécies de Sciaridae.

O objetivo deste trabalho foi, inicialmente, identificar a(s) espécie(s) de Sciaridae que ocorre(m) na cultura do morangueiro em sistema semi-hidropônico. Na sequência, testar algumas alternativas de controle como ácaros predadores e moléculas químicas, de modo a servir de suporte para o manejo das espécies encontradas. Por fim, verificar a compatibilidade de uso destes agentes de controle biológico (ácaros), com os produtos químicos (inseticidas e fungicidas) utilizados no manejo da cultura do morangueiro em sistema semi-hidropônico.

ARTIGO 1- Revista Brasileira de Entomologia

A new problem in semi-hydroponic strawberry crops in Brazil: Black fungus gnats (Diptera: Sciaridae)

Um novo problema na produção de morango semi-hidropônico no Brasil: Fungus gnats (Diptera: Sciaridae)

ADRIANE DA FONSECA DUARTE; JULIANO LESSA PINTO DUARTE; PATRICIA DA SILVA GRINBERG; UEMERSON SILVA DA CUNHA

2 Artigo I – A new problem in semi-hydroponic strawberry crops in Brazil: Black fungus gnats (Diptera: Sciaridae)

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Abstract

Soilless cultivation of strawberry is in expansion in Brazil due to factors like the reduction of phytosanitary problems and the possibility of extending the crop period, as well as the ergonometric facility for management of the culture. However, black fungus gnats (Sciaridae, Diptera) are ubiquitous pests in the greenhouse production system, causing direct and indirect damage to plants. The aim this study was to evaluate the occurrence of black fungus gnats species in semi-hydroponic cultivation of strawberry and to identify the species, as well as to describe the symptoms in infested plants. The work was developed in commercial productions of strawberry, in five municipalities (Arroio do Padre, Canguçu, Capão do Leão, Pelotas and Turuçu) during July to December 2017, where adult samples were collected twice a month, with petri dish traps containing water and neutral detergent placed inside the greenhouses, between the plants. A total of 2030 adult gnats belonging to two species were collected. *Bradysia* aff. *impatiens,* accounted with 90.79% of the total, and *Bradysia* aff. *ocellaris* accounted with 9.21%. In order to assist the correct determination of species, males of both species were identified and illustrated. With respect to the symptoms observed during the work, it was possible to verify since the wilted crown of plants to the death of plants, which were confirmed by the presence of larvae in the root system. As the occurrence of these insects is recent in strawberry, there is no pesticide registered in Brazil, so management strategies (cultural, physical, and sanitary) are discussed.

Key words: *Bradysia* aff. *impatiens*, *Bradysia* aff. *ocellaris*, management, identification.

Introduction

Strawberry planted area in Brazil reaches about 4.000 hectares and has an estimated production of more than 105 thousand tons per year (Resende, 2018). Although the country is not one of the top ten strawberry producers in the world, the culture has significant social and economic importance, aggregating family labor (Cecatto et al. 2013). The fruit has great nutritional appeal due to its functional properties as a good source of antioxidants, mainly given to its high vitamin C and phenolic contents (Pineli et al. 2011). In order to attend the consumer local market, it is necessary to increase production and sustainably produce, what improves consumer acceptance.

Strawberry may be cultivated in soil or soilless systems (semi-hydroponic system), being cultivated mainly in the soil by Brazilian producers. In this production system the susceptibility of most strawberry cultivars to soil-borne pathogens is high (Jafarnia et al. 2010; Palencia et al. 2016), what increases the excessive use of pesticides by producers (Cecatto et al. 2013). In the soilless systems, the support base for the substrate may be in tubular plastic packaging, fiber cement tiles, PVC gutters, or wood, where the substrates (organic, wooded rice husk, sand or coconut fiber) are placed free of soil pathogens (Gonçalves et al. 2016) besides providing a suitable microclimate for plants.

Therefore, soilless cultivation systems is an alternative to minimize these problems because the plants are cultivate inside greenhouses, protected from wind, hail, rain, frost and low temperatures. This system also reduces the prevalence of pests and diseases, and allows out of season production, increasing yield (Cecatto et al. 2013; Jafarnia et al. 2010). Nevertheless, this system is still subject to different issues that can affect the production.

In greenhouse crop-production systems, black fungus gnats (Diptera: Sciaridae) are ubiquitous pests (Braun et al. 2012). The small gnats of this family are extremely widespread and its species are adapted to a wide variety of climates (Colless and McAlpine 1991). These small flies are considered the major insect pests of greenhouse production systems (Cloyd 2015), since they cause direct (larval feeding) and indirect damage through the spread of fungi propagules such as *Fusarium*, *Phytium* and *Thielaviopsis* (Gardiner et al. 1990; El-Hamalawi 2008). Its larvae feed on a wide-range of horticultural crops such as tomatoes, sweet peppers, lettuces, onions and ornamental flowers (San-blas et al. 2017), *Eucalyptus* nurseries (Santos et al. 2012) besides to be the main pest in mushroom crops, nurseries and greenhouse rich in organic matter and moisture (Menzel et al. 2003; Cloyd 2008).

Mentioned in semi-hydroponic strawberry cultivation in Brazil, *Bradysia* sp. demonstrate to be an important pest in this system, since occasioned the dead plants (19.5% of plants) during forty five days (Radin et al. 2009), however the authors did not identify the species for Brazil. The correct identification of the species is extremely important to assist in the development of research on biology, ecology and control of the gnats.

There is no systematic survey of the strawberry sciarid pest species for the Neotropical region and literature on these species is scarce. More studies are needed in order to understand its biodiversity, mainly for economically important species. Therefore, the aim of this study was to identify the species of the black fungus gnats in semi-hydroponic strawberry cultivation, as well as to describe the symptoms showed by infested plants. Moreover, management practices are discussed in order to provide control measures to producers.

Material and Methods

I. Study sites

This study was carried out in strawberry commercial production facilities, located in five municipalities (Table 1) during five months (July to December 2017). Strawberry was planted in plastic bags filled with a mixture of burnt rice peels and commercial substrate and cultivated inside greenhouses. All others management practices (irrigation, fertilization and integrated pest management) were carried out in accordance with the technical recommendations and requirements of the culture.

Table 1. Information about the collection sites (municipality, geographical coordinates, strawberry cultivar and production system) of black fungus gnats in Rio Grande do Sul state

Municipality	Geographical coordinates	Cultivar
Arroio do Padre	31º29’15.1”S, 52º21’39.9”W	Albion; San Andreas
Canguçu	31º27’57.0”S, 52º35’58.0”W	Albion; San Andreas
Capão do Leão	31º48'10.8”S, 52º25'06.24”W	Aromas; Camarosa
Pelotas	31º38’57.7”S, 52º22’39.0”W	San Andreas
Turuçu	31º26’29.6”S, 52º13’31.0”W	Camino Real

II. Collection of black fungus gnats

Adult samples were collected inside the greenhouses twice a month. Petri dish traps containing water and neutral detergent, were placed amongst the plants in order to capture the gnats. The traps were removed 48 hours after the installation and sent to the Laboratory of Acarology (LabAcaro) at Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas (FAEM/UFPEL), Pelotas, Rio Grande do Sul state, where initially the black fungus gnats adults were examined and counted with the help of a stereoscopic microscope (40x) and then preserved in 70% alcohol.

III. Species determination

Male specimens were kept for approximately 120 minutes in a solution of 10% KOH for clarification, and then dissected and mounted as permanent slides using Hoyer's medium, (distiled water 40ml, glicerin 20ml, cloral hidrate 200g and arabic gome 30g) according to Moraes and Flechtmann (2008). Slides were kept in an incubator at 50°C for ten days in order to dry out and clarify the specimens. Species determination was done under phase contrast microscope following Lane (1959), Menzel et al. (2003), and Mohrig et al. (2012). Taxonomically relevant structures were photographed using a camera attached to the microscope. Photographs of males' hypoppygia were then enhanced by drawing over the images to highlight important morphological characters using Adobe Illustrator software.

Some specimens were photographed before clarification, in order to compare important taxonomic characteristics of the species found. Voucher specimens are deposited in the entomological collection Padre Jesus Santiago Moure, Departamento de Zoologia, Universidade Federal do Paraná (DZUP).

Results

A total of 2030 adults of sciarid gnats were collected in the strawberry production sites. All individuals belonged to *Bradysia* genus, with the species *Bradysia* aff. *impatiens* (Johannsen, 1912) accounting with 90.79% and *Bradysia* aff. *ocellaris* (Comstock, 1882) with only 9.21% (Table 2). In this work, *B.* aff. *ocellaris* was found in high quantity only in the municipality of Capão do Leão, accounting with 184 specimens collected (9.06% of the total). In the other collection sites, this species was absent or very rare, accounting with 0.00-0.10 percent of total (Table 2).

Tabela 2. Total number and proportion (%) in parenthesis of *Bradysia* aff. *impatiens* (Johannsen, 1912) and *Bradysia* aff. *ocellaris* (Comstock, 1882) found in strawberry production facilities

Municipality	*Bradysia* aff. *impatiens*		*Bradysia* aff. *ocellaris*		Total
	male	female	male	female	
Arroio do Padre	83 (4.09)	38 (1.87)	1 (0.05)	0 (0.00)	122 (6.01)
Canguçu	559 (27.54)	48 (2.36)	0 (0.00)	0 (0.00)	607 (29.90)
Capão do Leão	711 (35.02)	179 (8.82)	161 (7.93)	23 (1.13)	1074 (52.90)
Pelotas	40 (1.97)	40 (1.97)	2 (0.10)	0 (0.00)	82 (4.04)
Turuçu	114 (5.62)	31 (1.53)	0 (0.00)	0 (0.00)	145 (7.15)
Total	1507 (74.24)	336 (16.55)	164 (8.08)	23 (1.13)	2030 (100)

Symptoms of sciarid infestation included the wilting of the plants crown (Fig 1A), drying of the leaves edges (Fig 1B), wilting plants (Fig 1C) and death of the plants (Fig 1D).

Figure 1. Symptoms in strawberry plants due to the presence of *Bradysia* aff. *impatiens* (Johannsen, 1912) and *Bradysia* aff. *ocellaris* (Comstock, 1882) larvae. A: wilted plants crown; B: drying of the leaves edges; C: wilting plants; D: dead plants.

The two species of *Bradysia* found in strawberry plants in this study are very morphologically similar, but we provide the morphological characteristic that distinguishes both species, and these characteristics are illustrated in Figures 2 and 3.

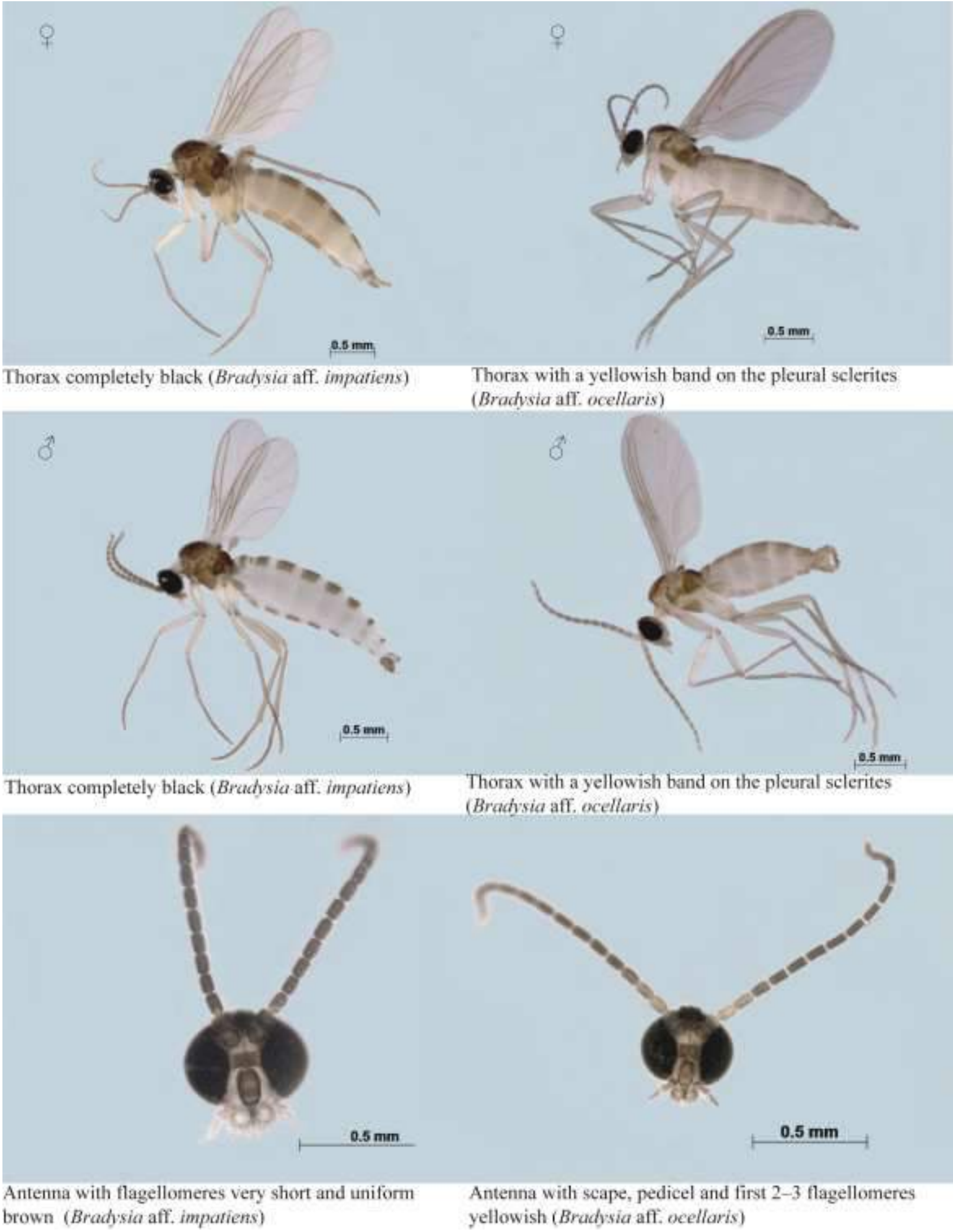

Figure 2. Morphological characters that distinguish *Bradysia* aff. *impatiens* (Johannsen, 1912) and *Bradysia* aff. *ocellaris* (Comstock, 1882) found on strawberry cultivation facilities in Brazil..

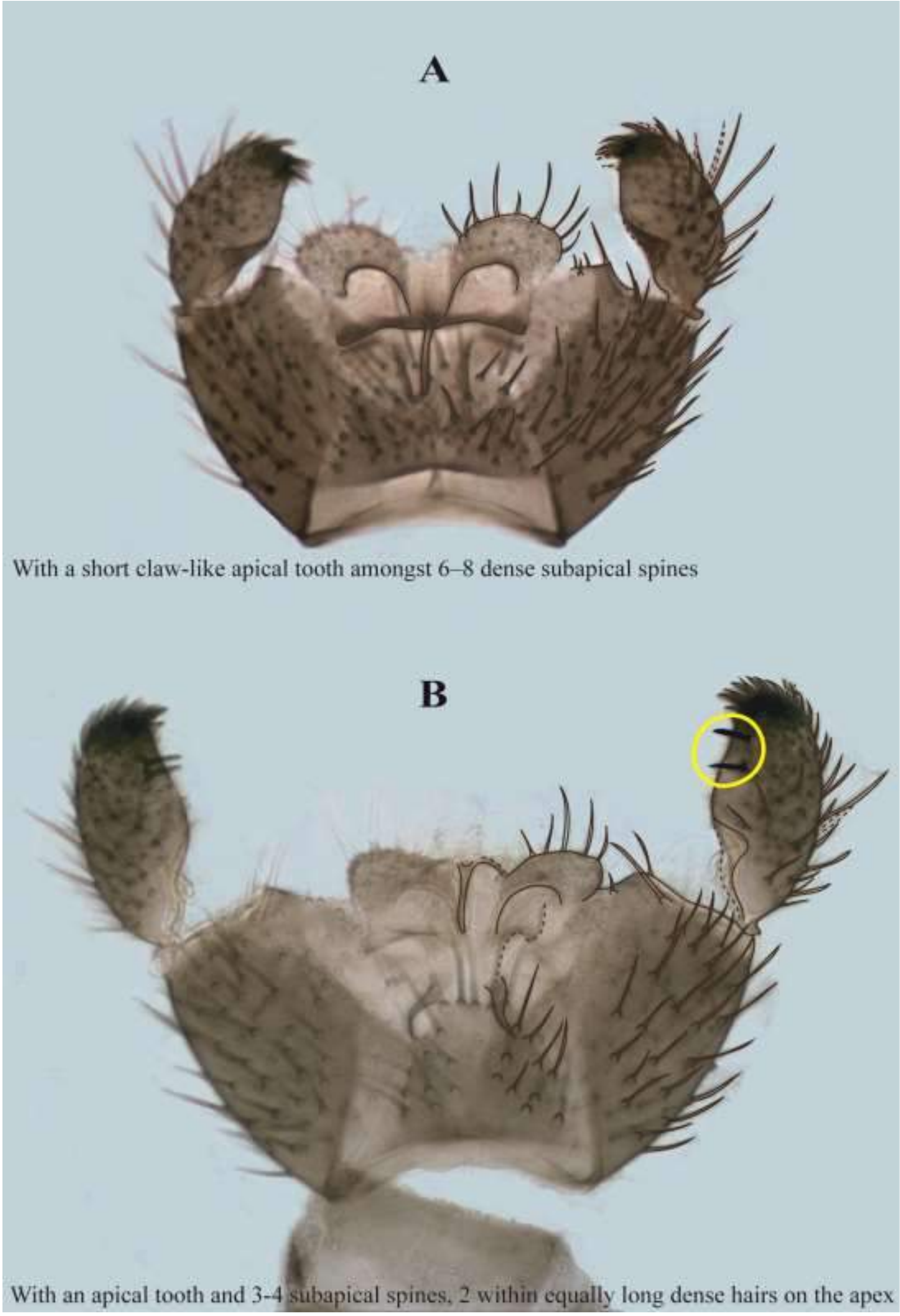

Figure 3. Hypopygium of *Bradysia* aff. *impatiens* (Johannsen, 1912) (A) and *Bradysia* aff. *ocellaris* (Comstock, 1882) (B) found on strawberry cultivation facilities in Brazil.

Discussion

Sciaridae gnats are very common in greenhouses, nurseries, others environments with high level of organic matter and mushroom farms, since sciarid larvae are major primary decomposers of plant debris (Mohrig et al. 2012). Thus, some *Bradysia* species are major insect pests in commercial greenhouses, especially during the propagation phase, when the plant root systems are starting to develop (Cloyd and Zaborski, 2004), as well as after the transplantation of seedlings. When the plants are still not well established, the root system is more susceptible to damage, so it is very important to reduce the population of sciarid gnats at this stage, strengthening the need to implement integrated pest management, through the monitoring with yellow adhesive traps.

Although symptoms of sciarid infestation are commonly observed in protect crops in Brazil, studies for species identification have not been performed. Radin et al. (2009) made the only study about semi-hydroponic strawberry cultivation regarding sciarids, but the authors did not identify the species of *Bradysia* they collected. Therefore, this is the first paper with species identification in semi-hydroponic strawberry cultivation. The species documented here were reported for the first time in Brazil in 2003, on cultivated mushroom *Agaricus blazei* (Menzel et al. 2003). However, in cultivated crops, this is the first record for Brazil. This is probably because the cultivation of strawberry inside greenhouses, with semi-hydroponic system, is relatively recent in the country. Also, farmers do not associate plant damage with the occurrence of gnats.

The correct identification of these species in strawberry is extremely important for management, because *B. impatiens* is a common greenhouse crop pest that causes economic losses worldwide (Cloyd, 2008) and was already reported in strawberry plants in other countries such Japan and Australia (Arimoto et al. 2018; Broadley et al. 2018). Among the sciarid species collected in cultivated plants inside greenhouses in Australia, *B.*

impatiens was the most frequently encountered species, corresponding to 62.6% of total (Broadley et al. 2018).

In greenhouse strawberry plants in Japan, for example, this species is considered the most important pest of the genus, because its larvae are known to damage the crowns of plants during feeding (Arimoto et al. 2018). Also, adults can contribute to the introduction and rapid spread of certain pathogens, because it can easily adhered to the flying adult insects (El-Hamalawi, 2008).

B. impatiens is a potential vector of *Fusarium* sp. to strawberry plants (Nam et al. 2017), therefore, it is very important to reduce the population of sciarid gnats inside greenhouses (Gorska-Drabik et al. 2011). Furthermore, the correct determination of the gnat species is essential to the proper management.

The two species found in this study, *B.* aff. *impatiens* and *B.* aff. *ocellaris*, are morphologically very similar (Menzel et al. 2003). The main characteristics that distinguish this two species are mentioned in table 3. Nevertheless, the management practices are similar for both species, although there are no registered pesticides for them in Brazil (Brasil, 2019). Therefore, it is very important to implement alternative management strategies that minimize damage and reduce the insect population, such as cultural, physical, and sanitation measures (Cloyd, 2015).

Humid and dark environments, rich in organic matter are ideal conditions for the development of their food source, since larvae of these insects feed on fungi, algae and decaying organic matter (Aguilera and Ortega 1996; Mansilla and Pastoriza 2001). So, management strategies that may be adopted to reduce the establishment of larvae are: installation of the greenhouses away from sources rich in organic matter and with good ventilation; maintenance of a clean environment inside the greenhouse, in order to avoid the establishment of fungi and algae; proper irrigation management, avoiding excess moisture in the substrate; and constant monitoring with yellow adhesive traps. Therefore, it is necessary to reduce these factors that contribute to the development of black fungus gnats.

Conclusions

According with morphological identification, two species of *Bradysia* were collected on semi-hydroponic strawberry cultivation, being *Bradysia* aff. *impatiens* more abundant than *Bradysia* aff. *ocellaris*.

The symptoms observed for sciarid infestation were the drying of the leaves edges, wilting and death of the plants.

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ARTIGO 2 – Experimental and Applied Acarology

Predatory mites as an alternative for the management of *Bradysia ocellaris* (Diptera: Sciaridae)

Ácaros predadores como uma alternativa para o manejo de *Bradysia ocellaris* (Diptera: Sciaridae)

ADRIANE DA FONSECA DUARTE; PRISCILLA COSTA GOBBI; LUCAS RAPHAEL DA SILVA; JULIANO LESSA PINTO DUARTE; UEMERSON SILVA DA CUNHA

3 Artigo II – Predatory mites as an alternative for the management of *Bradysia ocellaris* (Diptera: Sciaridae)

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Abstract

Bradysia species are the major insect pests of protected production systems, including strawberry crops in semi-hydroponic system. In Brazil, the species found in strawberry crops at moment were *Bradysia impatiens* and *Bradysia ocellaris* and they are management is difficult due to the absence of registered pesticides. The aim of this study was to evaluate the predatory efficiency of two species the mites [*Stratiolaelaps scimitus* (Womersley) and *Cosmolaelaps brevistilis* Karg] in laboratory conditions. The experiments were conducted at 25 ± 1ºC, 75 ± 3% RH, in the dark. Initially, a bioassay was performed in order to evaluate the predation potential of each species of mite on eggs, larvae (2-3 instar) and pupae of *B. ocellaris*. One adult female was used in each experimental unit, with thirty replicates, on which the predation rate, oviposition, and survivorship were evaluated for ten days. The second bioassay was performed in order to evaluate the efficiency of different densities of each predator species (2, 5, 7 and 10) with ten larvae of *B. ocellaris* in each experimental unit and eight replicates, being evaluated the emergence rate of flies. *Stratiolaelaps scimitus* was better than *C. brevistilis* in both bioassays, with the highest average predation rate of the 8.25 larvae/female/day, while in *C. brevistilis* it was of 4.45. Regarding to the different densities, the maximum predatory efficiency was 6.81 mite density for *S. scimitus*, while it was impossible to calculate it to *C. brevistilis* because difference between the densities was not significant.

Key words: *Stratiolaelaps scimitus*; *Cosmolaelaps brevistilis*; Strawberry; Fungus gnats

Introduction

Predatory mites are used in pest management for many years. The most studied family of predatory mites is Phytoseiidae, which is commercially available (Carrillo et al. 2015), and widely used for the control of phytophagous mites and small insects inhabiting the aerial parts of plants (Gerson et al. 2003). However, for the control of edaphic pest or pests that spend part of their life in the soil, the most studied family is Laelapidae (Acari: Mesostigmata) (Freire et al. 2007; Castilho et al. 2009; Moreira and Moraes 2015).

In this family, *Stratiolaelaps scimitus* (Womersley) has been widely used for the control of fungus gnats larvae in different protected crops in Europe (Freire et al. 2007). In Brazil, this predatory mite is registered as a commercial biological control agent for the control of *Bradysia matogrossensis* (Lane) larvae, a species commonly found in mushroom crops and nurseries (Brasil, 2019). However, in the recent years, other mite species, such as *Cosmolaelaps paulista* (Freire and Moraes) and *Cosmolaelaps brevistilis* (Karg) were found in Brazilian soil and have been studied for the control of edaphic pests, mainly larvae of Sciaridae flies, including *B. matogrossensis* (Freire and Moraes 2007; Duarte et al. 2018).

Commonly known as black fungus gnats, Sciaridae is the main family found in mushrooms, nurseries, and greenhouses with horticultural crops, rich in organic matter and moisture (Cloyd 2008; Shamshad 2010; Broadley et al. 2018). Within the genus *Bradysia* are the major species pests of protected production systems, feeding on a wide-range of horticultural crops (Cloyd 2015), including strawberry crops in the semi-hydroponic system. In Japan, *B. impatiens* (Johannsen) is the most important pest of the strawberry plants in greenhouses, due to the damage caused by larvae to the crowns of the plants (Arimoto et al. 2018). Morphologically very similar to *B. impatiens* is *B. ocellaris* (Comstock) (Menzel et al. 2003), which are found together in mushroom crops in Brazil (Menzel et al. 2003) and also in strawberry crops (Duarte et al. unpublished data) causing significant losses in production.

The management of this species in Brazil is very difficult due to the absence of pesticides registered in the Ministry of Agriculture, Livestock and Supply (Brasil, 2019). Therefore, the aim of this study was to evaluate under laboratory condition the potential of *S. scimitus* and *C. brevistilis* as agents of biological control of eggs, larvae, and pupae of *B. ocellaris*, besides defining the best density of *Stratiolaelaps scimitus*.

Material and Methods

This work was conducted in the Agricultural Acarology Laboratory (LabAcaro) of the Federal University of Pelotas, where the stock colonies of the predatory mites and *B. ocellaris* were maintained under environment conditions (20–27 °C and 50–90% RH). The experimental units were maintained in a rearing chamber at 25 ± 1 °C and 75 ± 3% RH, in the dark. All bioassays were conducted in a completely randomized design.

Stock colonies of Bradysia ocellaris

Adults and larvae of *B. ocellaris* were collected in a greenhouse with strawberry cultivation of the Aromas cultivar (31º48'10.8"S, 52º25'06.24"W), Capão do Leão. The insects larvae were bred in plastic containers (9.5 cm diameter, 6 cm high and capacity of 250mL) containing a mixture of pieces of potato, ground black beans and moist peat boss, which were kept in plastic cages (20 x 30 x 20cm; height, width and depth) and moistened once a week. For all bioassays, second and third instar larvae were collected with a brush.

Stock colonies of predatory mites

The predatory mites *C. brevistilis* and *S. scimitus*, have been kept in the LabAcaro since 2014, and are maintained in plastic containers (9.5 cm diameter, 6 cm high and capacity of 250mL), whose base was covered with a 3.0 cm thick layer of a solidified paste (9:1 of gypsum and activated charcoal, respectively). Colonies were maintained moist by addition of distilled water every two days, and fed ad libitum with a mixture of all stages of bacteriophagous nematode *Rhabditella axei* (Rhabditina: Rhabditidae), reared in the laboratory on pieces of rotting bean pods. For all studies, adult females with up to 96h, of each species of predator mites were used.

Experimental procedure

Two bioassay were conducted. The first one was performed in order to evaluate the predation potential of each species of mite on eggs, larvae (2-3instar), pupae of *B. ocellaris*, nematode (control) or mix of prey (larvae more nematode). The second bioassay was performed in order to evaluate the efficiency of each predator species in different densities.

Capacity of predation

The capacity of predation of each species of mite was evaluated, and for this, each experimental unit consisted of a transparent plastic Petri dish (2.7 cm diameter, 1.2 cm high), whose base was filled with a 0.5 cm thick layer of a solidified paste consisting of a mixture of gypsum and activated charcoal, maintained moist by daily addition of distilled water. The treatments were: T1- the nematode *Rhabditella axei* was used as control treatment, T2- 10 *B. ocellaris* eggs, T3- 10 *B. ocellaris* larvae, T4- 5 pupae of *B. ocellaris* and T5- a mixture of nematodes and 10 larvae. Thirty replicates were assembled for each treatment. One adult female predator were used in each experimental unit. The top end of each unit was sealed with a transparent plastic film (Magipac®) to prevent the organisms from escaping.

Evaluations were conducted during 10 days and the following parameters were evaluated: predation rate (daily number of prey consumed), oviposition, and survivorship of the predator. At each evaluation, consumed preys were replaced and predator eggs were discarded. The data of treatments (T3 and T5) obtained with *S. scimitus* have been published in previous works by Duarte et al. (2017) with only twenty replicates. Therefore, in this work, the number of replicates were increased for thirty, and they were used in this paper to compare with the efficiency of *C. brevistilis.*

Density of mites

The experiment was conducted in a bifactorial screening, where in the factor A was assigned the mite species (*C. brevistilis* and *S. scimitus*) and factor B the density of mites (0, 2, 5, 7 and 10). The experimental units consisted of a transparent plastic cup (9.5 cm diameter, 6 cm high and capacity of 250mL), whose base was covered with 20g of commercial substrate (MecPlant), 1g of ground black beans and 2mL of distilled water. Ten larvae were used in each experimental unit. Mites were released immediately after the release of the larvae. For each treatment eight replicates were used. The top end of each unit was sealed with the cap, on which a yellow adhesive trap (2.5 x 2.5 cm) has been set to monitor the emergence of adult flies. The evaluations were conducted during 15 days after the start of the flies' emergence, and the evaluated parameter was the number of emerged fly.

Statistical analysis

The normality and homoscedasticity of the data were evaluated by Shapiro–Wilk and Bartlett tests, respectively. For the first bioassay, as data did not fit the assumptions for parametric analyses, they were compared by non-parametric tests, using the Kruskal–Wallis test followed by Dunn's multiple comparison and Mann–Whitney test for comparison between two means. Comparison of survivorship was done by Fisher test ($p < 0.05$). For the second bioassay, after verification of the assumptions, the data followed parametric analyzes, the mites type treatment factor was compared by t-test ($p < 0.05$) and for the density factor regression models were applied ($p < 0.05$), adjusting quadratic equation, as follows: $y = y_0 + ax + bx^2$. Where: y = mites efficiency; x = density; and a, y_0 and b are estimated parameters of the equation; a is the difference between the maximum and minimum points of the variable; y_0 is the mites efficiency corresponding to the minimum or maximum point of the curve; and b, the slope of the curve. Model selection was based on low residue, low p-value, and high R^2 and R^2 adj. All these analyses were performed using the statistical software "R", version 3.4.1 (R Development Core Team 2018).

Results

Capacity of predation

For *C. brevistilis*, significant differences were observed in the predation rate (Kruskal–Wallis: $\chi 2 = 56.963$, df = 3, $p = 2.818 \times 10^{-12}$) with 4.45 larvae consumption per day, which did not differ from the treatment with the mix of two preys (larvae and *Rhabditella axei*) with 3.75 larvae consumption per day ($p = 0.16$). In the treatments with eggs and pupae, the consumption was lower, with 1.40 and 0.05 pupae/female/day respectively, with a significant difference between them ($p \leq 0.0006$) (Table 1).

The oviposition rate also presented significant differences amongst the treatments (Kruskal–Wallis: $\chi2 = 33.253$, df = 4, p = 1.06 x 10^{-6}). The highest oviposition rate was in the treatment with *Rhabditella axei* (0.95 egg/female/day), not differing significantly from the treatment in which the mix of two prey (*Rhabditella axei* and *B. ocellaris* larvae) was offered (p = 0.23). The lowest oviposition rate was in the treatment with pupae (0.23egg/female/day) which did not significantly differ from treatments with larvae and eggs ($p \geq 0.09$). When only larvae of *B. ocellaris* was offered, the oviposition rate was less than half of the control treatment, significantly differing (p = 0.0016) (Table 1). The survivorship of *C. brevistilis* ranged from 80.00-96.66% and, did not differ significantly from each other ($p \geq 0.05$) (Table 1).

Similar to *C. brevistilis*, the consumption of prey by *S. scimitus* was higher in the treatments with larvae and in the mix of the two preys, did not differing significantly from one another (p = 0.2824). Nevertheless, differences were observed in the others treatments ($\chi2 = 64.04$, df = 3, p = 8.048 x 10^{-14}), with a very low consumption rate (Table 1). *Stratiolaelaps scimitus* oviposited in all treatments, however significant differences were observed ($\chi2 = 41.317$, df = 4, p = 2.31 x 10^{-8}). The highest oviposition rate was verified in the treatments with the presence of *B. ocellaris* larvae (1.98-1.25 eggs/female/day) and the lowest one in the treatment with pupae (0.14egg/female/day), significantly differing from all treatments ($p \leq 0.003$) except for the eggs (p = 0.2322). The survivorship ranged from 80.00 to 93.33% and no significant differences were observed ($p \geq 0.05$).

The comparisons of the consumption rate of each prey between the predators did not differ only in the treatment with pupae of *B. ocellaris* (p = 0.1463), while in the other treatments, *S. scimitus* always demonstrate higher predation capacity than *C. brevistilis*, with the number of prey killed/day approximately twice higher (Table 1). The oviposition rate differed significantly on the treatments with larvae (p = 0.01649) and the mix of two prey (p = 2.066 x 10^{-6}). In the other treatment, the predation rate and oviposition was also higher for *S. scimitus* specie (Table 1) but did not significantly differ amongst the predatory mites ($p \geq 0.09$). Survivorship did not differ significantly amongst both predatory mites species ($p \geq 0.2538$).

Density of mites

When comparing the factor A (species of mites), the efficiency of *S. scimitus* were higher than *C. brevistilis*, and significantly differences were observed in all densities ($p \leq 2.08$ x 10^{-5}). The highest difference was observed when seven mites were released (p = 3.524 x 10^{-7}), with the mortality of larvae of *B. ocellaris* of 90% for *S. scimitus* and 21.25% for *C. brevistilis* (Figure 1). On the other hand, the smaller difference was observed in the treatment with the release of five mites ($p \leq 2.08$ x 10^{-5}) with mortality of 77.50 and 22.50% for *S. scimitus* and *C. brevistilis,* respectively. Yet, this difference was more than double.

Regarding the release of different densities of each mite species, no difference was observed for *C. brevistilis* ($p \geq 0.05$). The mortality ranged from 18.75 to 22.5%, therefore, a regression analysis was not performed , since the mortality rate was similar to the control in all predator densities.

The mortality rate (%) of *B. ocellaris* larvae in the tested densities of *S. scimitus* was significantly different (p = 2.644 x 10^{-5}) (Figure 2). The value of maximum efficiency was 6.81 mites. Considering that between the densities of 2 to 10 mites there was no significant differences (p = 0.58) and that an adult female of *S. scimitus* has a predatory capacity of 7.57 to 8.25 *B. ocellaris* larvae/day, with mixture of two prey or *B. ocellaris* larvae alone, respectively (Table 1), the release of only two mites was considered efficient in this volume of substrate (20g) and density of larvae.

Discussion

The biological control of black fungus gnats with predatory mites is widely studied in many countries (Cabrera et al. 2005) and there are species commercially available for use in it is pest management. In Brazil, *S. scimitus* was found in pots containing plants of *Canavalia ensiformis* (L.) infested by *Bradysia matogrossensis* (Lane) in São Paulo state (Freire et al. 2007), and provided effective control of a Brazilian population of *B. matogrossensis* in commercial scale experiments (Castilho et al. 2009). This mite is currently the only registered control measure for this pest in the country (Brasil, 2019). However, other species of black fungus gnats are found in greenhouse strawberry crops, such as *B. ocellaris* (Duarte et al., unpublished data). There is currently no pesticide registered for the management of this gnat (Brasil, 2019), and therefore, the discovery of tools for its management is very important.

Cosmolaelaps brevistilis was found in the soil of a native pasture of the Pampa biome, Brazil and was considered a good predator of *Caliothrips phaseoli* pupae (Hood) (Thripidae, Thysanoptera) (Duarte et al. 2018). Nevertheless, the high number of prey consumed by *S. scimitus* in the both bioassays of the present work indicates that the commercial species performed better than *C. brevistilis* for the control of *B. ocellaris*. According to preliminary observations, Brazilian populations of *S. scimitus* can consume up to 2.9 fourth-instar larvae of *B. matogrossensis* per day (Freire et al. 2007). In the present work, the consumption of *B. ocellaris* larvae by *S. scimitus* was higher (8.25 larvae/female/day).

In the second to evaluate the density of mites, *C. brevistilis* was not efficient, this is probably because *Cosmolaelaps* species are commonly found together with nematodes (Moreira et al. 2015), and the food source for the stock colonies in this study was a nematode species. This could have led to the satiation of the mites before they were inoculated in the experimental units with *B. ocellaris* larvae. Therefore, when exposed to larvae of *B. ocellaris*, the mites did not need to feed, since this species can survive several days without food (Duarte et al 2018). However, information on the specific food behavior of soil mites is scarce, mainly due to the great diversity of organisms and their very small size (Walter et al. 1988).

On the other hand, *S. scimitus* was efficient with the release of two mites, with significant predation rate (82.5%), did not being necessary to release higher densities. In mushroom production of *Agaricus blazei* Murill, naturally infested with *B. matogrossensis*, this same predator was evaluated in two densities, and demonstrated the ability to control the gnat, significantly reducing its population (Freire et al. 2007). However, the authors mentioned that more studies to determine lowest densities of the predators were needed, since lower predator densities might produce cheaper and acceptable results, increasing the chances of commercial implementation.

Furthermore, in commercial production of the mushroom *Agaricus bisporus* (Lange), this predator was evaluated with one and two releases and the authors concluded that *S. scimitus* can effectively control *B. matogrossensis*, with a reduction of 93% and 88% at the end of the observation period, respectively (Castilho et al. 2009). This result showed that, there were no differences for single or double releases. Moreover, the use of a single release incurs in lower costs in a control program involving this specie. At the end of the observation period, the number of predatory mites recovered was slightly higher in the treatment with two releases, suggesting an increase in its population in the course of the work (Castilho et al. 2009). Although, we did not count the number of predatory mites at the end of the experiment, it was possible to observe a large number of young mites regardless of the released density.

These results are important because biological control with predatory mites have long been used. Most recently the interest for this method of pest control has further increased (Castilho et al. 2015) since the interest of people in consuming the crops produced with minimum or no pesticide use has grown. Furthermore, the control of edaphic pests is very difficult, using conventional methods, and also cases of pest resistance to pesticides are continuously recorded (Moreira and Moraes 2015). Considering that in strawberry crops, the fruits are usually consumed *in natura,* the biological control of *B. ocellaris* with *S. scimitus* is an important alternative, because it enables the production with less chemical pesticides. However, more studies are necessary, mainly in greenhouse conditions, to verify if this efficiency persists in natural conditions.

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Table 1. Predation (mean ± SE number of prey killed/day), oviposition (mean ± SE number of eggs laid/ female/day) and survivorship (%) of *Stratiolaelaps scimitus* and *Cosmolaelaps brevistilis*, at 25 ± 1ºC, 75 ± 3% UR in the dark

Prey	Predation				Oviposition				% survival	
	S. scimitus		*C. brevistilis*		*S. scimitus*		*C. brevistilis*		*S. scimitus*^{ns}	*C. brevistilis*^{ns}
Rhabditella axei	-		-		1.53 ± 0.08	a	0.95 ± 0.22	a	83.33	86.67
Bradysia ocellaris (eggs)	3.10 ± 0.85	b A	1.40 ± 0.31	bB	0.31 ± 0.05	bc	0.33 ± 0.13	bc	80.00	93.33
Bradysia ocellaris (larvae)	8.25 ± 0.33	a A	4.45 ± 0.13	a B	1.25 ± 0.21	ab A	0.40 ± 0.05	bc B	86.67	83.33
Bradysia ocellaris (pupae)	0.09 ± 0.07	c ^{ns}	0.05 ± 0.03	c ^{ns}	0.14 ± 0.08	c ^{ns}	0.23 ± 0.10	c ^{ns}	93.33	80.00
Mixture of two food/prey	7.57 ± 0.31	a A	3.75 ± 0.50	a B	1.98 ± 0.19	a A	0.65 ± 0.21	ab B	90.00	96.67

–, not evaluated; ^{ns}, not significantly

Values followed by different letters (upper case in the row and lower case in the column) do not differ significantly (predation, oviposition: Kruskal–Wallis ANOVA; survivorship: Fisher test, all $p < 0.05$).

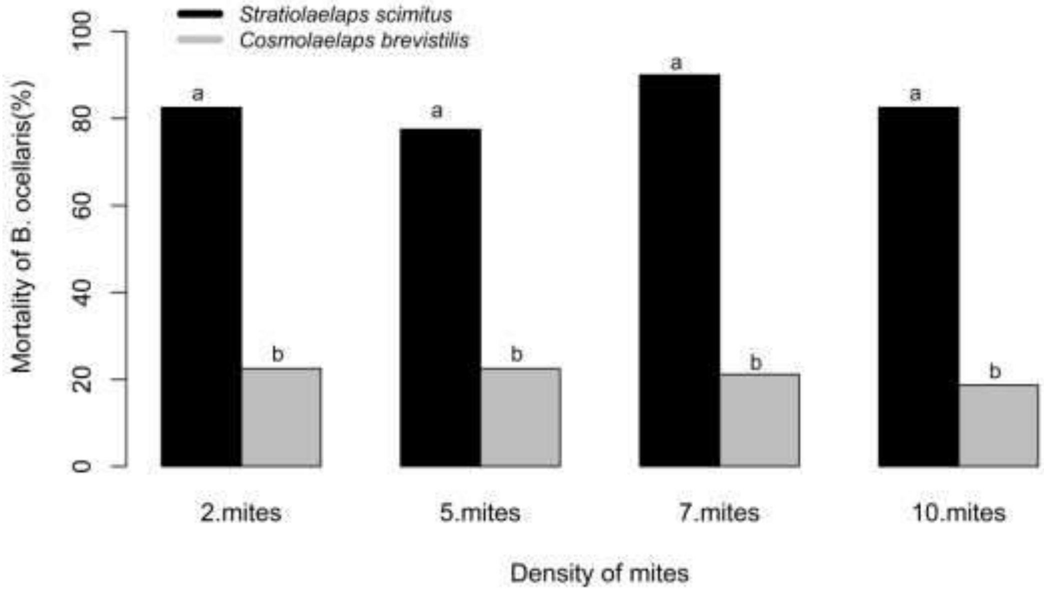

Figure 1. Mortality of *Bradysia ocellaris* (Comstock) in different density of mites, *Stratiolaelaps scimitus* and *Cosmolaelaps brevistilis*. Different letters between each density of mites showed significance by T-test ($p \leq 0.05$).

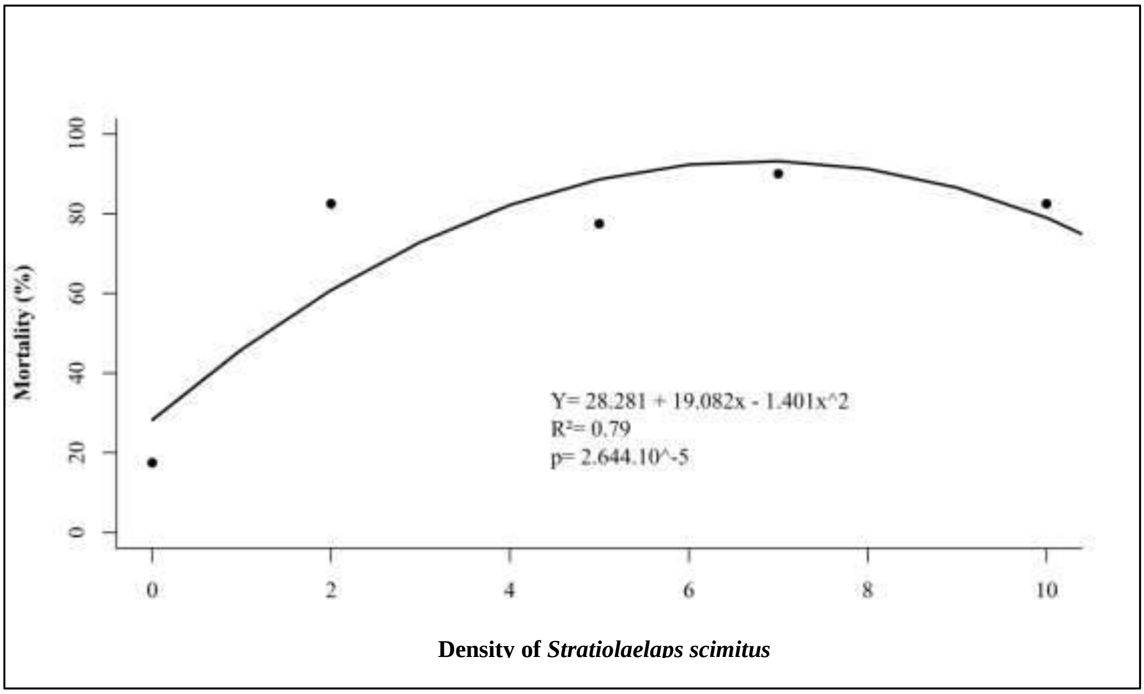

Figure 2. Mortality (%) of *Bradysia ocellaris* (Comstock) larvae, with the release of different densities of *Stratiolaelaps scimitus* (Womersley) adult mites in laboratory conditions (25 ± 1 °C and 75 ± 3% RH, in the dark).

ARTIGO 3 – Ecotoxicology (publicado)

Compatibility of widely used pesticides in strawberry crops with predatory mites *Stratiolaelaps scimitus* (Womersley) and *Cosmolaelaps brevistilis* (Karg)

Compatibilidade dos pesticidas utilizados em cultivos de morangueiro com os ácaros predadores *Stratiolaelaps scimitus* (Womersley) e *Cosmolaelaps brevistilis* (Karg)

ADRIANE DA FONSECA DUARTE, JULIANO LESSA PINTO DUARTE; JULIANO DE BASTOS PAZINI; LUCAS RAPHAEL DA SILVA; UEMERSON SILVA DA CUNHA.

4 Artigo III – Compatibility of pesticides used in strawberry crops with predatory mites *Stratiolaelaps scimitus* (Womersley) and *Cosmolaelaps brevistilis* (Karg)

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Abstract

Stratiolaelaps scimitus (Womersley) and *Cosmolaelaps brevistilis* (Karg) (Acari: Laelapidae) are predatory mites of soil-inhabiting pests, mainly small insects. Fungus gnats fly species are found in greenhouse strawberry production and may be controlled with predatory mites, being important to know their compatibility with the pesticides used in strawberry crops. In this study, the compatibility of seven commercial pesticides used in strawberry cultivation with the predatory mites *S. scimitus* and *C. brevistilis* was assessed in laboratory conditions. Survival and oviposition rates were evaluated between 0.5-120h after treatment (HAT). The results demonstrate that lambda-cyhalothrin treatment resulted in the lowest survival rate for both mites in the first evaluations, being moderately harmful, while spinetoran was slightly harmful to *C. brevistilis.* On the other hand, abamectin, azadirachtin, azoxystrobin + difenoconazole, iprodione and thiamethoxam were harmless for both mites and, oviposition rate was significantly different only at 72 and 120 HAT for *S. scimitus* and *C. brevistilis* respectively. These results may be used to develop guidelines for the adoption of selective pesticides in integrated pest management programs that conserves predatory mites.

Keywords: Soil predators; Laelapidae; *Bradysia* sp.; Integrated Pest Management.

Introduction

Strawberry crops has significant social and economic importance, primarily because it is usually cultivated in small rural properties, where it aggregates family workforce and provides an excellent source of income to the producers (Ponce et al. 2009). Strawberries are traditionally cultivated in the field soil, but conventional soil strawberry cultivation faces many sanitary problems. Soil-borne pathogens and damage caused by arthropods are the main issues on field-grown strawberries (Paranjpe et al. 2003).

For this reason, in many countries the traditional soil-based crop production has been replaced by soilless growing systems, which significantly reduces the issues with soil-borne pathogens and arthropod pests, reducing the need for chemical control (Cecatto et al. 2013). Furthermore, this system facilitates ease of harvest and can accommodate higher plant densities, increasing yields per area.

Nevertheless, this system is still subject to different issues that can impact production (Bernardi et al. 2012). The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), the cotton aphid *Aphis gossypii* Glover (Hemiptera: Aphididae), and the western flower thrip *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) are the main arthropod pests of greenhouse strawberries (Paranjpe et al. 2003). Besides those three species, sciarid flies from the genus *Bradysia* (Diptera: Sciaridae) has gained attention in this crop over the last years (Radin et al. 2009).

Sciarid *Bradysia* species are small flies, whose larvae are phytosaprophagous, mycetophagous and phytophagous (Broadley et al. 2018). They can become major pests inside greenhouses and nurseries, where they are one of the few insect pests in which the damaging life stage resides within the growing medium (Cloyd and Zaborski 2004). Its larvae can directly damage the roots of young seedlings and may cause indirect damage by creating wounds that allow the entry of soil-borne pathogens (Cloyd 2008).

They are mainly a problem under conditions of excessive moisture, which generally occurs during the propagation of seedlings (Cloyd and Zaborski 2004). In these situations, fly population inside the greenhouses can become massive, and considered that both larvae and adults of *Bradysia* are able to transmit pathogens from infected to healthy plants, the tolerance level for the presence of this pest should be very low, which means that intensive plant protection practices need to be implemented, including alternative management strategies.

The use of predatory mites to control this pest has been evaluated on different crops in various countries, especially using species from the family Laelapidae (Castilho et al. 2009). The species *Stratiolaelaps scimitus* (Womersley) (Acari: Laelapidae) is currently commercialized in Brazil in order to control populations of the species *Bradysia matogrossensis* (Lane) (Diptera: Sciaridae) in all crops where it is found including organic crops (Brasil, 2015), while *Cosmolaelaps brevistilis* (Karg) (Acari: Laelapidae) was evaluated as an alternative to control *B. matogrossensis* (Duarte et al. 2018) due to the scarcity of registered pesticides (Brasil, 2018).

Nevertheless, chemical control is still the most common strategy for managing pests in Brazilian agriculture (Zantedeschi et al. 2018). However, the intensive use of synthetic products has been compromising the effectiveness of the chemicals, through the development of resistance (Chen et al. 2017) and the decrease in the populations of biological control agents (Bernardi et al. 2012).

Integrated pest management enables the use of both chemical and biological control for a more efficient and sustainable agriculture. However, it is necessary to understand the compatibility of pesticides with biological control agents. The use of predatory mites might be viable to control *Bradysia* spp. in strawberry cultivation. Thus the aim of this work was to evaluate, under laboratory condition, the compatibility of the pesticides commonly applied in strawberry cultivation on *S. scimitus* and *C. brevistilis*.

Material and methods

Stock colonies

Specimens of *S. scimitus* was purchased from the company that markets the species in Brazil, while *C. brevistilis* were collected from the soil of a native pasture of the Pampa biome, in Aceguá, Rio Grande do Sul, Brazil (31°51′48″S, 54°10′04″W; 240 m a.s.l.). Since 2015, the colonies are maintained, in plastic containers (9.5 cm diameter x 6 cm high) whose base is covered with a 1.5 cm thick layer of a solidified paste (a mixture of nine parts gypsum to one part activated charcoal) (Abbatiello 1965). Colonies are maintained moist by daily addition of distilled water and fed *ad libitum* with a mixture of all stages of *Protorhabditis* sp. (Rhabditina: Rhabditidae) reared in laboratory on pieces of rotting bean pods.

Pesticides

Seven commercial formulations of strawberry-registered pesticides (Brasil, 2018) were used (Table 1). These agrochemicals are widely used against the main pests (diseases, mites, and insects) from Brazilian strawberry crops.

Tabela 1. Pesticides from Brazilian strawberry crops used in side-effect bioassays on *Stratiolaelaps scimitus* and *Cosmolaelaps brevistilis*

Active ingredient	Trade name	Class[a]	Chemical group	Concentration [Formulation][b]	Registered dose	
					a.i.[c]	c.p.[d]
Azoxystrobin + difenoconazole	Amistar Top	F	Strobilurim + triazole	20.0 + 12.5 [SC]		75
Iprodione	Rovral SC	F	Dicarboximide	50.0 [SC]		100
Abamectin	Vertimec 18 EC	A/I/N	Avermectin	1.8 [EC]		60
Azadirachtin	Azamax	A/I/N	Tetranortriterpenoid	1.2 [EC]		250
Lambda-cyhalothrin	Karate Zeon 50CS	I	Pyrethroid	5.0 [CS]		80
Spinetoram	Delegate	I	Spinosyn	25.0 [WG]		16
Thiamethoxam	Actara 250 WG	I	Neonicotinoid	25.0 [WG]		10
Control (water)	--	--	--	--		--

[a]Class: A= Acaricide; F= Fungicide; I= Insecticide; N= Nematicide; [b]Concentration in % [EC= emulsifiable concentrate, SC= suspension concentrate, CS= capsule suspension, WG= water dispersible granule]; [c]Registered dose for strawberry pest control (Brasil, 2018) in g a.i. and [d]g or mL of commercial product (c.p.)/100 L.

Pesticides side-effect biossays

Pesticides side-effect on predators *S. scimitus* and *C. brevistilis* were evaluated through the dry film-residue method used by Pazini et al. (2019). The experiment were kept in laboratory conditions [temperature: 25±1 °C; 70±10%RH; in the dark]. All bioassays were conducted in a completely randomized design with 10 replicates, each replicate corresponding to five female mites with < 24 h old, totalizing fifty females evaluated by treatment. Each species was studied in separate experimental units.

Glass vials (1.0 cm diameter x 8.0 cm height, 25.91 cm^2 surface area) were impregnated with 600 µL of each insecticidal mix at the respective field concentration (23.26$\mu L.cm^{-2}$). The control treatment was composed of distilled water. The mix was dried for approximately 24 h in rotating equipment to evenly distribute the insecticide in the vials. The mites were removed from the tubes after 4 h of exposure and placed in experimental unit consisting of a transparent plastic Petri dish (2.7 cm diameter x 1.2 cm high), whose base was covered with a 0.5 cm thick layer of the same mixture of gypsum and activated charcoal, maintained moist by daily addition of distilled water and fed all stages of *Protorhabditis* sp. The top end of each unit (glass tubes and plastic Petri dish) was sealed with a transparent plastic film (Magipac®) to prevent the organisms from escaping.

Survival rates were evaluated at 0.5; 1; 1.5; 2; 4; 8; 10; 22; 24; 48; 72; 96 and 120 hours after treatment (HAT). Predators incapable of moving after being stimulated with a fine-tipped brush were considered dead and were removed from the experimental units to avoid double counting. Oviposition was evaluated daily (eggs/replicate/day), considering the number of eggs/alive female/24h.

Based on mortality and fecundity, the total effect (E) was calculated for each treatment using the formula $E= 100\% - (100\% – M_a)*E_r$, where: M_a= corrected mortality calculated by Abbott's formula; and E_r = (the average oviposition of the treatment/the average oviposition of the control) (Reis and Sousa 2001; Silva and Oliveira 2006; Ahmad et al. 2009). These values were compared to the standards for laboratory ecotoxicological tests proposed by IOBC/WPRS in four categories: I: harmless ($E < 30\%$); II: slightly harmful ($30\% \leq E < 79\%$); III: moderately harmful ($80\% \leq E < 99\%$), and IV: highly harmful ($E > 99\%$).

Statistical analysis

The normality and homoscedasticity of the data were evaluated by Shapiro–Wilk and Bartlett tests, respectively. As all data did not fit the assumptions for parametric analyses, they were compared by non-parametric tests, using the Kruskal–Wallis test for variance analysis, and when significant the Dunn's test was applied for multiple comparisons between treatments (at 72 and 120 HAT for *S. scimitus* and *C. brevistilis* oviposition, respectively). The survival data of predator mites exposed to pesticides through time were subjected to survival analyses using Kaplan–Meier was adjusted and compared with the log-rank test ($p < 0.05$). All these analyses were performed using the statistical software "R", version 3.4.1 (R Development Core Team 2018).

Results

Differences were observed among treatments for *S. scimitus* (χ^2= 392, df= 7, $p < 0.0001$). The lowest survival rate was observed in lambda-cyhalothrin, which differed from all other pesticides ($p < 0.0001$). At the other extreme, significant differences were not observed between azoxystrobin+difenoconazole, spinetoram, iprodione, thiamethoxam, azadirachtin, abamectin and control ($p > 0.05$) (Fig. 1). During the evaluations, the lambda-cyhalothrin treatment caused the lowest survival rate in the first evaluations. At 10 hours after treatment (HAT) the survival rate of *S. scimitus* was 11.2% (Fig. 1). On the third day of evaluation (Fig. 1) the survival rate of *S. scimitus* in the lambda-cyhalothrin treatment was 8.6%, while in the second treatment with higher mortality (the iprodione treatment) survival rate was 87.8%.

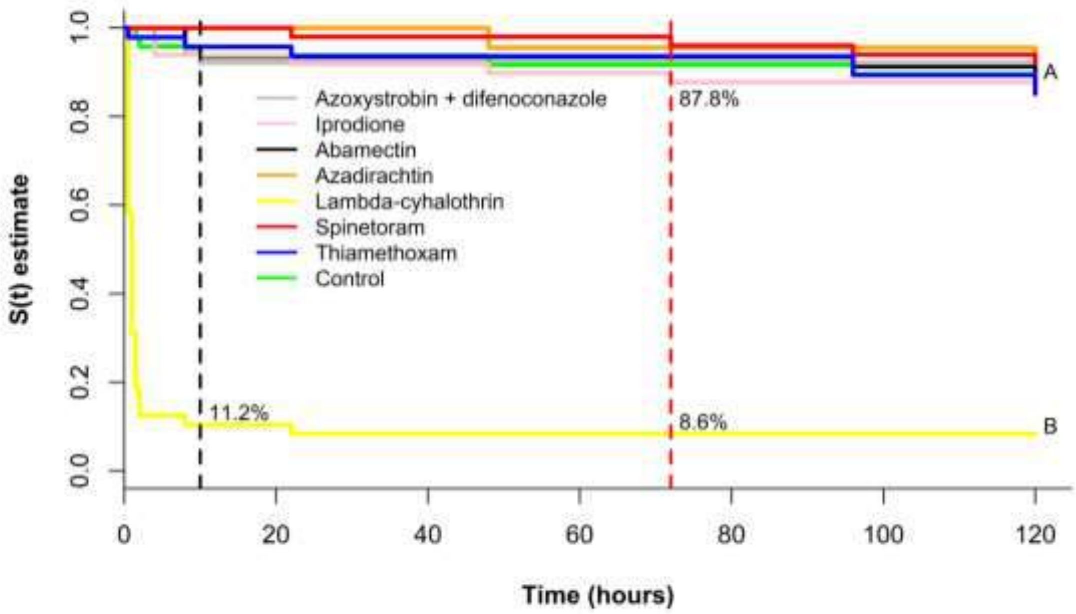

Figura 1. Survival curve of *Stratiolaelaps scimitus* adults after exposure to pesticides used in strawberry crop. Survival curves followed by different letters are significantly different by Log-rank test ($p < 0.05$). Black dashed line indicates 10 HAT and red dashed line indicates 72 HAT.

Lambda-cyhalothrin treatment resulted in the lowest survival rate for both mites (Fig. 1 and 2). However, unlike *S. scimitus*, females of *C. brevistilis* were affected when exposed to spinetoram, with significant differences between treatments (χ^2= 307, df= 7, $p < 2 \times 10^{-16}$), being that the survival rate was significantly different from the lambda-cyhalothrin ($p < 0.0001$) and abamectin ($p = 0.0084$) together with other pesticides ($p \leq 0.05$). However, abamectin, azoxystrobin+difenoconazole, iprodione, azadirachtin, thiamethoxam, and control treatment did not show significant differences ($p > 0.05$) (Fig. 2). Similar to the effect on *S. scimitus*, lambda-cyhalothrin also reduced the survival rate of *C. brevistilis* and at 10 HAT only 22.5% of females were alive (Fig. 2), this survival rate was maintained until 72 HAT, when the survival rate reached 20.0% on lambda-cyhalothrin and 60.2% on spinetoram (Fig. 2). Although lambda-cyhalothrin also reduced the survival of *C. brevistilis, S. scimitus* appears to be more sensitive to the pesticide, with only 8.6% survival rate at the end of the experiment (Fig 1).

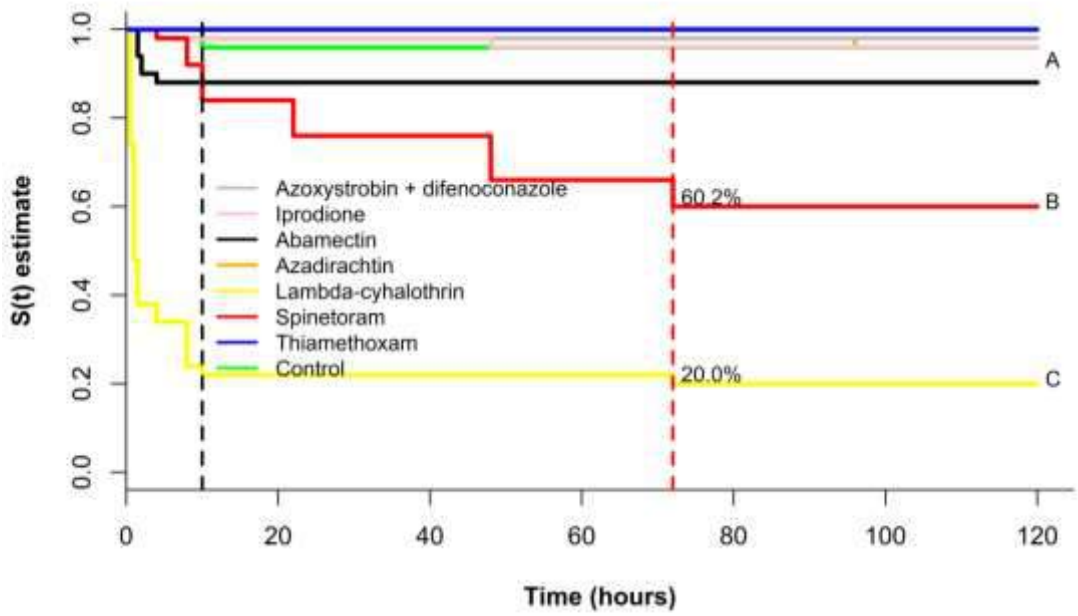

Figura 2. Survival curve of *Cosmolaelaps brevistilis* adults after exposure to pesticides used in strawberry crop. Survival curves followed by different letters are significantly different by Log-rank test ($p < 0.05$). Black dashed line indicates 10 HAT and red dashed lines indicates 72 HAT.

Stratiolaelaps scimitus oviposition significantly differed only at 72 HAT (χ^2 = 17.19; df = 7; p = 0.016) (Table 2). Lambda-cyhalothrin was the pesticide which most negatively affected the oviposition (none eggs was found), while the highest oviposition rate was on spinetoram treatment (0.70 egg/female) (p = 0.030) but did not differ from azadirachtin and azoxystrobin + difenoconazole treatment (p = 1.00). The control treatment, abamectin, thiamethoxan, and iprodione did not significantly differ ($p > 0.05$), showing an intermediary oviposition rate (Table 2). No significant difference was found in the other evaluated periods ($p \geq 0.05$).

Significant differences were observed for *C. brevistilis* oviposition only at 120 HAT (χ^2 = 12.29; df = 7; p = 0.037) when the highest oviposition rate was observed in the control treatment (0.52 egg/female), while azoxystrobin+difenoconazole, iprodione and spinetoram were the treatments that most negatively affected the oviposition rate, not differing significantly between them ($p > 0.05$). Lambda-cyhalothrin, thiamethoxam, azadirachtin and abamectin treatments reduced slightly less the oviposition, and did not differ significantly from each other ($p > 0.05$) with an intermediary oviposition rate (Table 2).

Tabela 2. Oviposition of Stratiolaelaps scimitus and Cosmolaelaps brevistilis (mean ± SE number of eggs laid/alive female/24h) after exposure to pesticides used in strawberry crop, in the dark, at 25 ± 1ºC and 80 ± 10% RH

Treatment	Time (hours)				
	24	48	72	96	120
	Stratiolaelaps scimitus				
Azoxystrobin + difenoconazole	0.19 ± 0.15 ns	0.45 ± 0.16 ns	0.60 ± 0.24 a*	0.30 ± 0.16 ns	0.37 ± 0.12 ns
Iprodione	0.20 ± 0.08	0.56 ± 0.13	0.33 ± 0.17 ab	0.17 ± 0.12	0.31 ± 0.15
Abamectin	0.16 ± 0.11	0.49 ± 0.20	0.37 ± 0.19 ab	0.36 ± 0.16	0.16 ± 0.13
Azadirachtin	0.40 ± 0.16	0.62 ± 0.26	0.66 ± 0.20 a	0.32 ± 0.23	0.28 ± 0.14
Lambda-cyhalothrin	0.25 ± 0.20	0.25 ± 0.20	0.00 ± 0.00 b	0.75 ± 0.30	0.00 ± 0.00
Spinetoram	0.26 ± 0.14	0.42 ± 0.17	0.70 ± 0.22 a	0.51 ± 0.27	0.36 ± 0.20
Thiamethoxam	0.17 ± 0.08	0.65 ± 0.17	0.37 ± 0.17 ab	0.34 ± 0.14	0.35 ± 0.16
Control	0.21 ± 0.11	0.49 ± 0.21	0.41 ± 0.15 ab	0.29 ± 0.19	0.29 ± 0.14
	Cosmolaelaps brevistilis				
Azoxystrobin + difenoconazole	0.14 ± 0.07 ns	0.10 ± 0.08 ns	0.31 ± 0.14 ns	0.31 ± 0.15 ns	0.29 ± 0.13 b*
Iprodione	0.16 ± 0.10	0.40 ± 0.12	0.25 ± 0.12	0.21 ± 0.11	0.25 ± 0.14 b
Abamectin	0.14 ± 0.09	0.45 ± 0.22	0.11± 0.10	0.14 ± 0.11	0.34 ± 0.17 ab
Azadirachtin	0.05 ± 0.04	0.29 ± 0.18	0.29 ± 0.09	0.35 ± 0.15	0.35 ± 0.16 ab
Lambda-cyhalothrin	0.27 ± 0.21	0.27 ± 0.21	0.30 ± 0.14	0.20 ± 0.15	0.40 ± 0.22 ab
Spinetoram	0.45 ± 0.18	0.26 ± 0.14	0.28 ± 0.21	0.25 ± 0.16	0.19 ± 0.13 b
Thiamethoxam	0.31 ± 0.11	0.37 ± 0.14	0.35 ± 0.13	0.31 ± 0.11	0.37 ± 0.11 ab
Control	0.19 ± 0.16	0.17 ± 0.07	0.23 ± 0.11	0.15 ± 0.08	0.52 ± 0.20 a

[ns]Not significant; [*]Significant by the variance analysis and by the Kruskal-Wallis test (72 hours for *S. scimitus* and 120 hours for *C. brevistilis*). Values followed by same letters in the column do not differ significantly (Dunn test $p < 0.05$).

Of the tested pesticides, spinetoram was classified as slightly harmful (class II) to *C. brevistilis*, and lambda-cyhalothrin was classified as moderately harmful (class III) to both predators with a reduction coefficient of 97.97 and 83.91% (Table 3) for *S. scimitus* and *C. brevistilis* respectively. The others pesticides were classified as harmless (class I) because they did not cause a reduction in the survival and oviposition rates of *S. scimitus* and *C. brevistilis.*

Tabela 3. *Stratiolaelaps scimitus* and *Cosmolaelaps brevistilis* total oviposition (mean ± SE number of eggs laid/alive female), corrected mortality (Ma), effect on reproduction (Er), and total effect (E) after exposure to pesticides used in strawberry crop, in the dark, at 25 ± 1ºC and 80 ± 10% RH

	Treatment	Oviposition	M_a (%)	E_r	E (%)	Class[1]
S. scimitus	Azoxystrobin + difenoconazole	1.78 ± 0.24 a*	0.00	1.29	0	I
	Iprodione	1.57 ± 0.24 ab	10.64	1.14	0	I
	Abamectin	1.26 ± 0.21 ab	14.89	0.91	22.29	I
	Azadirachtin	2.06 ± 0.26 a	12.77	1.49	0	I
	Lambda-cyhalothrin	0.33 ± 0.16 b	91.49	0.24	97.97	III
	Spinetoram	2.08 ± 0.28 a	6.38	1.51	0	I
	Thiamethoxam	1.48 ± 0.20 ab	12.77	1.07	6.45	I
	Control	1.38 ± 0.21 ab				
C. brevistilis	Azoxystrobin + difenoconazole	1.16 ± 0.15 ab	0.00	0.90	10.08	I
	Iprodione	1.20 ± 0.16 ab	2.04	0.93	8.88	I
	Abamectin	1.06 ± 0.19 b	10.20	0.82	26.21	I
	Azadirachtin	1.32 ± 0.18 ab	4.08	1.02	1.85	I
	Lambda-cyhalothrin	1.13 ± 0.27 ab	81.63	0.88	83.91	III
	Spinetoram	0.90 ± 0.17 b	28.57	0.70	50.17	II
	Thiamethoxam	1.70 ± 0.15 a	0.00	1.32	0.00	I
	Control	1.29 ± 0.19 ab				

*Significant by the variance analysis and by the Kruskal-Wallis test. Values followed by same letters in the column do not differ significantly (Dunn test $p < 0.05$). [1]Toxicity categories proposed by the IOBC/WPRS for pesticide side effect studies on natural enemies: I: harmless (E < 30%); II: slightly harmful (30% ≤ E < 79%); III: moderately harmful (80% ≤ E < 99%), and IV: highly harmful (E > 99%).

Discussion

The release of predatory mites to control invertebrate pests inside greenhouses is an important phytosanitary measure. In this regard, the knowledge of the effects of pesticides on beneficial arthropods, is essential to successful integrate biological and chemical control strategies into IPM programs (Pazini et al. 2019). Our results showed that the toxicity levels of the pesticides here evaluated were variable for the different predators and chemical group.

According to our findings, all fungicides here studied were harmless for both predators as well as abamectin, azadirachtin, and thiamethoxam. The harmlessness of fungicide was not surprising since most of them have no known site of action in mites. Most other studies also observed none or minor effects of fungicides that belong to the chemical groups Triazole, Strobilurin and Dicarboximide against other beneficial arthropods important for biological control and IPM (Castilho et al. 2013; Magano et al. 2015; Ditillo et al. 2016; Bueno et al. 2017; Pazini et al. 2017b).

Azadirachtin has shown high potential for use against pests in different agroecosystems due to its high insecticide and acaricide activities and rapid degradation in the environment (Biondi et al. 2012). Our finding of its low acute effects on *S. scimitus* and *C. brevistilis* was similar to previous studies where it was reported safe such as to some phytoseiid mite predators (Brito et al. 2006; Bernardi et al. 2012; Schlesener et al. 2013; Lima et al. 2016), which are essential for strawberry pest management through a combined use with chemical control or preservation of existing natural enemies. The low toxicity of neem-based compounds to predatory mites can be justified due to the action of enzymes (e.g. esterases, glutathione S-transferases, and oxidative enzymes) that work in the detoxification of xenobiotics (Vidal and Kreiter 1995).

However, in the studies of Brito et al. (2006), Bernardi et al. (2012), and Lima et al. (2016), the authors mentioned that abamectin compromised phytoseiid mites performance, differently from what was found in our results for *C. brevistilis* and *S. scimitus*. It is important to highlight that despite abamectin presents toxic action against several mite species, the levels of the acute toxicity of the pesticides are not interchangeable. The differential susceptibility observed in toxicity studies may have resulted from the specific characteristics of insecticides and mite species. The difference in thickness and chemical composition of the cuticle among species can significantly facilitate the pesticide penetration, increasing toxicity to certain arthropod pest species (Bueno et al. 2017). Other intrinsic particularities that can affect the acute effect are age, body weight and detoxification capacity (Vidal and Kreiter 1995; Brittain and Potts 2011). On this regard, Arnó and Gabarra (2011) underlined the need to conduct specific experiments for each natural enemy to enable the successful integration of pesticides in biological control to IPM programs.

With respect to thiamethoxam, when Zanardi et al. (2017) evaluated sublethal effects of pyrethroid and neonicotinoid insecticides on *Iphiseiodes zuluagai* Denmark and Muma (Acari: Phytoseiidae), they found similar results, and mentioned that thiamethoxam did not affect the adult survival and fecundity of females, being classified as harmless to the predators. This insecticide belongs to the neonicotinoids group, which have little contact action and need to be ingested by the target pest and therefore, are more specific (Tomizawa and Casida 2005; Stecca et al. 2017).

On the other hand, spinetoram was slightly harmful to females *C. brevistilis*. Nevertheless, spinetoram was considered safe for several non-target arthropods according to the World Health Organization (Who, 2009). Spinosyns are part of a new class of pesticides derived from biological substances produced by the soil actinomycete *Saccharopolyspora spinosa* Mertz and Yao (Bacteria: Actinobacteridae) (Sparks et al. 1998). Spinetoram is a spinosyn derived from spinosad and consists of 3'-O-ethyl-5.6-dihydro-spinosyn J and 3'-O-ethylspinosyn L (Galm and Sparks 2016). In the mite *Galendromus occidentalis* (Nesbitt) (Acari: Phytoseiidae), this insecticide was also very toxic when applied on eggs and larvae of the western predatory mite, with 100% of mortality of females after 72 h of exposure (Lefebvre et al. 2011). This result demonstrates that the low adverse effects of the spinosyn chemical group on non-target arthropods can be overestimated. This fact may be related to the neurotoxic action of these insecticides, which act through direct contact with the surface of the mite's body or through ingestion involving the disruption of acetylcholine and aminobutyric acid (GABA) receptors in the arthropod nervous system (Kirst 2010).

Lambda-cyhalothrin was moderately harmful to both predators, reducing greatly the oviposition mainly of *S. scimitus*. Similar to our result, this insecticide also negatively affected the populations of *T. urticae* and of its predator, *G. occidentalis*, causing high mortality, reduction in fertility and viability (Schmidt-Jeffries and Beers 2018) probably because the pyrethroids act basically by direct contact, due to their high lipophilicity and their affinity with the chemical composition of arthropod's cuticle (Hall and Thacker 1993). Lipophilicity of the insecticides is inversely proportional to their solubility in water, thus, compounds of higher lipophilicity can penetrate arthropod bodies at higher rates due to its similarity to the cuticle (Stecca et al. 2017). In this work, a "shock effect" was found for both predators. According to Hall and Thacker (1993), this is due to fast nervous cell depolarization of the central and peripheral nervous systems, causing hyperexcitation, irritability, feeding activity inhibition and death of arthropods.

Our findings show that except for lambda-cyhalothrin none of the tested insecticides and fungicides used in Brazilian strawberry crops were significantly harmful to *C. brevistilis* and *S. scimitus* under controlled conditions even in drastic pesticide-exposure where predatory mites experienced residual contact with treated glass surfaces. Acute toxic impacts of these pesticides on *C. brevistilis* and *S. scimitus* should further decrease under field conditions due to the heterogeneous distribution of pesticides in the plant canopy, the interactions expected between plants and pesticides, their deterioration by abiotic agents, and the escape of mites to non-treated areas due pesticide-repellent properties (Jansen 1999, Madbouni et al. 2017, Pazini et al. 2017a). Thus, the insecticides abamectin, azadirachtin, spinetoram, and thiamethoxam, and the fungicides azoxystrobin + difenoconazole and iprodione are compatible with adults of *C. brevistilis* and *S. scimitus*, being necessary to evaluate the effect these pesticides in others phases of the life these predatory mite. These results could be useful for pesticide selection and their use in IPM programs for strawberries in Brazil.

Compliance with ethical standards

All procedures performed in studies were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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ARTIGO 4 – Phytoparasitica

Efficacy of insecticides for control of *Bradysia ocellaris* (Diptera: Sciaridae) larvae under laboratory

Eficácia de inseticidas para o controle de larvas de *Bradysia ocellaris* (Diptera: Sciaridae) em laboratório

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5 Artigo IV – Efficacy of insecticides for control of *Bradysia ocellaris* (Diptera: Sciaridae) larvae under laboratory

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Abstract

In greenhouse rich in organic matter and moisture, and nursery crops, one of the main problems is black fungus gnats larvae. The species *Bradysia ocellaris* is commonly found pestering the crops, mainly strawberry in semi-hydroponic system. The control of the pest in this system in Brazil, is difficult, due an absence of registered insecticide, so the aim of this work was to evaluate the effect of some insecticides on *B. ocellaris* larvae, as well as to found the lethal concentrations (LC_{50}) of the acetamiprid and novaluron. During the experiments, it was also possible to verify that the insecticide novaluron, insect growth regulator (IGR), caused the deformation of the insects. Among all pesticides evaluated, malationa, and thiamethoxam showed good mortality rate, with 96 and 86%, respectively. Considering that these are not very selective to natural enemies and bees, we opted to continue the bioassays with the third and fourth more efficient insecticide acetamprid (78%) and novaluron (44%). The lethal concentrations (LC_{50}) were 19.18mg a.i.L^{-1} at 48h to acetamiprid and 1.24mg a.i.L^{-1} at 120h to novaluron, however when the larvae were fed on potato pieces treated with novaluron, independently of the dose, the mortality rate was of 100%, since that no larvae could complete the development cycle of none larvae. In conclusion, among all insecticides evaluated, only acetamiprid and novaluron, indicated that are possible tools for the management of the *B. ocellaris* larvae, however further studies are necessary.

Keywords: Acetamiprid, Novaluron, Black fungus gnats, Chemical control.

Introduction

Inside greenhouses, favorable conditions as excessive watering and organic substrates can lead to a rapid increase of Sciaridae fly populations (Broadley et al. 2018; Gorska-Drabik et al. 2011). The presence of these small gnats can lead to the occurrence of direct and indirect (Cloyd 2008). The direct damage can be occasioned by larvae, when they are feeding on decaying organic matter, fungi and algae inhabiting the substrate (Cloyd 2015), and also on small roots, root hairs, and tender lower stems (San-blas et al. 2017). In the start of development, the seedlings and plants are more susceptive to the attack of insects, because the root system is not yet fully developed and it is critical for plant production (Gorska-Drabik et al. 2011).

Indirectly, the larvae may predispose plants to further attack by soil-borne plant pathogens (Cloyd 2015). The adults also are responsible to indirect damage, by the spreading of certain pathogens adhered to the flying adult insects (El-Hamalawi 2008). These flies are considered the major insect pest of nurseries and greenhouse production systems throughout the world (Cloyd 2008, 2015).

In the genus *Bradysia*, the species *B. impatiens* (Johannsen, 1912), *B. coprophila* (Lintner, 1895) and *B. ocellaris* (Comstock, 1882) are the most commonly found and the most serious economic pests of greenhouse crops (Menzel et al. 2003; Sohier et al. 2012), being also an important pest in mushroom (Shamshad et al. 2009; Shin et al. 2015) and, more recently, in strawberry crops in semi-hydroponic system (Duarte et al., unpublished data).

The control of *B. ocellaris* in Brazil is difficult due to the absence of insecticides registered in the Ministry of Agriculture, Livestock and Supply (Brasil, 2019). The aim of this study was to evaluate the effect of insecticides in the control of *B. ocellaris* larvae under laboratory conditions.

Material and methods

Stock colonies

The colony of *B. ocellaris*, was established with larvae and adults collected from strawberry plants. The insects were maintained in plastic cups (250mL) containing a mixture of pieces of potato, ground black beans and moist peat boss, which were kept in plastic containers (30 x 20 x 18 cm; height, weight and depth) moistened once a week, and maintained in the laboratory conditions, at 20–27 °C and 50–90% RH. For all studies, second and third instar larvae were collected with a brush.

Bioassays

The bioassays were performed under a rearing chamber at 25 ± 1 °C and 75 ± 10% RH, in the dark. All bioassays were conducted in a completely randomized design, with fifty larvae by treatment. The experimental units consisted of a transparent plastic Petri dish (2.7 cm diameter, 1.2 cm high), whose base was covered with three filter paper discs, which were moistened with 0.1ml distilled water every 48h.

Toxicity of insecticides

Initially, to evaluate the control efficiency, eleven commercial formulations of insecticides were used (Table 1). These agrochemicals are widely used against the main pest insects from Brazilian strawberry crops or similar crops. All active ingredients of the eleven insecticides were prepared using distilled water, which served also as the control treatment. Potato pieces (0.5 cm^2) were dipped into each test solution for 10 seconds with gentle agitation and then air-dried at room temperature. Two treated potato pieces were transferred to an experimental unit, and, after that, five larvae were introduced, being considered each experimental unit as a single replicate, with ten replicates for each treatment (fifty larvae per treatment in total).

The evaluations were done every 24h, until the fifth day (120h), being that the larvae were considered dead if they were unable to move when touched by a moist brush.

Effect of Acetamiprid and novaluron on the larvae

After the first bioassay, two insecticides were chosen (Acetamiprid and Novaluron), according with the efficiency results and with the selectivity to natural enemies and bees. For these two insecticides, the lethal concentrations necessary to dead 50% of populations (LC_{50}) was calculated with similar procedures to first bioassay, with the only difference being the concentrations evaluated (200.00, 80.00, 30.00, 20.00, 5.00, 2.00 and 0.00 mg a.i.L^{-1} for Acetamiprid and 20.00, 10.00, 1.00, 0.05, 0.01 and 0.00 mg a.i.L^{-1} for Novaluron). Mortality was assessed if the larvae were unable to move when touched by a moist brush at 48 and 120h to acetamiprid and novalurom, respectively, to calculate the response dose. To evaluate the total effect, larvae was considered dead when they could not pupate and emerge. The deformations resulting from the treatments were photographed. Survival was considered, only when adults emerged.

Statistical analysis

The normality and homoscedasticity of the data were evaluated by Shapiro–Wilk and Bartlett tests, respectively. The mortality data of *B. ocellaris* larvae exposed to insecticides through time were compared by non-parametric tests, using the Kruskal–Wallis test followed by Dunn test for multiple comparison ($p < 0.05$) since data did not fit the assumptions for parametric analysis. The number of pupae and the emergence of the flies, were evaluated by variance analysis, followed by Duncan test ($p < 0.05$), as the data fit the assumptions for parametric analysis. These analyses were performed using the statistical software “R”, version 3.4.1 (R Development Core Team 2018).

The LC_{50} was calculated and with probit analysis, their 95% fiducial limits and the slope of the line relating probit mortality to the log dose, by gompertz distribution model using the statistical software SAS University Edition 9.4 software, SAS Institute (Der & Everitt, 2015).

Results

Toxicity of insecticides

Differences were observed between the pesticides during all period evaluated ($p \leq 0.05$), however malathion and thiamethoxam never differed significantly between themselves ($p \geq 0.05$), with the higher mortality rate of 72 and 42% at 24h and 96 and 86% at 120h, respectively. Acetamiprid was the third insecticide with higher mortality rate, but at 24 and 72h after the evaluations, this pesticide did not differ significantly from lambda-cyhalothrin ($p \geq 0.06$), as well at 96 and 120h, where it did not differ from *Bacillus thuringiensis* var. *kurstaki* linhagem HD-I 17.600 (Btk) and novaluron ($p \geq 0.06$) (Table 2).

Regarding, novaluron mainly after 72h, there was an increase the mortality rate, and in the 120h, it did not differ from the pesticides with a good mortality in this period ($p \geq 0.20$) with 44% of mortality, being among the four most efficient pesticide (Table 2). After the end of the bioassay (120h) it was possible to observe that most of the pre-pupae were dead, and considering that this pesticide is a growth regulator, more bioassay were conducted to evaluate this, as well as to find the LC_{50} for novaluron and acetamiprid, considering that these pesticides provided a good mortality and are more selective to natural enemies.

Effect of acetamiprid and novaluron on the *B. ocellaris* larvae

Regarding the pesticide novaluron, none larvae could complete the cycle at any concentrations, however at 120h after the evaluations, the LC_{50} was 1.24mg a.i.L^{-1}. The mortality rate in different concentrations of acetamiprid ranged from 72–10% at 48h, with LC_{50} of the 19.18mg a.i.L^{-1} (Table 3).

However at the end of the bioassay, the pupae formations in different concentrations of novaluron did not observe, while in the acetamiprid treatment, it was observe, and, the number of pupae and emergence was possible at doses of 20mg.L^{-1} or less of acetamiprid (Fig 2A and 2B). The pupae formations at lower concentrations of acetamiprid (20-2mg.L^{-1}) was observed, and did not differ significantly between themselves, as well of the control ($p \geq 0.01$) (Fig 1A).

Regarding the emergence, all concentrations (20-2mg.L^{-1}) did not differ significantly ($p \geq 0.01$) (Fig. 1B), but it is important to highlight that, the number of survivors was high, with emergence rate of the 37.7 - 43.6% corresponding to the pupal viability of 100% at 20mg treatment (Figs. 1A and 1B). At doses greater than 20mg. L^{-1}, no larvae became pupae.

Novaluron ingestion affects pre-pupae, pupation and adult emergence

During both bioassays, it was possible to observe that the development cycle of *B. ocellaris* was not completed when the larvae ingested potato pieces treated with novaluron.

This fact was also observe in the different concentrations, being that between 0.01-20.00mg of active ingredient per liter, there was total insect mortality. Besides the fact that the larvae did not move when stimulated, some of the larvae turned transparent. It was also possible to verify the presence of internal white masses and dark spots in the cuticle (Figure 2A), head and abdomen distention and wing dysplasia, not forming pre-pupae and pupae with success (2B – 2D). None of these symptoms were observed in the control treatment.

Discussion

According to our results, it was observed that in the evaluated concentrations most of the eleven insecticides tested did not show potential to control *B. ocellaris* larvae. The two best insecticides were malathion and thiamethoxam, however, these belong to organophosphate and neonicotinoid chemical group, respectively.

Organophosphate insecticide, acts basically by contact, primarily by phosphorylation of the acetylcholinesterase enzyme (AChE) at cholinergic nerve endings, in the synaptic regions, this result a loss of available AChE and accumulation of acetylcholine (Fukuto 1990). Considering, that the enzyme is essential to the control of nerve impulse transmission from nerve fibers to smooth and skeletal muscle cells, the insects have an excessive stimulation of cholinergic receptors (Casida and Durkin 2013).

Malathion for example, is toxic to insects owing generally low concentrations of carboxylesterases in insects (Fukuto 1990), being highly toxic to *Apis mellifera* (Rinkevich et al. 2015) and bees as *Plebeia emerina* (Friese) and *Tetragonisca fiebrigi* (Schwarz) (Hymenoptera: Apidae: Meliponini) (Padilha et al. 2019). These insects are important pollinators of angiosperm plants, as strawberry crops in greenhouses (Antunes et al. 2007). Therefore, the use of this kind of insecticide is not a good option for the control of *B. ocellaris* larvae in strawberry crops.

On the other hand, neonicotinoid insecticides have a systemic action for crop protection against piercing-sucking pests (Tomizawa and Casida 2005) acting as nicotinic acetylcholine receptor agonists in post-synaptic neurons of the central nervous system, as mimics of the neurotransmitters that interact with the insect nicotinic acetylcholine receptors (Jacob et al. 2019a). They are widely used in agricultural system (Tavares et al. 2015) and in our study provided a good mortality rate of *B. ocellaris*. However, among those evaluate, thiamethoxam was more efficient to control of *B. ocellaris* larvae (86% at 120h) than acetamiprid (78% at 120h), it is more toxic to honeybees, and can affect the larval development of *A. mellifera* (Tavares et al. 2015). Furthermore, it is extremely dangerous to *T. angustula* (Latreille) (Jacob et al. 2019a), but considering that the honeybees and native bees, start to visit the strawberry crops after the flowering period and black fungus gnats are problem in the rooting period, this insecticide need be more studied.

Belonging to the neonicotinoid group, the acetamiprid is the safest neonicotinoid for *T. angustula*, *T. fiebrigi*, *P. emerina* and *Scaptotrigona postica* (Arena and Sgolastra 2014; Jacob et al. 2019a; Jacob et al. 2019b; Padilha et al. 2019). Together with *A. mellifera* these insects are essential to the pollination of strawberry plants and help them reach physiological maturity, produce fruits and seeds (Piovesan et al. 2019). Considering this, more studies were conducted with acetamiprid in order to determine the best concentration for the control of *B. ocellaris* larvae.

According to the results this work, it was possible to verify that in the lowest dose of acetamiprid (20-2mg.L^{-1}) there were survivors of *B. ocellaris*, whereas a female can lay up to 57-116 eggs (Sari and Amelia 2015), there will be survivors in the environment. At 48h after the exposure to *B. ocellaris* larvae, the LC_{50} was 19.18mg a.i.L^{-1}, this concentration is good for controlling of *B. ocellaris* larvae and has little action on pollinators (Padilha et al. 2019; Piovesan 2018), however more studies to discovery the LC_{90} is necessary, mainly to reduce the number of survivors in the environment.

The best results were obtained when the larvae were submitted to potato pieces treated with novaluron, an insect growth regulator insecticide (IGR) belonging to benzoylurea. Mortality rates were high after 120h. This result was already expected, since that IGR acts as chitin-synthesis inhibitor, disrupting the formation of chitin and is very effective against immature insects (Zhang et al. 2017). The IGRs, as chlorfuazuron for example, are highly effective against lepidopterous insect pests, with relatively slow but strong activity (Perveen 2000) and novaluron can provide control of several dipteran pests (Cutler and Scott-Dupree 2007).

After 120h of the evaluation of the novaluron, it was possible to observe that independently of the dose, the larvae did not complete the developmental cycle, and presented deformed pre-pupae and pupae. Similar results was found when larvae of *Haematobia irritans* and *Musca domestica* (Muscidae, Diptera) were submitted to different concentrations of the novaluron (Lohmeyer and Pound 2012).

According to the mode of action of novaluron, there is a physiological selectivity in favor of adult insects since it targets chitin synthesis, which predominately occurs at the molts in juvenile insect stages (Cutler and Scott-Dupree 2007).This fact indicates that novaluron has good selectivity to beneficial insects since the pollinators found in greenhouses with strawberry are adult insects. Thus, novaluron can be used for the management of the *B. ocellaris* larvae, without affecting the populations of pollinators and predators. Nevertheless, more studies are necessary to evaluate the efficiency of this in the semi-field conditions and in the greenhouse.

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Tabela 1. Insecticides used in preliminary bioassay in order to evaluated the effect on *Bradysia ocellaris* larvae

Active ingrediente	**Trade name**	**Chemical group**	**Concentration [Formulation]**[a]	**Registered dose**	
				strawberry[b]	**c.p.**[c]
Abamectin	Vertimec 18 EC	Avermectin	1.8 [EC]	Yes	60
Acetamiprid	Mospilan	Neonicotinoid	20.0 [WG]	Not	30
Azadirachtin	Azamax	Tetranortriterpenoid	1.2 [EC]	Yes	250
Bacillus thuringiensis Cepa HD1 (Bt)	BtControl	Microbiological	7.0 [SC]	not	300
Bacillus thuringiensis var. *kurstaki* linhagem HD-I 17.600 (Btk)	Dipel	Microbiological	3.36 [SC]	not	100
Deltamethrin	Decis 25 EC	Pyrethroid	2.5 [EC]	not	40
Lambda-cyhalothrin	Karate Zeon 50CS	Pyrethroid	5.0 [CS]	yes	80
Malathion	Malathion 1000 EC	Organophosphate	100.0 [EC]	not	250
Novaluron	Rimon Supra	Benzoylurea	10.0 [SC]	yes	40
Spinetoram	Delegate	Spinosyn	25.0 [WG]	yes	16
Thiamethoxam	Actara 250 WG	Neonicotinoid	25.0 [WG]	yes	10
Control	--	--	--		--

[a]Concentration in % [EC= emulsifiable concentrate, SC= suspension concentrate, CS= capsule suspension, WG= water dispersible granule]; [b]Registered dose for strawberry pest control (Brasil, 2019) in g a.i. and [c]g or mL of commercial product (c.p.)/100 L.

Tabela 2. Percentage accumulated mortality (mean ± SE) of *Bradysia ocellaris* larvae, after exposure to the pesticides, in the labor conditions, at 25 ± 2 ºC, 75 ± 10%RH, in the dark

Treatment	**Exposure time (Hours)**									
	24*		**48***		**72***		**96***		**120***	
Abamectin	6.00 ± 1.36	c	12.00 ± 2.38	b	16.00 ± 2.92	c	26.00 ± 2.99	bc	26.00 ± 2.99	c
Acetamiprid	38.00 ± 3.11	ab	72.00 ± 2.73	a	72.00 ± 2.73	ab	76.00 ± 2.31	ab	78.00 ± 2.08	ab
Azadirachtin	6.00 ± 1.36	c	10.00 ± 2.49	b	10.00 ± 1.49	c	12.00 ± 1.46	c	12.00 ± 1.46	c
Bacillus thuringiensis Cepa HD1	4.00 ± 1.19	c	10.00 ± 2.40	b	12.00 ± 2.38	c	18.00 ± 2.31	c	20.00 ± 3.26	c
Bacillus thuringiensis var. *kurstaki* linhagem HD-I 17.600	8.00 ± 1.46	b	20.00 ± 2.98	b	20.00 ± 2.98	c	26.00 ± 2.68	bc	30.00 ± 2.40	bc
Deltamethrin	6.00 ± 1.36	c	18.00 ± 2.08	b	18.00 ± 2.08	c	18.00 ± 2.08	c	22.00 ± 2.47	c
Lambda-cyhalothrin	14.00 ± 1.90	bc	20.00 ± 2.30	b	22.00 ± 2.81	bc	24.00 ± 3.21	c	26.00 ± 3.27	c
Malathion	72.00 ± 2.73	a	92.00 ± 1.97	a	92.00 ± 1.97	a	94.00 ± 1.36	a	96.00 ± 1.19	a
Novalurom	4.00 ± 1.19	c	12.00 ± 1.97	b	18.00 ± 2.47	c	28.00 ± 2.38	bc	44.00 ± 2.23	abc
Spinetoram	4.00 ± 1.78	c	6.00 ± 1.90	b	8.00 ± 2.97	c	12.00 ± 2.38	c	12.00 ± 2.38	c
Thiamethoxam	42.00 ± 2.47	a	70.00 ± 2.00	a	82.00 ± 2.08	a	84.00 ± 1.78	a	86.00 ± 1.36	ab
Control	4.00 ± 1.19	c	8.00 ± 1.46	b	12.00 ± 1.97	c	12.00 ± 1.97	c	12.00 ± 1.97	c

* Significant by the variance analysis and by the Kruskal-Wallis test, values followed by same letters in the column do not differ significantly (Dunn test $p < 0.05$).

Tabela 3. Acute lethal toxicity of formulations of acetamiprid (48h) and novaluron (120h) (LC_{50} mg a.i.L-1) exposed to larvae of *Bradysia ocellaris* at 25ºC ± 2, 75% RH, in the dark

Insecticide	*n*	Slope ± SE	LC_{50}[a] (95% CI)	χ^2	*P value*[b]
Acetamiprid	350	2.10 ± 0.36	19.18 (10.31 – 29.44)	33.70	<0.0001
Novaluron	300	0.41 ± 0.18	1.24 (0.09 – 5.14)	5.15	0.0232

[a]Lethal concentrations 50. Insecticide doses that causes 50% mortality of the population (mg a.i.L^{-1})

[b]p-value derived from Chi square test larger than 0.05 indicates the gompertz model provides acceptable description of data

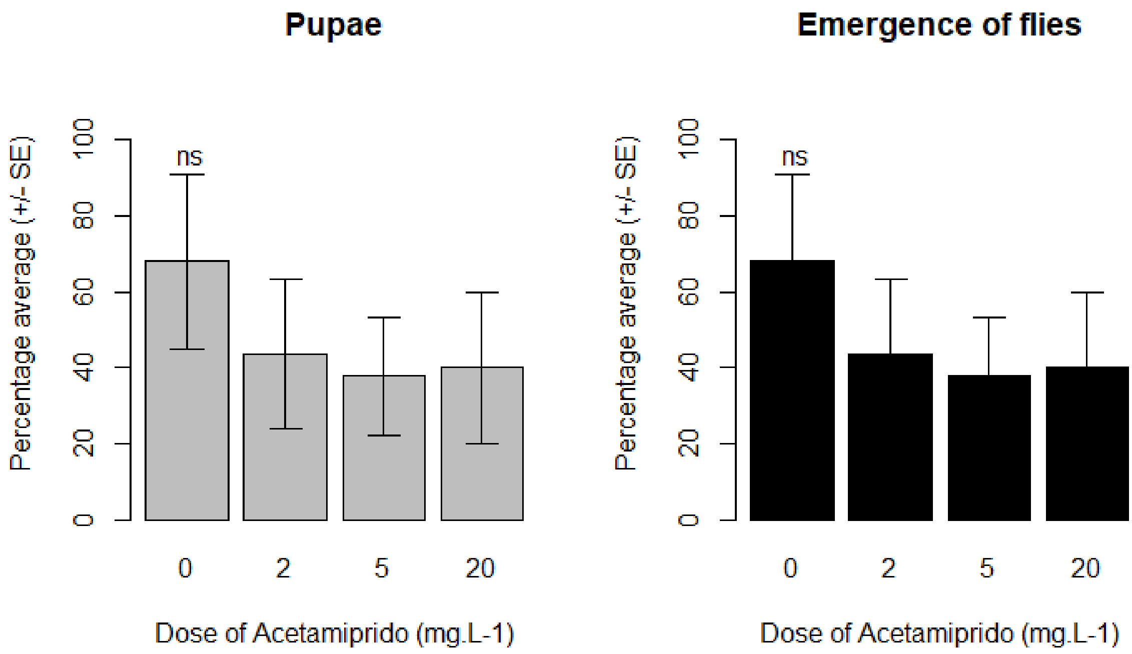

Figure 1. Total effect of the acetamiprid, with percentage average (±SE) of pupae and emergence of *Bradysia ocellaris* flies in different concentrations ($mg.L^{-1}$). [ns]Not significantly, by Duncan test ($p < 0.01$).

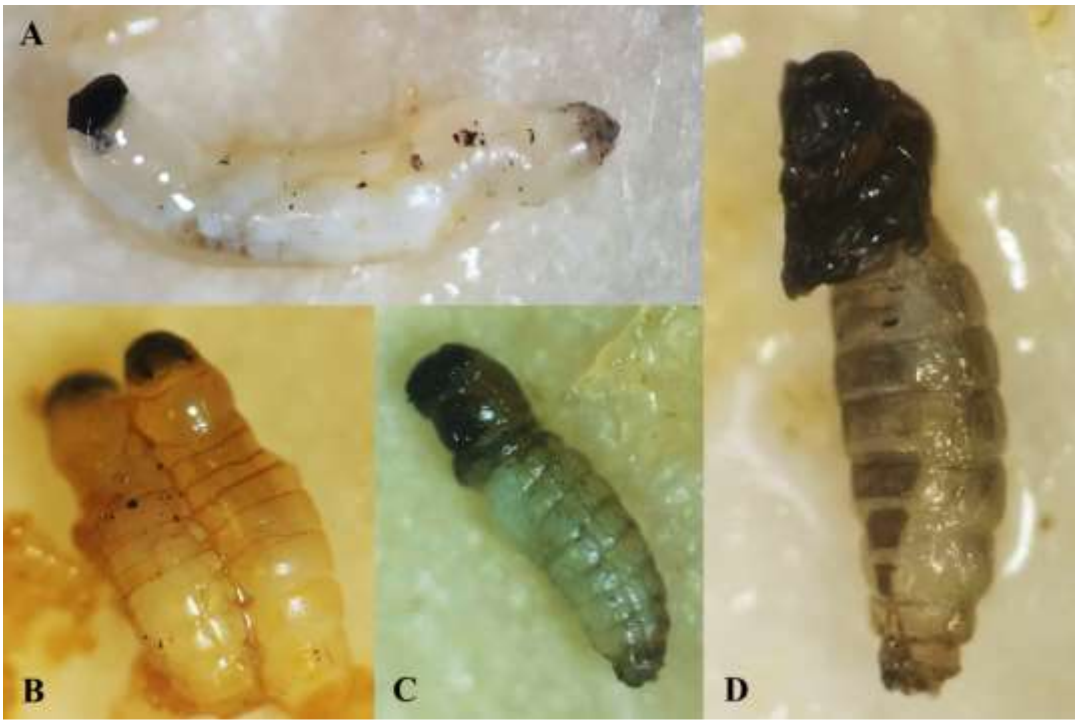

Figura 2. Effect of novaluron on the development of *Bradysia ocellaris* in laboratory bioassay. (A) larvae showing the white internal masses and the dark spots in the cuticle; (B and C) pre-pupae showing deformation and the dark spots; (D) dead deformed pupae.

6. Considerações finais

Em cultivo de morangueiro semi-hidropônico no sul do Rio Grande do Sul, as espécies de Sciaridae encontradas foram *Bradysia impatiens* e *Bradysia ocellaris*, sendo que os principais danos foram observados na fase inicial do ciclo do morangueiro (enraizamento). Com relação ao uso de ácaros predadores, *Stratiolaelaps scimitus* demonstrou um bom potencial de controle sobre larvas de *B. ocellaris* e, adultos deste predador são pouco afetados pelos agroquímicos utilizados no manejo de pragas e doenças da cultura do morangueiro. Dentre os pesticidas avaliados sobre os ácaros predadores *S. scimitus* e *C. brevistilis*, lambda-cialotrina foi o mais prejudicial, pertencendo à classe III (moderamente perigoso), enquanto espinetoram foi levemente perigoso (classe II) apenas para *C. brevistilis*. Com relação aos inseticidas avaliados sobre larvas de *Bradysia ocellaris*, acetamiprido e novaluron foram os que demonstraram resultados promissores, no entanto mais estudos ainda são necessários. Os resultados obtidos neste trabalho são importantes e promissores, uma vez que além de indicar as espécies de Sciaridae encontradas em cultivos de morangueiro semi-hidropônico no sul do Rio Grande do Sul, fornecem alguns indicativos de ferramentas de manejo, que poderão auxiliar no manejo deste insetos além de indicar estudos futuros que precisam ser conduzidos.

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