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Dissertação

Inventário de visitantes florais na cultura do morangueiro e efeitos de inseticidas sobre as abelhas nativas *Melipona quadrifasciata* e *Tetragonisca fiebrigi* (Hymenoptera: Apidae)

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Inventário de visitantes florais na cultura do morangueiro e efeitos de inseticidas sobre as abelhas nativas *Melipona quadrifasciata* e *Tetragonisca fiebrigi* (Hymenoptera: Apidae)

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(Mario Quintana)

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Resumo

PIOVESAN, Bruna. **Inventário de visitantes florais na cultura do morangueiro e efeitos de inseticidas sobre as abelhas nativas *Melipona quadrifasciata* e *Tetragonisca fiebrigi* (Hymenoptera: Apidae).** 2018. 107f. Dissertação (Mestrado) - Programa de Pós-Graduação em Fitossanidade. Universidade Federal de Pelotas, Pelotas.

A polinização é de grande importância na produção de várias culturas agrícolas. No morangueiro, é facilitada com o auxílio de insetos, como as abelhas, que desempenham papel fundamental na redução das taxas de deformação dos frutos. Os objetivos deste trabalho foram: a) realizar um inventário dos visitantes florais associados ao morangueiro da cultivar Albion cultivado no sistema semi-hidropônico na região da Serra Gaúcha e identificar potenciais espécies de abelhas nativas polinizadoras; b) determinar os efeitos letais (mortalidade), subletais (atividade locomotora), além do perfil de detoxificação de inseticidas e acaricidas utilizados na cultura do morangueiro sobre as abelhas nativas sem ferrão *Melipona quadrifasciata* e *Tetragonisca fiebrigi*; c) realizar uma revisão sistemática seguida de meta-análise abordando os efeitos dos inseticidas sobre abelhas. No primeiro estudo, os insetos presentes na cultura do morangueiro foram capturados as 9h30min, 12h30min e 15h30min, em três cultivos comerciais localizados nos municípios de Bento Gonçalves e Farroupilha. No segundo estudo, as abelhas foram expostas a formulação comercial dos inseticidas novalurom (Rimon Supra®), espinetoram (Delegate®) e tiametoxam (Actara® 250 WG) e do acaricida abamectina (Vertimec® 18 EC), via oral e tópica, determinando a CL_{50} e DL_{50}, respectivamente. O efeito comportamental sobre a atividade locomotora das abelhas foi avaliado após a exposição a doses subletais (CL_{10} e CL_{50}). A inibição da enzima esterase foi avaliada em *T. fiebrigi* após a aplicação de tiametoxam. No terceiro estudo, foram investigados trabalhos que avaliaram os efeitos dos grupos dos organofosforados, piretróides, neonicotinóides, inibidores da síntese de quitina e espinosinas em abelhas. As flores do morangueiro foram visitadas por 47 espécies de insetos sendo *Apis mellifera* a mais abundante, constante, dominante e frequente. Doze espécies de abelhas nativas foram identificadas sendo *Tetragonisca fiebrigi*, *Plebeia emerina* e *Plebeia remota* as com maior potencial para polinização dirigida do morangueiro em cultivo protegido, devido a abundância e facilidade de manejo. O efeito secundário dos inseticidas variou conforme a forma de exposição e espécie de abelha. Na via oral, *M. quadrifasciata* foi mais sensível que *T. fiebrigi* a todos os inseticidas, com exceção para abamectina, enquanto na via tópica, *T. fiebrigi* foi mais susceptível. Tiametoxam seguido por

espinetoram e abamectina foram os mais letais, para ambas as espécies e formas de exposição, enquanto novalurom não foi prejudicial. A atividade locomotora das abelhas foi alterada quando expostas a doses subletais de abamectina, espinetoram e tiametoxam. Os ensaios enzimáticos indicaram que as esterases podem estar envolvidas no processo de detoxificação do tiametoxam em *T. fiebrigi*. Nas condições avaliadas, espinetoram e abamectina podem ser tão tóxicos como tiametoxam para *M. quadrifasciata* e *T. fiebrigi*. Estes resultados devem ser confirmados em experimentos de campo para definir a possibilidade do gerenciamento integrado de pragas e polinizadores. Os neonicotinoides são o grupo de inseticidas mais estudado no mundo e mais comumente relatados por causar efeitos subletais em abelhas. Muitas lacunas sobre os impactos dos inseticidas ainda permanecem, especialmente em relação as abelhas nativas sem ferrão e a susceptibilidade em condições de campo.

Palavras-chave: *Fragaria x ananassa* Duch; polinização entomófila; abelhas sem ferrão; produção de alimentos; toxicidade de agrotóxicos

Abstract

PIOVESAN, Bruna. **Floral visitors inventory on strawberry crop and effects of insecticides on native bees *Melipona quadrifasciata* and *Tetragonisca fiebrigi* (Hymenoptera: Apidae).** 2018. 107f. Master of Science - Graduate Program in Plant Protection. Federal University of Pelotas, Pelotas.

Pollination is of great importance in the production of various agricultural crops. In the strawberry, it is facilitated with the aid of insects, such as bees, which play a fundamental role in reducing the rates of deformation of the fruits. The objectives of this study were: a) make an inventory of floral visitors associated with the strawberry of the Albion cultivar grown in the semi-hydroponic system in the Serra Gaúcha region and to identify potential species of native pollinating bees; b) determine the lethal effects (mortality), sublethal (locomotor activity), as well as the detoxification profile of insecticides and acaricides used in strawberry cultivation on native stingless bees *Melipona quadrifasciata* and *Tetragonisca fiebrigi*; c) conduct a systematic review followed by meta-analysis addressing the effects of insecticides on bees. In first study, the insects present in the strawberry crop were captured at 9:30 am, 12:30 a.m. and 3:30 p.m., in three commercial crops located in Bento Gonçalves and Farroupilha. In second study, bees were exposed to the commercial formulation of the insecticides novaluron (Rimon Supra®), spinetoram (Delegate®) and thiamethoxam (Actara® 250 WG) and acaricide abamectin (Vertimec® 18 EC), through the oral and topical route, to determine the LC_{50} and LD_{50}, respectively. The behavioral effect on locomotor activity of bees was evaluated after exposure to sublethal doses (CL_{10} and CL_{50}). Inhibition of the esterase enzyme was evaluated in *T. fiebrigi* after application of thiamethoxam. In third study, we investigated the effects of organophosphates, pyrethroids, neonicotinoids, inhibitors of chitin synthesis and spinosyns in bees. The flowers of the strawberry were visited by 47 species of insects being *Apis mellifera* the most abundant, constant, dominant and frequent. Twelve species of native bees were identified as *Tetragonisca fiebrigi*, *Plebeia emerina* and *Plebeia remota* as those with greater potential for directed pollination of the strawberry in protected cultivation due to abundance and ease of handling. The secondary effect of insecticides varied according to the form of exposure and species of bee. In the oral route, *M. quadrifasciata* was more sensitive than *T. fiebrigi* to all insecticides, except for abamectin, whereas in the topical route *T. fiebrigi* was more susceptible. Thiamethoxam followed by spinetoram and abamectin were the most lethal, for both species and forms of exposure, while novaluron was not harmful. The locomotor activity of bees was altered when exposed to sublethal doses of abamectin, spinetoram

and thiamethoxam. Enzyme assays indicated that the esterases may be involved in the detoxification process of thiamethoxam in *T. fiebrigi*. Under the conditions evaluated, spinetoram and abamectin may be as toxic as thiamethoxam for *M. quadrifasciata* and *T. fiebrigi.* These results should be confirmed in field experiments to define the possibility of integrated pest and pollinator management. Neonicotinoids are the most studied group of insecticides in the world and most commonly reported to cause sublethal effects on bees. Many gaps on the impacts of insecticides still remain, especially in relation to native stingless bees and susceptibility under field conditions.

Key-words: *Fragaria x ananassa* Duch; entomophilic pollination; stingless bees; food production; toxicity of pesticides

Lista de Figuras

Artigo 1

Artigo 2

Artigo 3

Lista de Tabelas

Artigo 1

Artigo 2

Sumário

INTRODUÇÃO GERAL

Aproximadamente 75% das 240.000 espécies de plantas existentes no mundo dependem da polinização, seja pelo vento, água, animais, insetos ou outros agentes polinizadores (SCHULP et al., 2014; WITTER et al., 2014). Dentre estas, encontram-se diversas culturas de interesse agrícola, como morango, canola, girassol, tomate e melancia (KREMEN et al., 2002; GARIBALDI et al., 2011; BOMMARCO et al., 2012; ABROL et al., 2017).

O morangueiro (*Fragaria x Ananassa* Duch.) é uma planta pertencente à família Rosaceae, cultivada em diferentes sistemas de cultivo e nas mais variadas regiões do mundo (CHANG et al., 2001). Os morangos resultam do desenvolvimento do receptáculo da flor, originando a polpa comestível, enquanto que o verdadeiro fruto é denominado botanicamente de aquênio (WITTER et al., 2014). Além da produção em sistema convencional no solo, os morangos podem ser cultivados em sistemas suspensos (com ou sem solo) e protegidos, como é o caso do sistema semi-hidropônico (BORTOLOZZO et al., 2007). A melhoria das condições de manejo, assim como menores problemas fitossanitários para a cultura são algumas vantagens deste sistema (BORTOLOZZO et al., 2007; GONÇALVES et al., 2016). A maioria das cultivares comerciais de morangueiro apresentam flores hermafroditas, ou seja, possuem órgãos masculinos e femininos na mesma flor, entretanto dificilmente ocorre coincidência da liberação do pólen com a receptividade do estigma, tornando-se necessário a fecundação cruzada, que sofre influência de diversos agentes, como o vento e insetos polinizadores (ANTUNES et al., 2007; WITTER et al., 2014).

A polinização por insetos desempenha papel fundamental na redução das taxas de malformação dos frutos (FREE, 1993), aumentando o lucro para o produtor em função dos melhores preços obtidos com a comercialização do fruto *in natura*. As abelhas são os principais polinizadores, pois devido à presença de estruturas

adaptadas no corpo (corbícula, escopa ou cerdas) acabam transportando o pólen entre as flores durante a coleta de alimento (SILVEIRA et al., 2002).

A espécie *Apis mellifera* Linnaeus, 1758 (Hymenoptera: Apidae) está entre os polinizadores mais utilizados em cultivos comerciais, no entanto diversas outras espécies, como as abelhas nativas sem ferrão vem sendo utilizadas como alternativa (SLAA et al., 2006). Estas abelhas são consideradas um grupo muito diversificado e abundante tanto em regiões tropicais como subtropicais do planeta (SOMMEIJER; RUIJTER, 1999). Por apresentarem facilidade de manejo, são utilizadas com sucesso em um grande número de plantas, incluindo aquelas cultivadas em sistemas protegidos. Flores de tomate-cereja e pimentão polinizadas por *Melipona quadrifasciata* produziram frutos de melhor qualidade, mais pesados e maiores em comparação à autopolinização natural (ROSELINO; SANTOS; BEGO, 2010; MEYRELLES, 2013). Efeitos significativos na frutificação de cultivares de morangueiro produzidos em sistema protegido foram observados com a polinização pelas espécies *Plebeia nigriceps* e *Tetragonisca fiebrigi* (ANTUNES et al., 2007; WITTER et al., 2012). Malagodi-Braga e Kleinert (2004) verificaram que uma colônia de *Tetragonisca angustula* em estufa com 1350 plantas de morango foi adequada para polinização das flores primárias da cultivar Oso Grande.

Durante a condução da cultura do morangueiro, geralmente são utilizados agrotóxicos para o manejo de insetos, ácaros ou doenças que, se não controlados, podem prejudicar a produção (BERNARDI et al., 2015). No entanto, estes produtos podem apresentar efeitos negativos em organismos não alvo, como inimigos naturais e polinizadores (SPADOTTO et al., 2004). As abelhas podem ser expostas a estes compostos através do contato direto, com a superfície do corpo, ou indireto, por meio da ingestão. Neste último caso, a atratividade das flores em pleno florescimento que estejam contaminadas com resíduos de determinados grupos de inseticidas é apontada como uma das principais causas da mortalidade de abelhas (BORTOLOTTI et al., 2003; FREITAS; PINHEIRO, 2010). O manejo integrado de artrópodes-praga e polinizadores nas culturas é, portanto, um aspecto que não deve ser negligenciado, dada a contribuição dos últimos para a produção de alimentos (KREMEN, 2004; POTTS et al., 2010).

A toxicidade de inseticidas para as abelhas tem como base a determinação de uma dose média letal (DL_{50}) ou uma concentração média letal (CL_{50})

representando, respectivamente, uma dose ou concentração capaz de provocar a mortalidade de 50% da população experimental (LOURENÇO, 2012). Além de considerar a mortalidade letal (a curto prazo), é importante analisar os efeitos causados a longo prazo, denominados subletais ou crônicos que não provocam diretamente a mortalidade, mas que podem estar associadas ao comprometimento das atividades dos indivíduos e consequentemente ao declínio da colônia (BORTOLOTTI et al., 2003; THOMPSOM; MAUS, 2007; FREITAS; PINHEIRO, 2010). Recentemente, diversos estudos têm demonstrado graves distúrbios tanto a nível de colônia como de indivíduo decorrente da exposição prolongada a baixas doses ou concentrações de inseticidas (BARON et al., 2017; FRIOL et al., 2017; ALKASSAB; KIRCHNER, 2018).

Dentre os principais grupos químicos de inseticidas e/ou acaricidas com registro no Ministério da Agricultura para uso na cultura do morangueiro no Brasil, destacam-se as avermectinas, as espinosinas, os inibidores da síntese de quitina e os neonicotinóides (AGROFIT, 2018).

Os neonicotinóides apresentam ação neurotóxica capaz de interferir na transmissão dos impulsos nervosos dos insetos. A principal causa de investigação desta classe de inseticidas está relacionada à sua ação sistêmica e residual na planta, pois a substância ativa é capaz de translocar até o pólen e néctar das flores, entrando em contato com organismos não alvos, como os polinizadores (BLACQUIÉRE et al., 2012). A exposição contínua de tiametoxam na alimentação proporcionou decréscimo no consumo de alimento e na longevidade de duas espécies de abelhas (*Bombus terrestris* e *Scaptotrigona bipunctata*) (ROSA, 2014). De acordo com Faria (2009), tremores e morte são sintomas característicos de intoxicação por inseticidas neonicotinóides.

Recentemente, produtos denominados “bioinseticidas” estão sendo utilizados no controle de pragas como alternativa aos produtos já existentes no mercado (VILLAVERDE et al., 2014). O grupo das espinosinas se destaca, e pode ser encontrado sob a forma dos princípios ativos espinetoram e espinosade (AGROFIT, 2018). Espinosade é derivado da fermentação aeróbica do actinomiceto *Saccharopolyspora spinosa* Mertz e Yao, 1990, e espinetoram é oriundo da modificação sintética do espinosade, ambos com capacidade para controle de diversas espécies de insetos (SPARKS et al., 1998). Alguns trabalhos destacam que

inseticidas deste grupo apresentam baixa toxicidade residual, em relação a outros inseticidas e maior seletividade aos insetos benéficos (WILLIAMS; VALLE; VIÑUELA, 2003; RUIZ et al., 2008). Entretanto, os riscos impostos por novos inseticidas, principalmente bioinseticidas, são em grande parte desconhecidos, apesar do aumento da utilização e sua segurança percebida do ambiente, que se baseia na sua origem natural. Para a abelha *Melipona quadrifasciata*, espinosade apresentou maior toxicidade oral do que o inseticida neonicotinóide imidacloprido, além de afetar a capacidade de voo, o que pode comprometer a atividade de forrageamento, e consequentemente resultar em graves efeitos para a colmeia (TOMÉ et al., 2015).

Inseticidas reguladores do crescimento dos insetos também podem exercer efeitos sobre polinizadores, especialmente ao agirem sobre fases imaturas (MOMMAERTS; STERK; SMAGGHE, 2006; CUTLER; SCOTT-DUPREE, 2007).

Os acaricidas representam uma das ferramentas de manejo mais utilizadas pelos agricultores para o controle de ácaros na cultura do morango (MORAES; FLECHTMANN, 2008). A resistência de ácaros a muitos desses produtos fez com que doses mais altas e aplicações mais frequentes em misturas com outros produtos fossem adotadas, no sentido de aumentar a eficiência de controle dos ácaros-praga (MORAES; FLECHTMANN, 2008). No entanto, parecem provocar diversos efeitos sobre insetos benéficos. Carvalho et al. (2009) verificaram que abamectina foi extremamente tóxica para adultos de *A. mellifera*, independentemente do modo de exposição. Em relação aos efeitos deste grupo sobre as abelhas nativas, poucas informações estão disponíveis (DEL SARTO et al., 2014).

Além de mudanças observadas visualmente no comportamento das abelhas após exposição a estas substâncias, é fundamental o conhecimento de como elas agem no interior do seu corpo, a nível de órgãos, células e enzimas, que são importantes para a sobrevivência do indivíduo. Como a maioria dos outros insetos, as abelhas dependem de um conjunto de enzimas de detoxificação para metabolizar agrotóxicos (JOHNSON et al., 2012). Análise do genoma de espécies de abelhas realizadas até o momento, como é o caso de algumas do gênero *Bombus*, *Megachile rotundata* e *A. mellifera*, demonstram a presença de menor número de genes envolvidos com a detoxificação e respostas a estresses comparado a outros insetos, como por exemplo, *Drosophila melanogaster* (Diptera: Drosophilidae) o que pode explicar a alta susceptibilidade das abelhas (CLAUDIANOS et al., 2006; XU et al.,

2013). No entanto, apesar do avanço das técnicas moleculares, trabalhos nesta linha ainda são escassos para a maioria das espécies de abelhas nativas. Neste sentido, na tentativa de entender um pouco mais sobre o sistema de defesa destas abelhas, algumas ferramentas têm sido utilizadas. A aplicação de inibidores enzimáticos previamente a exposição de inseticidas pode fornecer informações a respeito do perfil metabólico envolvido no processo de detoxificação de substâncias, o que tem importância para a questão da susceptibilidade (IWASA et al., 2004). Esterases e glutationas, por exemplo, parecem auxiliar na integridade do sistema nervoso e metabolismo de *A. mellifera* (CARVALHO et al., 2013).

Pesquisas com o tema “abelhas, polinização e agrotóxicos” até então eram pouco realizadas no mundo. A importância econômica destes insetos devia-se a produção de mel, primeiro alimento açucarado de origem animal conhecido desde os tempos pré-históricos (CRANE, 1999). No entanto, estima-se que o valor das abelhas como polinizadoras seja maior do que como produtoras de mel, movimentando grandes valores econômicos em todo planeta (GALLAI et al., 2009; GIANNINI et al., 2015). O desaparecimento de grandes populações em alguns países da Europa e nos Estados Unidos, conhecida por Desordem do Colapso de Colônias (DCC), no entanto, trouxe preocupação para toda sociedade e impulsionou o crescente desenvolvimento de pesquisas na busca das possíveis causas (VAN ENGELSDORP et al., 2010). Atualmente, a relação com inseticidas é um dos fatores mais estudados, uma vez que são utilizados em várias culturas polinizadas por abelhas em todo o mundo (BLACQUIÈRE et al., 2012). Diversas informações estão disponíveis na literatura, no entanto, muitas são contraditórias, o que torna necessário um debate global sobre o tópico, compilando todos os dados até então obtidos.

Este trabalho foi realizado com os seguintes objetivos: a) conhecer os visitantes florais associados ao morangueiro da cultivar Albion cultivado no sistema semi-hidropônico na região da Serra Gaúcha e identificar potenciais espécies de abelhas nativas polinizadoras; b) avaliar os efeitos letais e subletais dos inseticidas/acaricidas abamectina, espinetoram, novalurom e tiametoxam sobre as abelhas nativas *Melipona quadrifasciata* e *Tetragonisca fiebrigi;* c) realizar uma revisão sistemática seguida de meta-análise abordando os efeitos dos inseticidas sobre abelhas.

Artigo 1- Horticultura Brasileira

Visitantes florais e potenciais polinizadores da cultura do morangueiro em cultivo semi-hidropônico

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Visitantes florais e potenciais polinizadores da cultura do morangueiro em cultivo semi-hidropônico

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RESUMO

O cultivo do morangueiro no sistema semi-hidropônico tem aumentado na região Sul do Brasil por facilitar o manejo, além de evitar adversidades climáticas, pragas e doenças. Nesse sistema, o ambiente protegido pode dificultar o acesso de insetos polinizadores, fundamentais para a produtividade da cultura. Este trabalho teve como objetivo conhecer os visitantes florais associados ao morangueiro da cultivar Albion cultivado no sistema semi-hidropônico e identificar potenciais espécies de abelhas nativas polinizadoras. Os insetos foram capturados em diferentes períodos do dia (9h30min, 12h30min e 15h30min) em três cultivos comerciais localizados nos municípios de Bento Gonçalves e Farroupilha, Rio Grande do Sul. Em cada área de estudo foram realizados três dias de amostragens durante janeiro de 2017. As flores foram visitadas por 47 espécies de insetos. *Apis mellifera* foi a espécie mais abundante, constante, dominante e frequente. Doze espécies de abelhas nativas foram identificadas: *Tetragonisca fiebrigi*, *Tetrapedia* sp., *Trigona spinipes*, *Schwarziana quadripunctata*, *Dialictus* sp.1, *Dialictus* sp.2, *Augochloropsis* sp.1, *Augochloropsis* sp.2, *Augochlora* sp.1, *Plebeia emerina*, *Plebeia remota* e *Bombus pauloensis*. Todas essas espécies são potenciais polinizadoras da cultura. As espécies nativas *Tetragonisca fiebrigi*, *Plebeia emerina* e *Plebeia remota* apresentam potencial para polinização dirigida do morangueiro em cultivo protegido devido a abundância e facilidade de manejo.

Palavras-chave: *Fragaria x ananassa* Duch., polinização, agricultura, diversidade, abelhas nativas.

ABSTRACT

Floral visitors and potential pollinators of the strawberry crop in semi-hydroponic system

Strawberry cultivation in the semi-hydroponic system has increased in the Southern Region of Brazil for facilitating the management, besides avoiding climatic adversities, pests and diseases. However, in this system the protected environment can hamper the access of insects pollinators, fundamental for the productivity of the crop. This work aimed to know the floral visitors associated with the strawberry of the cultivar Albion cultivated in the semi-hydroponic system and to identify potential species of native pollinating bees. The insects were captured at different times of the day (9:30 a.m., 12:30 a.m. and 3:30 p.m.) in three commercial crops located in Bento Gonçalves and

Farroupilha, RS. In each study area, three days of sampling were carried out during January 2017. The flowers were visited by 47 species of insects. *Apis mellifera* was the most abundant, constant, dominant and frequent species. Twelve species of native bees were identified: *Tetragonisca fiebrigi*, *Tetrapedia* sp., *Trigona spinipes*, *Schwarziana quadripunctata*, *Dialictus* sp.1, *Dialictus* sp.2, *Augochloropsis* sp.1, *Augochloropsis* sp.2, *Augochlora* sp.1, *Plebeia emerina*, *Plebeia remota* and *Bombus pauloensis*. All these species are potential pollinators of the crop. The native species *Tetragonisca fiebrigi*, *Plebeia emerina* and *Plebeia remota* present potential for directed pollination of the strawberry in protected crop due to abundance and ease of handling.

Keywords: *Fragaria x ananassa* Duch., pollination, agriculture, diversity, native bees.

INTRODUÇÃO

No grupo das pequenas frutas, o morangueiro (*Fragaria x ananassa* Duch.) é a cultura mais difundida no mundo, sendo cultivado em praticamente todos os países de clima temperado a tropical (Galvão, 2014). No Brasil, o cultivo concentra-se em diversas regiões, com maior destaque nos estados de Minas Gerais, Paraná, Rio Grande do Sul, São Paulo, Espírito Santo, Santa Catarina e o Distrito Federal (Antunes & Peres, 2013). A produtividade média é de cerca de 30 t/ha, ocorrendo diferença acentuadas entre regiões, em função do local e sistema de cultivo adotado (Antunes *et al*., 2015).

Nas últimas décadas, a utilização do sistema semi-hidropônico em estufa do tipo túnel alto e o melhoramento genético das plantas representaram importantes avanços tecnológicos que contribuíram para ampliação da área cultivada (Calvete *et al*., 2007; Antunes *et al*., 2015). O sistema semi-hidropônico apresenta como uma das principais vantagens a facilidade na realização dos tratos culturais comparado ao sistema convencional, uma vez que consiste em bancadas que sustentam embalagens com substratos ligados ao sistema de irrigação (Gonçalves *et al*., 2016). O desenvolvimento de cultivares indiferentes ao fotoperíodo (dia-neutro), ampliou o período de produção, possibilitando o cultivo em locais de temperaturas amenas durante o florescimento (10-20ºC) e ao longo de todo o ano (Costa *et al*., 2014). No Rio Grande do Sul, a Serra Gaúcha é uma importante região produtora de morangos em sistemas protegidos com esta característica fotoperiódica (Costa *et al*., 2014) com destaque para as cultivares Aromas e Albion (Antunes *et al*., 2011).

Na cultura do morangueiro, a polinização é fundamental para atingir a maturidade fisiológica, gerar frutos e sementes (Chagnon *et al*., 1989; Witter *et al*., 2014). Embora a maioria das cultivares comerciais sejam autoférteis, algumas apresentam variações na sua capacidade de autopolinização (Witter *et al*., 2014). Em muitos casos o órgão reprodutivo feminino (gineceu) torna-se receptivo antes do órgão masculino estar disponível (androceu), favorecendo a polinização cruzada (Nye & Anderson, 1974; Roselino *et al*., 2009). Agentes polinizadores como as abelhas apresentam capacidade de transportar o pólen entre diferentes plantas, promovendo a polinização (Zebrowska, 1998).

O cultivo em ambiente protegido, no entanto, pode funcionar como uma barreira física à ação de insetos polinizadores (Antunes *et al*., 2007). Quantidades insuficientes de pólen associado à falta de indivíduos para transportá-lo entre as flores resultam em imperfeita fertilização do óvulo, o que diminui a produção de hormônios, principalmente auxinas, que promoveriam o crescimento da área do receptáculo, próximo ao aquênio (Nitsch, 1950; Abrol *et al*., 2017). Tais fatores contribuem para elevadas porcentagens de deformações dos frutos e menor rendimento (Zebrowska, 1998; Malagodi-Braga, 2002; Witter *et al*., 2012). Em função da importância mundial desta frutífera e do elevado investimento na sua implantação e condução, é fundamental levar em consideração a polinização. Estudos sobre as espécies de visitantes florais em morangueiro foram desenvolvidos em cultivo aberto (Malagodi-Braga, 2002; Albano *et al*., 2009; Connelly *et al*., 2015; Abrol *et al*., 2017), enquanto em ambientes protegidos até então não existem informações disponíveis.

Os objetivos deste trabalho foram conhecer os visitantes florais associados ao morangueiro da cultivar Albion cultivado no sistema semi-hidropônico na Região da Serra Gaúcha e identificar potenciais espécies de abelhas nativas polinizadoras.

MATERIAL E MÉTODOS

Áreas de estudo

As espécies de visitantes florais associados à cultura do morangueiro foram inventariadas durante a safra 2016/17 em cultivos comerciais da cultivar Albion. As coletas foram realizadas em três áreas (uma no município de Bento Gonçalves/RS e duas no município de Farroupilha/RS) totalizando três dias de amostragem por localidade, no

mês de janeiro de 2017 (Figura 2 e Tabela 1). Nos três locais, o sistema de produção utilizado foi o semi-hidropônico em estufa do tipo túnel alto. Durante o período do estudo, foram realizados os manejos tradicionais na cultura: retiradas de estolões, folhas secas e em excesso, aplicação de agrotóxicos e colheita dos frutos. Todas as coletas foram realizadas em dias ensolarados, com ventos amenos e temperatura maior ou igual a 15ºC.

Amostragem dos visitantes florais

Foram escolhidos aleatoriamente quatro transectos/linhas de cultivo com 25 metros de comprimento (Adaptado de Vaissiéri *et al*., 2011). Os espécimes foram identificados visualmente dentro da área de estudo durante três vezes ao longo do dia (09h30min, 12h30min e 15h30min). Dois coletores previamente treinados percorreram ao mesmo tempo cada transecto pré-determinado durante 15 minutos (7,5 min de ida e 7,5 min de volta). Para captura foi utilizada rede entomológica e câmara mortífera, composta por tubo falcon (50 mL) contendo algodão umedecido com acetato de etila.

Conservação dos indivíduos

No laboratório de Entomologia da Embrapa Uva e Vinho (Bento Gonçalves, RS) os insetos foram contados, triados e montados em alfinetes entomológicos etiquetados (Malagodi-Braga, 2002). A identificação dos espécimes foi realizada utilizando microscópio estereoscópico, com a ajuda de chaves dicotômicas de literatura especializada para cada família (Silveira *et al*., 2002). Após separados por família, os insetos foram identificados com auxílio de taxonomistas.

Análise dos dados

A diversidade de insetos nas áreas de estudo foi avaliada por meio do perfil de diversidade de Hill (1973), utilizando o programa estatístico R. Análise faunística foi realizada para definir as classes de abundância, constância, dominância e frequência das espécies de abelhas, conforme descrito por Silveira Neto *et al*. (1976).

RESULTADOS E DISCUSSÃO

Uma grande variedade de insetos (47 espécies) foi coletada visitando as flores do morangueiro da cultivar Albion em ambiente protegido, incluindo espécies das ordens Hymenoptera, Diptera, Coleoptera, Hemiptera e Lepidoptera (Tabela 2).

Ao comparar as áreas de estudos (A, B e C) através da análise de série de Hill, observou-se maior diversidade de visitantes florais na área B, seguida da área A, quando comparadas a área C (0= riqueza de espécies; 1= Índice de Shannon Wiener; 2= Índice de Simpson) (Figura 1). No entanto, independente da diversidade de insetos e de as áreas serem particularmente diferentes, observou-se que à medida que os valores de equabilidade foram acrescentados à série de Hill (à direita da curva) as comunidades acabaram comportando-se de modo muito semelhante (Figura 1), indicando similar distribuição das espécies. Embora o manejo de agrotóxicos tenha sido parecido nas três áreas durante o período de estudo, na área C os históricos de aplicações ao longo do desenvolvimento da cultura demonstraram a adoção de manejo mais intenso em relação as outras áreas. Isto pode ser explicado em função da ocorrência neste local de importantes insetos prejudiciais à cultura, como a broca-do-morango (*Lobiopa insularis*) (Castelnau, 1840) (Coleoptera: Nitidulidae) e tripes (*Frankliniella occidentalis*) (Pergande) (Thysanoptera: Thripidae). A primeira espécie se alimenta preferencialmente de frutos maduros, enquanto a segunda, danifica as estruturas reprodutivas das flores e frutos verdes, provocando manchas de coloração bronzeada ao redor dos aquênios (Bernardi *et al*., 2015).

Além das questões de manejo da cultura, as diferenças nos valores de abundância e diversidade podem estar diretamente associadas a desigualdade entre as paisagens próximas aos cultivos. A presença de maiores fragmentos de floresta no entorno da área B pode explicar o maior índice de diversidade, uma vez que estes locais servem como abrigo e nidificação para várias espécies, especialmente as nativas (Figura 2 (B)). Na área A, apesar de pequenos fragmentos, estes estavam presentes em maior quantidade e mais próximos aos cultivos protegidos do que na área C (Figura 2 (A) e (C)). A presença de um fragmento lateral apenas com plantas de eucalipto e o entorno com grandes cultivos de frutíferas (caquizeiros, videiras e pessegueiros) pode ter influenciado a menor diversidade observada na área C (Figura 2 (C)). Segundo Ricketts *et al*. (2008) as

alterações nas paisagens devido a intensificação da agricultura ameaçam diretamente a biodiversidade. Diversos trabalhos têm demonstrado que a paisagem circundante exerce efeito sobre a estabilidade dos serviços ecossistêmicos, como é o caso da polinização por insetos (Klein *et al*., 2007; Halinski *et al*., 2015). Garibaldi *et al*. (2011) observaram que a riqueza de abelhas visitantes florais, a taxa de visitação e a produção de frutos de 21 culturas agrícolas estudadas em 15 países foi reduzida com o aumento da distância das áreas naturais. Cabe destacar, que ao longo dos anos, locais e regiões as populações de insetos podem variar e, portanto, a extrapolação de resultados para outras condições deve considerar tais fatores.

A ordem Hymenoptera foi a mais abundante em ambas as áreas de estudo, seguida da ordem Diptera nas áreas A e C e da ordem Coleoptera na área B (Figura 3 (A), (B) e (C)). A maior abundância de Coleoptera nesta área, provavelmente ocorreu devido à alta população de *Diabrotica speciosa* (Germar, 1824) (Coleoptera: Chrysomelidae). O predomínio de insetos destas três ordens também foi observado em flores de morangueiro (cv. Campinas e cv. Dover) cultivado em sistema aberto (Malagodi-Braga, 2002). Isto indica que mesmo sob cultivo protegido, os insetos vão até as flores em busca de recursos alimentares, e as abelhas, principais representantes dos himenópteros, se destacam, pois delas retiram néctar e grandes quantidades de pólen para alimentação das crias.

Em relação à riqueza de espécies, a ordem Diptera se destacou das demais nas áreas A e B. Outros estudos também observaram alta riqueza de insetos desta ordem em flores de morangueiro das cultivares Chandler e Camarosa (Albano *et al*., 2009; Abrol *et al*., 2017). O hábito predador e generalista da maioria das espécies de Diptera e Coleoptera coletadas, pode explicar a presença nas flores, pois além das presas que eventualmente possam aparecer, também se alimentam do pólen, excelente alimento protéico (Casari & Ide, 2012).

A família Syrphidae (Diptera) apresentou várias espécies, dentre elas as do gênero *Toxomerus*, *Eristalis*, *Palpada* e *Quichuana*. Pelo fato de quando adultos mimetizarem abelhas e vespas e se alimentarem quase exclusivamente de pólen e néctar é possível que estas espécies contribuam para a polinização do morangueiro, uma vez que ao visitar as flores carregam pólen aderido às suas cerdas (Marinoni *et al*., 2007). Outras espécies das famílias Faniidae, Sarcophagidae, Calliphoridae e Sepsidae (Diptera) são considerados

apenas visitantes florais, pois a maioria desenvolve-se em matéria orgânica em decomposição (Couri *et al*., 2005).

Coleópteros das famílias Curculionidae, Cantharidae, Chrysomelidae (*Diabrotica speciosa*), Tenebrionidae (*Lagria villosa*) (Fabricius, 1783) e Scarabaeidae (*Macraspis dichroa* Mannerheim, 1829) estavam presentes nas flores em busca de alimento. *D. speciosa* foi observada danificando as estruturas reprodutivas do morangueiro, no entanto, já foi relatada como polinizadora em algumas culturas como *Momordica charantia* Linnaeus (1763) (Cucurbitaceae) (Lenzi *et al*., 2005). *Eriopis connexa* (Germar, 1824) (Coleoptera: Coccinellidae) e *Coleomegilla quadrifasciata* (Schöenherr, 1808) (Coleoptera: Coccinellidae) são espécies predadoras, e assim como os Hemipteras das famílias Geocoridae e Miridae estão presentes no ambiente desempenhando importante papel no controle biológico de insetos-pragas. Rhyparochromidae (Hemiptera) e as espécies *Hylephila phylaeus phylaeus* (Drurys, 1773), *Conga iheringii* (Mabile, 1891) (Lepidoptera: Hesperiidae), assim como *Duponchelia fovealis* Zeller, 1847 (Lepidopetra: Carambidae) foram considerados visitantes florais, pois estavam presentes apenas em busca de alimento e algumas vezes causando danos ao morangueiro. *Duponchelia fovealis* é uma espécie exótica, que se tornou praga-chave do morangueiro no Brasil, pelo fato de causar severas injúrias às folhas e frutos (Zawadneak *et al*., 2016).

A riqueza de espécies visitantes florais da ordem Hymenoptera foi semelhante em ambas as áreas de estudo, com oito espécies nas áreas A e C, e sete espécies na área B, compostas por abelhas pertencentes às famílias Apidae e Halictidae (Tabela 2). A espécie mais abundante foi *Apis mellifera*, representando 62,7% dos himenópteros visitantes florais amostrados na área A, 72,4% na área B e 90,1% amostrados na área C (Figura 4). De acordo com a análise faunística, esta espécie foi classificada como a mais abundante, constante, dominante e muito frequente em todas as áreas amostradas (Tabela 3). Resultados semelhantes foram observados em flores de morangueiro da cultivar Chandler (Abrol *et al*., 2017). Esta abelha é facilmente encontrada e representa a principal espécie manejada para polinização de culturas agrícolas no mundo inteiro (Minussi *et al*., 2007).

Diversas espécies de abelhas nativas visitaram as flores (Tabela 2). Foram encontradas um total de 12 espécies potencialmente polinizadoras da cultura. Na área A, *Dialictus* sp.1 e *Tetragonisca fiebrigi* foram as espécies mais abundantes (18,2% e 13,6%, respectivamente), seguidas de *Trigona spinipes* (1,8%) e *Tetrapedia* sp., *Schwarziana*

quadripunctata, *Dialictus* sp.2 e *Augochloropsis* sp.1 (todas com 0,9%) (Figura 4). As espécies de abelhas nativas mais abundantes encontradas nas flores de morangueiro na área B foram, *Plebeia remota* (8,1%), *Dialictus* sp.1 (6,5%) e *Plebeia emerina* (6,5%), seguidas de *Trigona spinipes* (4,9%), *Tetragonisca fiebrigi* e *Dialictus* sp.2 (ambas com 0,8%). Enquanto na área C, as abelhas nativas representaram apenas cerca de 10% dos himenópteros visitantes florais coletados (Figura 4). Porém, entre estas observou-se riqueza de espécies, equivalente a observada nas demais áreas. Espécies pertencentes a família Apidae, como *Trigona spinipes*, *Plebeia emerina*, *Plebeia remota* e *Bombus pauloensis* e a família Halictidae, incluindo *Dialictus* sp.1, *Augochlora* sp.1 e *Augochloropsis* sp.2 foram encontradas visitando as flores. Isto indica que apesar da menor quantidade de mata nativa no entorno e do manejo desta área, as abelhas nativas estavam presentes no ambiente e conseguiram chegar às flores do morangueiro, embora com menor intensidade que *A. mellifera*.

Além da visita às flores em busca de alimento (pólen e néctar), as abelhas polinizam as plantas através do transporte de pólen em estruturas do seu corpo. *Tetragonisca fiebrigi*, *Plebeia remota* e *Plebeia emerina* são facilmente encontradas nos biomas do Rio Grande do Sul (Camargo & Pedro, 2013) e em função de se adaptarem bem ao manejo racional têm grande potencial para uso em larga escala em cultivos protegidos. *Tetragonisca angustula* e *Plebeia nigriceps*, pertencentes aos mesmos gêneros das espécies observadas neste trabalho, mostraram-se eficientes na polinização de flores de morangueiro cultivado em ambiente protegido, reduzindo principalmente o percentual de frutos deformados (Antunes *et al*., 2007; Witter *et al*., 2012). *T. spinipes* e *B. pauloensis* também realizam a polinização, porém são espécies com maior dificuldade de manejo, pois constroem seus ninhos em árvores e cavidades pré-existentes, respectivamente (Marsaro Júnior *et al*., 2017).

Dialictus sp.1 foi a abelha nativa mais abundante nas três áreas. O gênero *Dialictus*, assim como os gêneros *Augochlora* e *Augochloropsis* abrangem as abelhas solitárias ou semi-sociais, conhecidas como abelhas-metálicas (Silveira *et al*., 2002). Estas abelhas são frequentemente encontradas polinizando diversas culturas, estando entre os gêneros com maior riqueza de espécies no Sul do Brasil (Halinski *et al*., 2015). Apesar da facilidade de serem encontradas nos agroecossistemas, e do importante serviço de polinização que desempenham, pouco se conhece sobre sua biologia e o fato de serem

poucos sociais dificulta o manejo racional para polinização. Cabe, portanto, adotar medidas de conservação destas espécies no próprio habitat natural, através da manutenção dos fragmentos florestais próximo aos cultivos do morango.

O horário de amostragem com maior abundância e riqueza de abelhas nas flores foi às 12h30min em ambas as áreas, estas representadas exclusivamente por espécies pertencentes às famílias Apidae e Halictidae (Figura 5). A diminuição da visitação ao longo da tarde pode ter sido influenciada pela redução da disponibilidade dos recursos florais. Segundo Polatto *et al*. (2014), após um certo período do dia, a escassez de recursos florais produzidos pela maioria das plantas pode estimular as abelhas a forragear flores no início dos dias subsequentes. Imperatriz-Fonseca *et al*. (1985) apontam a disponibilidade de recursos como um dos fatores externos reguladores da atividade de voo das abelhas.

Maior número de indivíduos e espécies de abelhas nativas visitaram as flores até às 12h30min, com declínio das atividades após este período (Tabela 2). Comportamento semelhante foi verificado em plantas de pimenta (*Piper hispidinervum*) e melão (*Cucumis melo*) onde as abelhas nativas preferiram visitar as inflorescências para a coleta de pólen e néctar durante o período da manhã (até as 12 horas) (Thomazini *et al*., 2002; Tschoeke *et al*., 2015). Apesar da abundância de indivíduos da espécie *Apis mellifera* também ser maior às 12h30min, esta manteve-se mais ativa que as demais espécies após as 15h30min (Tabela 2). Segundo Chang *et al*. (2001), o seu padrão de forrageamento está entre 10 e 16 horas. Tschoeke *et al*. (2015) verificaram que *Apis mellifera* manteve sua atividade durante o período da tarde, porém apenas para a coleta de néctar.

Diferenças interespecíficas nas atividades de forrageamento podem estar diretamente relacionadas a fatores abióticos (temperatura, luminosidade e umidade relativa do ar) e ao hábito generalista de *Apis mellifera*, a qual possui enxames populosos com alta necessidade de recursos alimentares, tornando-a eficiente no recrutamento de indivíduos (Giannini *et al.,* 2015).

O conhecimento de particularidades entre as espécies pode contribuir na adoção de estratégias de manejo para polinização dirigida na cultura do morangueiro. Uma possibilidade para otimização deste serviço ecossistêmico, é a associação de *A. mellifera,* manejada em colmeias próximas aos cultivos, com abelhas nativas, que podem ser instaladas no interior dos ambientes protegidos e preservadas nas matas do entorno da

cultura. A ação complementar de diferentes espécies de abelhas torna ainda mais efetivo o processo de polinização no morangueiro, auxiliando principalmente na melhoria da formação dos frutos (Malagodi-Braga & Kleinert, 2007).

Para beneficiar simultaneamente a cultura e a preservação das espécies polinizadoras é recomendado que a aplicação de agrotóxicos, quando necessária, seja realizada no fim da tarde, procurando coincidir com os horários de menor atividade dos polinizadores. Além disso, a manutenção de matas nativas no entorno dos cultivos protegidos é fundamental para a reprodução natural das espécies.

Em síntese, o cultivo do morangueiro em sistema semi-hidropônico recebe grande diversidade de visitantes florais, incluindo insetos das ordens Hymenoptera, Diptera, Coleoptera, Hemiptera e Lepidoptera, sendo Hymenoptera a mais frequente e abundante. As 13 espécies de abelhas pertencentes as famílias Apidae (*Apis mellifera*, *Tetragonisca fiebrigi*, *Tetrapedia* sp., *Trigona spinipes*, *Schwarziana quadripunctata*, *Plebeia emerina*, *Plebeia remota* e *Bombus pauloensis*) e Halictidae (*Dialictus* sp.1, *Dialictus* sp.2, *Augochloropsis* sp.1, *Augochloropsis* sp.2, *Augochlora* sp.1,) são potenciais polinizadoras na cultura do morangueiro, pois apresentam adaptações para o transporte de pólen. Nos municípios de Bento Gonçalves e Farroupilha, a introdução de colmeias de *T. fiebrigi*, *P. emerina* ou *P. remota* no interior de cultivos protegidos de morangueiro pode ser uma alternativa ao déficit de polinização devido a abundância e facilidade de manejo.

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FIGURAS E TABELAS

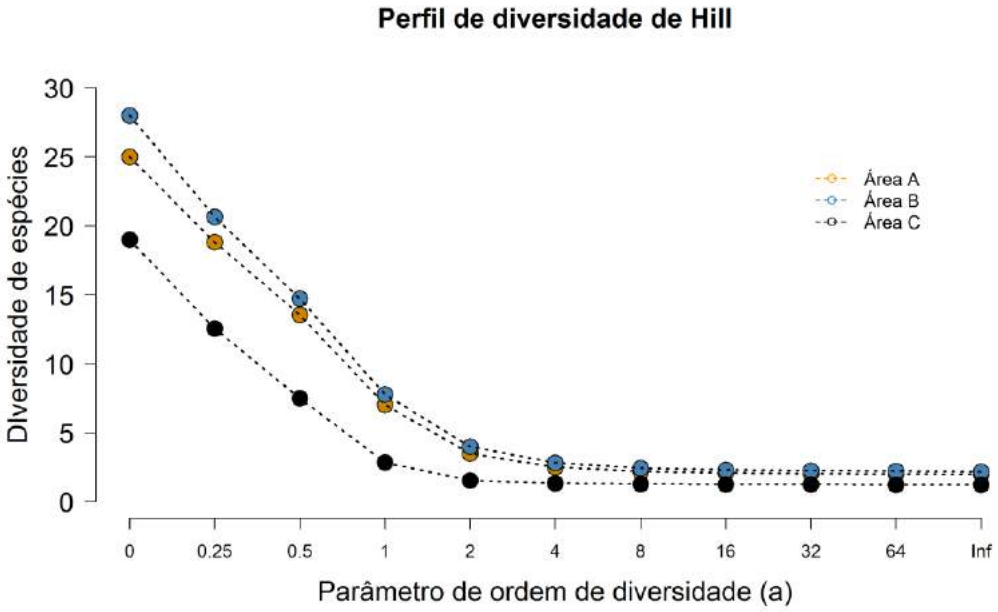
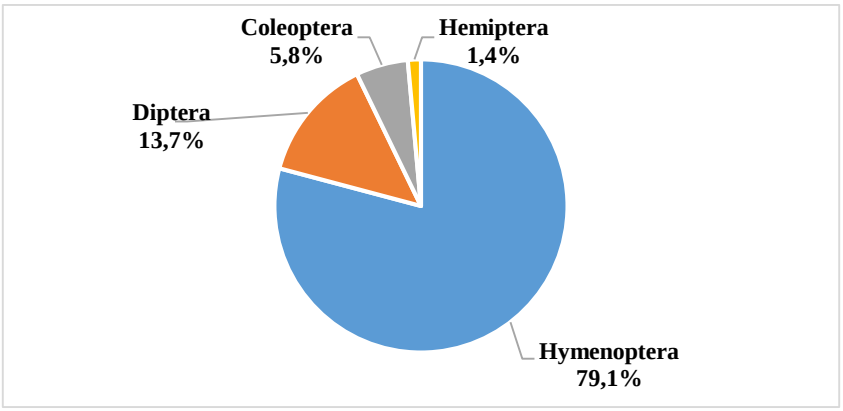


Figura 1. Perfil de diversidade (série de Hill) de insetos em três pontos amostrais (área A: Bento Gonçalves/RS, área B e C: Farroupilha/RS). À medida que se desloca para a esquerda do eixo *x* uma maior importância é dada às espécies raras. Quando se desloca para o lado direito as espécies raras tornam-se menos importantes, e há um maior peso à equabilidade das espécies de insetos. Índices de diversidade extraídos do eixo *x*: 0 = *riqueza de espécies*; 1 = *índice de Shannon*; 2 = *índice de Simpson*; Inf = *índice de Berger-Parker*. Diversity profile (Hill series) of insects at three sample points (area A: Bento Gonçalves/RS, area B and C: Farroupilha/RS). As it moves to the left of the *x*-axis, greater importance is given to rare species. When moving to the right side rare species become less important, and there is a greater weight to the equability of insect species. Diversity indexes extracted from the x-axis: 0 = *species richness*; 1 = *Shannon index*; 2 = *Simpson index*; Inf = *Berger-Parker index,* Universidade Federal de Pelotas, 2017.

Figura 2. Imagem aérea dos locais de realização das coletas: (A) Bento Gonçalves, (B) e (C) Farroupilha. Google Earth, 2017 (Aerial image of collection sites): (A) Bento Gonçalves, (B) and (C) Farroupilha, Universidade Federal de Pelotas, 2017.



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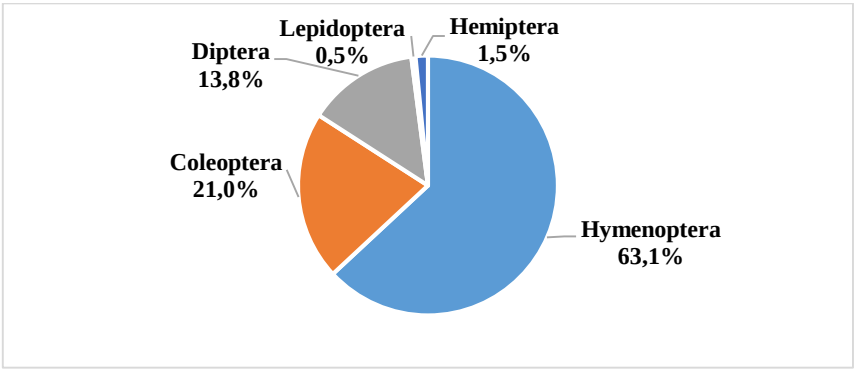

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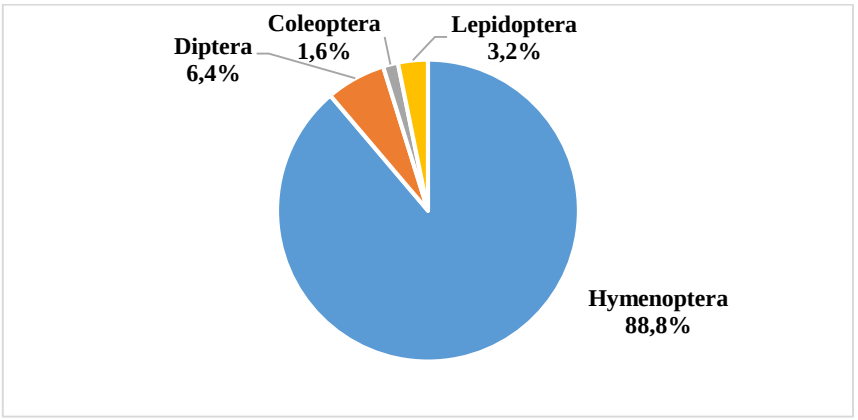
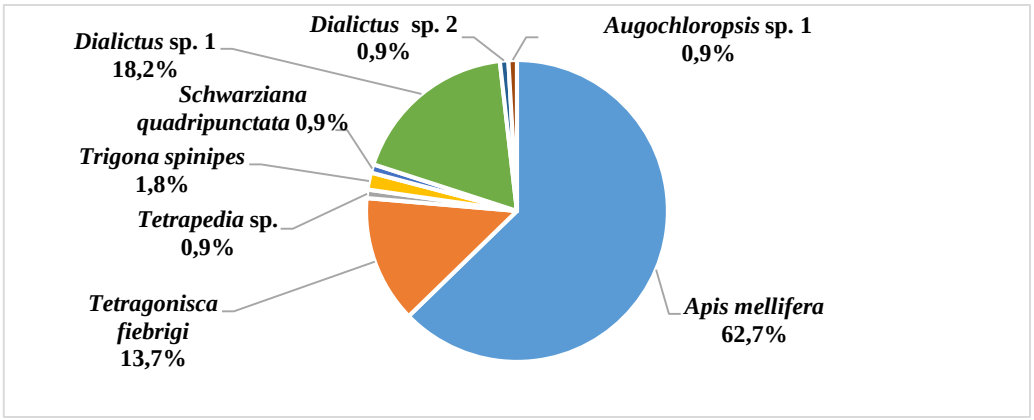


(C)

Figura 3- Abundância relativa das ordens de insetos coletados nas flores de morangueiro da cultivar Albion nas áreas de estudo: (A) Bento Gonçalves, (B) e (C) Farroupilha (Relative abundance of insect orders collected in Albion cultivar strawberry flowers in the study areas): (A) Bento Gonçalves, (B) and (C) Farroupilha, Universidade Federal de Pelotas, 2017.



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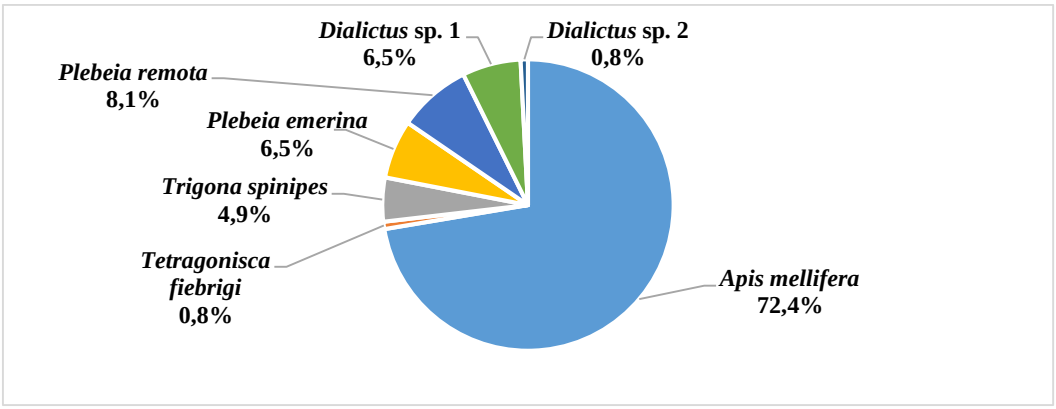

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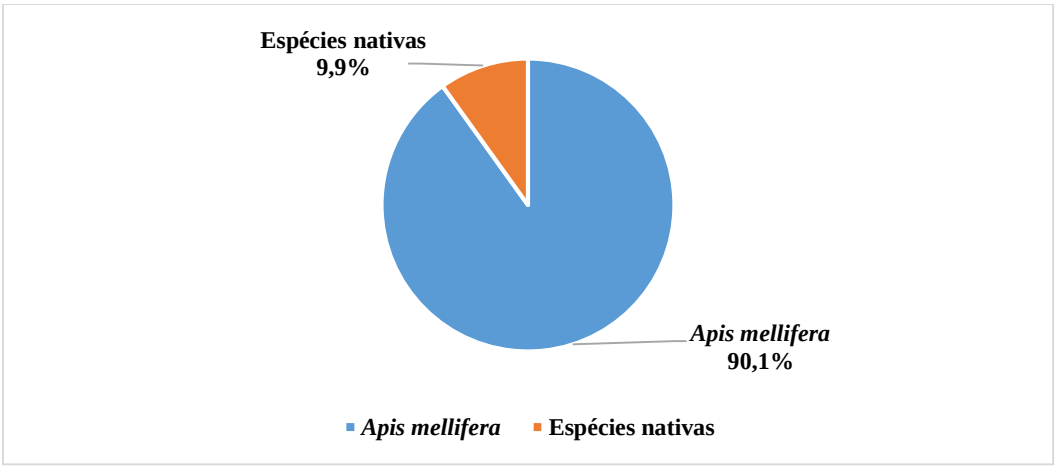

(C)

Figura 4- Abundância relativa de himenópteros, representados por espécies de abelhas em flores de morangueiro da cultivar Albion, nas áreas: (A) Bento Gonçalves, (B) e (C) Farroupilha (Relative abundance of hymenoptera, represented basically by species of bees in flowers of the strawberry of Albion, in the areas: (A) Bento Gonçalves, (B) and (C) Farroupilha, Universidade Federal de Pelotas, 2017.

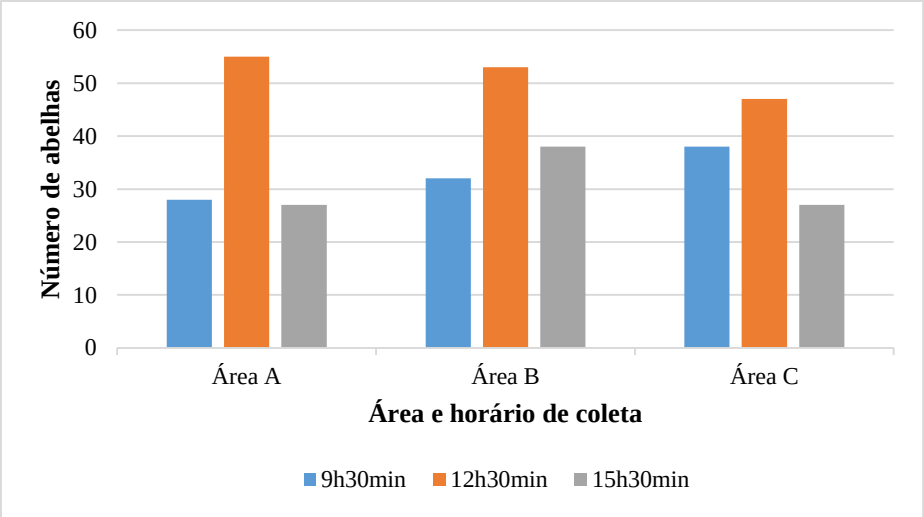

Figura 5- Número total de abelhas coletadas visitando flores de morangueiro em diferentes períodos do dia, nas áreas de estudo: (A) Bento Gonçalves, (B) e (C) Farroupilha (Total number of bees collected visiting strawberry flowers at different times of the day, in study áreas: (A) Bento Gonçalves, (B) and (C) Farroupilha, Universidade Federal de Pelotas, 2017.

Tabela 1. Área, localização, idade e espaçamento entre plantas onde realizaram-se as amostragens de visitantes florais associados à cultura do morangueiro (Area, location, age and spacing between plants where sampling of floral visitors associated with strawberry culture was carried out). Bento Gonçalves/ Farroupilha, Universidade Federal de Pelotas, 2017.

Área[1]	Município	Latitude	Longitude	Altitude (m)	Data plantio	Idade plantas (anos)	Espaçamento plantas (cm)
A	Bento Gonçalves	29º10’47.97’’S	51º24’44.20’’O	573	jun/16	1	12
B	Farroupilha	29º10'45.81’’S	51º21'28,21’’O	583	jun/15	2	12
C	Farroupilha	29º08'35.37’’S	51º21'59.82’’O	565	jun/15	2	7

[1]Área cultivada em cada propriedade

Tabela 2. Número de indivíduos obtidos e seus respectivos horários de coletas observados nas áreas (A) Bento Gonçalves, (B) e (C) (Farroupilha) (Number of individuals obtained and their respective collection times observed in the areas (A) Bento Gonçalves, (B) and (C) (Farroupilha), Universidade Federal de Pelotas, 2017.

Ordem/Família	Gênero/Espécie	**Horário/Área**											
		9h30min				**12h30min**				**15h30min**			
		A	**B**	**C**	**Total**	**A**	**B**	**C**	**Total**	**A**	**B**	**C**	**Total**
Hymenoptera													
Apidae	*Apis mellifera*	11	19	33	**63**	37	39	40	**116**	21	31	27	**79**
	Tetragonisca fiebrigi	6	-	-	**6**	5	1	-	**6**	4	-	-	**4**
	Tetrapedia sp.	1	-	-	**1**	-	-	-	**-**	-	-	-	**-**
	Trigona spinipes	1	3	1	**5**	1	1	2	**4**	-	2	-	**2**
	Schwarziana quadripunctata	1	-	-	**1**	-	-	-	**-**	-	-	-	**-**
	Plebeia emerina	-	1	-	**1**	-	6	1	**7**	-	1	-	**1**
	Plebeia remota	-	8	1	**9**	-	-	1	**1**	-	2	-	**2**
	Bombus pauloensis	-	-	1	**1**	-	-	-	**-**	-	-	-	**-**
Halictidae	*Dialictus* sp. 1	6	1	1	**8**	12	5	1	**18**	2	2	-	**4**
	Dialictus sp. 2	1	-	-	**1**	-	1	-	**1**	-	-	-	**-**
	Augochloropsis sp.1	1	-	-	**1**	-	-	-	**-**	-	-	-	**-**
	Augochloropsis sp.2	-	-	-	**-**	-	-	1	**1**	-	-	-	**-**
	Augochlora sp.1	-	-	1	**1**	-	-	-	**-**	-	-	-	**-**
Diptera													
Syrphidae	*Toxomerus* sp. MORFO 1	1	2	1	**4**	3	10	-	**13**	1	2	-	**3**
	Toxomerus sp. MORFO 2	-	-	-	**-**	-	1	-	**1**	-	1	1	**2**
	Toxomerus sp. MORFO 3	-	-	-	**-**	-	1	1	**2**	1	-	1	**2**
	Toxomerus sp. MORFO 4	-	-	1	**1**	-	-	-	**-**	-	-	-	**-**
	Toxomerus sp. MORFO 5	-	-	-	**-**	-	-	1	**1**	-	-	-	**-**
	Toxomerus sp. MORFO 6	-	1	-	**1**	-	-	-	**-**	-	-	-	**-**
	Eristalis sp. MORFO 1	3	-	-	**3**	1	-	-	**1**	-	-	1	**1**
	Eristalis sp. MORFO 2	-	-	-	**-**	-	-	-	**-**	1	-	-	**1**
	Palpada sp.	2	1	-	**2**	-	-	-	**-**	-	-	-	**-**
	Quichuana sp.	-	-	-	**-**	-	-	-	**-**	-	-	1	**1**
Fanniidae	MORFO 1	1	1	-	**2**	-	-	-	**-**	1	1	-	**2**
	MORFO 2	-	-	-	**-**	-	1	-	**1**	-	-	-	**-**
	MORFO 4	-	-	-	**-**	-	1	-	**1**	-	-	-	**-**

Cont.

Ordem/Família	Gênero/Espécie	**Horário/Área**											
		9h30min				**12h30min**				**15h30min**			
		A	**B**	**C**	**Total**	**A**	**B**	**C**	**Total**	**A**	**B**	**C**	**Total**
Sarcophagidae	*Archytas* sp.	-	-	-	-	1	-	-	**1**	1	1	-	**2**
	MORFO 1	-	1	-	**1**	-	-	-	-	-	-	-	-
	MORFO 2	-	-	-	-	-	1	-	**1**	-	-	-	-
Calliphoridae	MORFO 1	-	-	-	-	-	-	-	-	1	-	-	**1**
	Lucilia sp.	-	-	-	-	-	-	-	-	-	1	-	**1**
Sepsidae		-	-	-	-	-	-	-	-	1	-	-	**1**
Coleoptera													
Curculionidae		1	-	-	**1**	-	-	1	**1**	-	-	-	-
Cantharidae		-	1	-	**1**	1	-	-	**1**	1	-	-	**1**
Chrysomelidae		-	-	-	-	1	-	-	**1**	-	-	-	-
	Diabrotica speciosa	-	9	1	**10**	1	17	-	**18**	1	8	-	**9**
Coccinellidae	*Coleomegilla quadrifasciata*	-	-	-	-	-	-	-	-	-	1	-	**1**
	Eriopis connexa	-	-	-	-	1	-	-	**1**	1	3	-	**4**
Tenebrionidae	*Lagria villosa*	-	1	-	**1**	-	-	-	-	-	-	-	-
Scarabaeidae	*Macraspis dichroa*	-	-	-	-	-	-	-	-	-	1	-	**1**
Hemiptera													
Geocoridae		1	-	-	**1**	-	-	-	-	-	-	-	-
Miridae	MORFO 1	1	-	-	**1**	-	-	-	-	-	-	-	-
	MORFO 2	-	-	-	-	-	-	-	-	-	1	-	**1**
Rhyparochromidae		-	-	-	-	-	1	-	**1**	-	1	-	**1**
Lepidoptera													
Hesperiidae	*Hylephila phylaeus phylaeus*	-	-	-	-	-	1	-	**1**	-	-	-	-
	Conga iheringii	-	-	1	**1**	-	-	-	-	-	-	-	-
Crambidae	*Duponchelia fovealis*	-	-	2	**2**	-	-	-	-	-	-	1	**1**
SUBTOTAL		38	49	44	**131**	64	87	49	**200**	37	59	32	**128**
TOTAL													**459**

- = espécie ausente (- = ausent specie). Bento Gonçalves/Farroupilha, UFPEL, 2017.

Tabela 3. Análise faunística das espécies de abelhas amostradas em três localidades com produção semi-hidropônica de morangueiro (Fauna analysis of bees species sampled in three localities with semi-hydroponic production of strawberry). Bento Gonçalves/Farroupilha, Universidade Federal de Pelotas, 2017.

ESPÉCIES/ÁREAS	**ÁREA**											
	A				**B**				**C**			
	A	**C**	**D**	**F**	**A**	**C**	**D**	**F**	**A**	**C**	**D**	**F**
Apis mellifera	ma	w	d	mf	ma	w	d	mf	ma	w	d	mf
Tetragonisca fiebrigi	ma	w	d	mf	c	z	nd	f	-	-	-	-
Tetrapedia sp.	c	z	nd	f	-	-	-	-	-	-	-	-
Trigona spinipes	c	z	nd	f	c	y	d	f	c	y	d	f
Schwarziana quadripunctata	c	z	nd	f	-	-	-	-	-	-	-	-
Dialictus sp. 1	ma	w	d	mf	ma	w	d	mf	c	z	d	f
Dialictus sp. 2	c	z	nd	f	c	z	nd	f	-	-	-	-
Augochloropsis sp.1	c	z	nd	f	-	-	-	-	-	-	-	-
Augochloropsis sp.2	-	-	-	-	-	-	-	-	c	z	nd	f
Plebeia emerina	-	-	-	-	c	y	d	f	c	z	nd	f
Plebeia remota	-	-	-	-	c	y	d	f	c	z	d	f
Augochlora sp.1	-	-	-	-	-	-	-	-	c	z	nd	f
Bombus pauloensis	-	-	-	-	-	-	-	-	c	z	nd	f

A= Abundância: ma = muito abundante; c = comum; C = Constância: w= constante; y= acessória; z = acidental; D = Dominância: d= dominante; nd = não dominante; F = Frequência: mf = muito frequente; f = frequente; - = ausente (A= Abundance: ma= very abundant; c= common; C= Constancy: w=constant; y= accessory; z= accidental; D= Dominance: d= dominant; nd= non-dominant; F= Frequency: mf= very frequent; f= frequent; - = ausent). Bento Gonçalves/Farroupilha, UFPEL, 2017.

Artigo 2- Journal of Economic Entomology

Effects of insecticides used in strawberries on stingless bees *Melipona quadrifasciata* and *Tetragonisca fiebrigi* (Hymenoptera: Apidae)

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Effects of insecticides used in strawberries on stingless bees *Melipona quadrifasciata* and *Tetragonisca fiebrigi* (Hymenoptera: Apidae)

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Abstract- The use of pesticides is considered one of the most important threats to pollinators, especially since they are widely used in agriculture for pest control. In the last years a number of studies have been reported severe secondary effects on various bee species, including exotic and native bees. In this study, lethal (mortality) and sublethal (locomotor activity) effects, beyond the detoxification profile of insecticides and acaricides used in strawberries in Brazil (abamectin, novaluron, spinetoram and thiamethoxam) were evaluated on the native stingless bees *Melipona quadrifasciata* and *Tetragonisca fiebrigi*. The results showed that the effects varied significantly according to the pesticide, type of exposure (oral or topical) and bee species. Through the oral exposure, *M. quadrifasciata* was more susceptible for all insecticides with the exception of abamectin, while in topical exposure, *T. fiebrigi* was more sensitivity. Thiamethoxam followed by spinetoram and abamectin were the most lethal, regardless species or exposure route; novaluron was not harmful at highest tested dose. The locomotor activity of bees was altered in the presence of sublethal doses (LC_{10} and LC_{50}) of insecticides abamectin, spinetoram and thiamethoxam. The enzyme assays indicated that esterases might be involved

in the detoxification process of thiamethoxam in *T. fiebrigi*. Spinetoram and abamectin can be as much as toxic as thiamethoxam against *M. quadrifasciata* and *T. fiebrigi* in laboratory experiments. These findings should be confirmed in field experiments to define possibilies to combine pest control and pollinator management. In crops like strawberries, the selectivity of native pollinators should be considered.

Keywords: Acute toxicity, behavior effects, native bees, pesticides, *Fragaria x ananassa* Duch

Introduction

Bees perform an important service as pollinators in a large number of agricultural interest crops as well wild plants (Klein et al. 2007; Cresswell 2011). The honeybee *Apis mellifera* L., 1758 is the most widely used pollinator in commercial crops worldwide and the most often used as a model organism for non-target toxicity studies (Minussi et al. 2007; Brittain and Potts 2011). However, in recent years, many beekeepers from different countries have been reporting an unusual bee mortality resultind in high losses of honey bee hives (Bortolotti et al. 2003). Therefore, the use of other bees, such as the stingless bees, has been encouraged, since these bees also contribute to pollination in agricultural crop areas (Slaa et al. 2006).

Among these, are the *Melipona quadrifasciata* Lepeletier, 1836 known as “mandaçaia”, with similar size of *Apis mellifera*, found in parts of Argentina, Brazil and Paraguay, and *Tetragonisca fiebrigi* (Schwarz, 1938), known as “jataí”, a small bee, found in parts of the Argentina, Brazil, Paraguay and Bolivia (Camargo and Pedro 2013). Several studies have showed that these bees play an important role in the pollination of crops in protected

environments such tomatoes and strawberries as they improve the production and the quality of fruits (Free 1993; Del Sarto et al. 2005; Antunes et al. 2007).

The strawberry is a plant cultivated and much appreciated worldwide (Witter et al. 2012). Although most commercial cultivars are hermaphrodite and self-pollinated, the lack of pollinators during flowering of the crop associated with insufficient amounts of pollen results in deformations of the fruits and lower yields (Zebrowska 1998; Witter et al. 2014). The ease of management of *M. quadrifasciata* and *T. fiebrigi* combined with the absence of functional sting makes these species suitable for the pollination of strawberries in protected environments and with that they aid in the pollination deficit (Slaa et al. 2006). In addition, because these bees are different in size, they may have a complementary effect on flower pollination, since medium to large bees fold at the top of the receptacle and pollinate the apical stigmas, while small bees circulate in the stamens and around of the receptacle, mainly to pollinate the basal stigmas (Chagnon et al. 1993; Malagodi-Braga and Kleinert 2007).

However, in these crops the application of pesticides is a common practice in the management of pest arthropods (Bernardi et al. 2015). Abamectin, thiamethoxam, spinetoram and novaluron are used to control mites, aphids, thrips and caterpillars, respectively during strawberry crop production in Brazil (Agrofit 2018). Thus, during the pollination activity, stingless bees can also be exposed directly or indirectly to these products (Talebi et al. 2008; Mullin et al. 2010). Directly, through the contact of the body of the insect with chemical molecules suspended in the air and indirectly through the ingestion of pesticide residues present in pollen, nectar or water (Girolami et al. 2009; Johnson et al. 2010). Several studies have demonstrated the occurrence of serious lethal and sublethal effects in native bees exposed to

insecticides (Tomé et al. 2015; Pitts-Singer and Barbour 2016; Dorneles et al. 2017), however little information on most of species is available.

Therefore, due to the importance of pollination for strawberry production, it is necessary to know the toxicity of pesticides on these bees. The objectives of this study were to stablish the lethal dose and lethal concentration (LD_{50} and LC_{50}), as well to evaluate the sublethal effects (locomotor activity) and detoxification profile of insecticides used in strawberry on species of the native bees *M. quadrifasciata* and *T. fiebrigi*.

Material and methods

Bee collection

Foragers bees of *M. quadrifasciata* and *T. fiebrigi* were collected from three different colonies in the Meliponary at Federal University of Pelotas (UFPel), (Pelotas, RS, Brazil) and maintained in disposable plastic cages of 250 mL. After the collection, the bees were transported to the laboratory (28ºC ± 1°C temperature, 70% ±5% relative humidity and scotophase of 24 hours) for 24 hours.

Toxicity bioassays

The tested insecticides were the following: abamectin (Vertimec® 18 EC 1.8% a.i.; Syngenta Crop Protection LTDA; São Paulo, SP, Brazil), thiamethoxam (Actara® 250 WG 25.0% a.i.; Syngenta Crop Protection LTDA; São Paulo, SP, Brazil), spinetoram (Delegate® 25.0% a.i.; Dow AgroSciences Industrial LTDA; São Paulo, SP, Brazil) and novaluron (Rimon Supra® 10.0% a.i.; Adama Brazil S/A; Londrina, PR, Brazil) all registered for the strawberry crop in Brazil. Susceptibility of *M. quadrifasciata* and *T. fiebrigi* was assessed through oral and topical exposure. The experiments were conducted in two steps using a combined

methodology adapted from Felton et al. (1986), OECD (1998a and b) and Medrzycki et al. (2013).

1) *Preliminary tests*: serial dilutions were performed (1:10) with insecticide stock concentration (1000 ng a.i./µL) in distilled water. After establishing the interval of doses with changes in mortality, some intermediate doses were established at extremes response (0 to 100% mortality).

2) *Final tests*: the insecticide stock concentration (1000 ng a.i./µL) was diluted in distilled water to establish 6 to 8 doses of increasing concentrations.

Six replicates with ten adult bees from different colonies were used for each treatment. The bioassays were conducted under a randomized design. Bioassays with mortality higher than 10% in the control treatment were not considered for analysis.

Determination Lethal Oral Concentration (LC_{50})

The commercial formulations of selected insecticides were diluted in sucrose solution (50% v/v). In order to stimulate consumption, the insects were starved for a period of two hours prior the experiments. Each group of bees as fed with 1 mL of insecticide solution for six hours. Subsequently, the insecticide solution was replaced by sucrose solution *ad libitum*. The concentrations of thiamethoxam, spinetoram, abamectin and novaluron ranged from 0.1 – 100 ng a.i./µL diet; 0.1 – 50 ng a.i./µL diet; 0.1 – 100 ng a.i./µL diet and 1.0 – 50000 ng a.i./µL diet para *T. fiebrigi* and from 0.01 – 1.0 ng a.i./µL diet; 0.1 – 10 ng a.i./µL diet; 2.0 – 50 ng a.i./µL diet and 1.0–50000 ng a.i./µL diet to *M. quadrifasciata*, respectively. The bees from the control group were fed with sucrose solution only. Dead insects as well as abnormal symptoms were recorded during 48 hours after initial exposition.

Determination Lethal Topical Dose (LD_{50})

120 The commercial formulations of selected insecticides were diluted in distilled water and
121 acetone (50% v/v) with a range of concentrations. Prior topical application, the bees were
122 anesthetized with CO_2 for 10 seconds. Using a microapplicator (Burkard Scientific, UK) a drop
123 with 0.5 μL (*T. fiebrigi*) or 1.0 μL (*M. quadrifasciata*) was deposited on the pronotum of each
124 bee. The concentrations of thiamethoxam, spinetoram and abamectin ranged from 0.5 – 50 ng
125 a.i./bee; 0.5 – 50 ng a.i./bee and 0.25 – 125 ng a.i./bee to *T. fiebrigi* and from 0.1 – 100 ng
126 a.i./bee; 1.0 – 500 ng a.i./bee; and 1.0 – 1000 ng a.i./bee to *M. quadrifasciata*, respectively.
127 Control bees received a drop of distilled water and acetone (50% v/v). Bees were fed with a
128 sucrose solution (50%) *ad libitum*. Mortality and abnormal behavior were recorded 48 hours
129 after initial exposure.
130
131 **Behavioral bioassays**
132 *Locomotor activity*
133 Bees were exposed to sublethal concentrations LC_{10} and LC_{50}. The treatments were
134 conducted as described previous for determine the oral LC_{50}. The insects were individually
135 released at one end of a silicone tube with a total length of 60 cm (Figure 1). A fluorescent lamp
136 was used at the opposite end of tube to stimulate the bees. The time that each individual bee
137 spent to walk a distance of 50 cm toward the light source was recorded. Based on
138 untreated/control bees, the maximum walking period was about 1 minute. Subsequently, the
139 average speed for each bee was calculated. The bioassays using 30 bees per treatment were
140 performed under 28 ± 1 ºC, at 4 and 24 hours after initial exposure.
141
142 *Insecticide synergism*
143 Thiamethoxam and the synergist *S,S,S*-tributylphosphorotrithioate (DEF) in technical
144 grade were used to investigate the role of the esterases in the detoxification of insecticide. The

synergist was dissolved in acetone. A drop of 0.5 μL of DEF (1000 mg L^{-1}) was applied topically on the pronotum of each bee (*T. fiebrigi)*. After 4 hours, a drop with 0.5, 2.5, 5, 10, 25 and 50 ng a.i./bee of thiamethoxam were topically applied. In the control treatment only the synergist and acetone were applied. Mortality assessments and dose response curve (LD_{50}) were performed 48 hours after initial exposition.

Statistical analyses

Statistical analyses to determine the LC_{50} and LD_{50} values were performed with "four-parameter log-logistic function" of the ''drc'' package (Analysis of Dose-Response Curves using the statistical software R® (Ritz and Streibig 2005). Toxicity was assessed by comparing the LC_{50} and LD_{50} values between the insecticides for each species of bee and also comparing these values among the species. In both cases, the values of the LC_{50} and LD_{50} confidence intervals were used, being significantly different when no overlap occurred in the confidence intervals, at 95% probability. For locomotor activity, analysis of variance was performed using the Kruskal-Wallis test and when statistic obtained a significant p-value (<0.05), the Dunn test was applied to 95% probability.

Results

Toxicity bioassays

Acute oral toxicity

The calculated values to LC_{50} (48h) of thiamethoxam, spinetoram and abamectin to *T. fiebrigi* were 2.05 ng a.i./μL, 2.72 ng a.i./μL, and 3.53 ng a.i./μL diet, respectively (Figure 2). The insecticide thiamethoxam has a higher toxicity to *T. fiebrigi* when compared to abamectin, but did not differ from spinetoram. Likewise, the LC_{50} for abamectin and spinetoram did not present a significant difference (Table 1). In the bioassays performed with *M. quadrifasciata*

the LC_{50} (48h) of thiamethoxam, spinetoram and abamectin were 0.18 ng a.i./μL, 2.45 ng a.i./μL and 8.81 ng a.i./μL diet, respectively (Figure 3). Non-overlapping in the LC_{50} confidence intervals indicated that there was a significant difference between the toxicity of these insecticides. Thiamethoxam presented a higher lethal effect, with an LC_{50} value considered extremely low when compared to spinetoram and abamectin (Table 1).

When comparing the toxicity of the insecticides among the species in this route of exposure, *M. quadrifasciata* presented greater susceptibility to insecticides than *T. fiebrigi* (except for abamectin). The overlapping in the confidence interval of LC_{50} for spinetoram indicated that the two species did not differ in susceptibility to this insecticide. For both species, the most toxic insecticides when ingested were thiamethoxam, spinetoram and abamectin, respectively.

Novaluron caused a low percentage of mortality for adults of *T. fiebrigi* (10%) and *M. quadrifasciata* (16%) at the maximum concentration 50000 ng a.i./μL diet and not possible to construct the dose-response curve. Due to this result, it was not used in the other assays.

Acute topical toxicity

Topical treatments in *T. fiebrigi* with thiamethoxam, spinetoram and abamectin resulted in LD_{50} values of 5.50 ng a.i./bee, 5.79 ng a.i./bee and 8.07 ng a.i./bee, respectively (Figure 4). Although the thiamethoxam insecticide showed LD_{50} (48 h) lower than the other insecticides, they did not differ significantly, since the confidence intervals overlapped (Table 2). The values for the topical LD_{50} of the thiamethoxam, spinetoram and abamectin for *M. quadrifasciata* were as the following: 9.06 ng a.i./bee, 26.27 ng a.i./bee and 237.74 ng a.i./bee, respectively (Figure 5). Confidence interval values indicated a significant difference in susceptibility of *M. quadrifasciata* to topically applied insecticides (Table 2). Thiamethoxam

194 presented higher toxicity for the species, with an LD_{50} value about 26 times lower than
195 abamectin. Spinetoram was the second most toxic.

196 In this route of exposure, *T. fiebrigi* was the species most susceptible to the insecticides
197 studied, according to the values of the confidence intervals of each LD_{50}. For the two species,
198 the most toxic insecticides were thiamethoxam, spinetoram and abamectin, respectively.

199

200 **Behavioral bioassays**

201 *Locomotor activity*

202 Exposure to insecticides caused changes in the locomotor activity of the bees, varying
203 according to species, concentration, insecticide and period evaluated. Thiamethoxam
204 significantly reduced the average speed of *T. fiebrigi* (X^2=52.48; df=2; p<0.0001) and *M.*
205 *quadrifasciata* (X^2=30.76; df=2; p<0.0001) 4 hours after the oral exposure. However, after 24
206 hours, no statistically significant difference was observed, either for *T. fiebrigi* (X^2=0.09; df=2;
207 p=0.9538) as for *M. quadrifasciata* (X^2=5.18; df=2; p=0.07). When comparing the average
208 speed of *T. fiebrigi* bees from the control treatment with the speed of bees exposed to LC_{10} and
209 LC_{50} of thiamethoxam after 4 hours, a statistically significant difference was observed, since
210 the control group walked the established distance with a higher velocity than the others (2.20
211 cm/s) (Figure 6A). Bees of the LC_{10}, although presenting a lower average speed in relation to
212 the control (1.85 cm/s), were less impaired than those exposed to LC_{50}, which presented reduced
213 locomotor activity (0.52 cm/s). *M. quadrifasciata* presented similar behavior and after 4 hours,
214 the average speed of bees exposed to LC_{10} (2.19 cm/s) and LC_{50} (1.85 cm/s) differed statistically
215 from the control group (4.18 cm/s), but there was no difference between bees of LC_{10} and LC_{50}.
216 No significant difference was observed at 24 hours, of the control group (3.59 cm/s) (Figure
217 6B).

Bees exposed to LC_{50} and LC_{10} of spinetoram showed a significant difference in average speed values compared to control group bees at 4 hours (*T. fiebrigi*: X^2=10.57; df=2; p=0.005; *M. quadrifasciata*: X^2=28.75; df=2; p<0.0001) and 24 hours after (*T. fiebrigi*: X^2=28.01; df=2; p<0.0001; *M. quadrifasciata*: X^2=25.35; df=2; p<0.0001). Bees from the control treatment, walked the established distance with higher speed (*T. fiebrigi*: 2.13 cm/s (4 horas) and 2.01 cm/s (24 horas); *M. quadrifasciata*: 2.52 cm/s (4 horas) and 3.45 cm/s (24 horas)) than bees submitted to feeding with the insecticide, which presented minimum average speed of 1.27 cm/s (*T. fiebrigi*) and 1.24 cm/s (*M. quadrifasciata*) after 24 hours exposed to LC_{50} (Figure 7A and B).

When the species were exposed to abamectin, there was a significant difference between the groups for the two evaluated periods (4 hours= *T. fiebrigi*: X^2=34.27; df=2; p<0.0001; *M. quadrifasciata*: X^2=9.52; df=2; p=0.008; 24 hours= *T. fiebrigi*: X^2=12.03; df=2; p=0.002; *M. quadrifasciata*: X^2=16.36; df=2; p=0.0002). The average speed of *T. fiebrigi* bees belonging the control after 4 hours (1.54 cm/s) and 24 hours (1.66 cm/s) was statistically higher than the speed of bees exposed to LC_{10} and LC_{50} (Figure 8A). For *M. quadrifasciata*, at 4 hours, the average speed of the control bees (3.49 cm/s) did not differ statistically from the speed of bees exposed to feeding with LC_{50} (3.13 cm/s), while both differed and were higher than those under LC_{10} (2.32 cm/s). On the other hand, after 24 hours of feeding, both the average speed of bees exposed to LC_{50} (2.73 cm/s) and LC_{10} (1.95 cm/s) differed from the control (3.51 cm/s) showing lower average speeds. At this time, it was also observed that the bees exposed to LC_{50} presented higher average speed compared whit LC_{10} bees (Figure 8B).

Enzymatic Inhibition

The application of enzymatic inhibitor of esterase (*S,S,S*-tributylphosphorotrithioate-DEF) whit insecticide thiamethoxam resulted in significant reduction of the LC_{50} value for *T.*

fiebrigi of 5.50 ng a.i./bee in the absence of the inhibitor to 3.37 ng a.i./bee in the presence (Figure 9 and Table 3).

Discussion

The results have shown that the toxicity of the insecticides and the acaricide abamectin vary according to the route of exposure and species. In the oral exposure to thiamethoxam the LC_{50} for *M. quadrifa*sciata was 0.18 ng a.i./µL diet, considered lower than other insecticides. The high toxicity of neonicotinoids to bees has been reported in several studies (Biddinger et al. 2013; Soares et al. 2015; Jiménez and Cure 2016). *Melipona scutellaris* exposed by feeding with imidacloprid presented oral LC_{50} of 0.81 ng a.i./µL diet (Costa et al. 2015). For *A. mellifera*, independent of the route of exposure, thiamethoxam was extremely toxic (Costa et al. 2014). The LC_{50} oral for this specie was 0.12 ng a.i./µL diet (48 hours) (Laurino et al. 2011), which corroborates the results observed in this study.

Neonicotinoids cause alterations in the nerve impulses of insects, since they interfere in the nicotinic acetylcholine receptors (nAChR) (Brown et al. 2006; Tan et al. 2007). The high toxicity of thiamethoxam compared to other insecticides may be related to the presence of nitro-substituted compounds in the chemical structure of the molecule, which gives high insecticidal activity compared to other neonicotinoids such as acetamiprid and thiacloprid (Iwasa et al. 2004).

Comparing the LC_{50} obtained in this study with the recommended concentrations of insecticides for strawberry cultivation in Brazil (Agrofit 2018), it was verified that higher concentrations are used. In the case of thiamethoxam, an insecticide that has been shown to be most toxic to both species and routes of exposure, the recommended concentration is 25 ng a.i./µL water (10 g/100 L water) which represents about 12 and 140 times the oral LC_{50} value for *T. fiebrigi* and *M. quadrifasciata*, respectively. This scenario becomes even more worrying,

since neonicotinoid insecticides, once absorbed by the plant, can translocate up to the water of guttation, nectar and pollen of the crops, which in the flowering stage attract several bees (Krupke et al. 2012). In cucurbit crop, the foliar application and drip irrigation of thiamethoxam during flowering resulted in high average levels of residues in pollen (122 ng/g) and nectar (17,6 ng/g) (Dively and Kamel 2012). It should be noted, however, that under field conditions insecticides are influenced by environmental conditions such as temperature, humidity, light and plant metabolism, which may favor the degradation of some of the molecules (Pinheiro and Freitas 2010). According Dively and Kamel (2012), environmental conditions have a significant effect on overall residue levels, and therefore further studies are needed to determine the real dose that bees may be exposed in the pollen and/or nectar of the crops.

Despite the biological origin of the insecticides of the spinosyns group, the species *T. fiebrig*i showed high susceptibility to spinetoram, being equivalent to thiamethoxam. This fact may be related to the neurotoxic action of these insecticides (Jeschke et al. 2011). A similar result was observed with spinosad (also belonging to the spinosyns) that presented a higher lethal effect than the imidacloprid insecticide for *M. quadrifasciata* (Tomé et al. 2015). Spinosad and spinetoram were found to be dangerous for *Megachile rotundata* in contact with adults, causing changes also in the immature stages of this species (Gradish et al. 2012). These results demonstrate that the lower adverse effects of this group on beneficial insects may be overestimated, and it is therefore important to emphasize the importance of non-exemption of new bio-insecticidal molecules from the risk assessment analysis for bees.

T. fiebrigi was more susceptible to abamectin than *M. quadrifasciata* in the two exposure routes evaluated. Del Sarto et al. (2014) observed that *A. mellifera* also presented lower tolerance of this acaricide than *M. quadrifasciata*. The oral administration of abamectin significantly reduced the survival of *A. mellifera*, with a lethal time about three times less than

292 deltamethrin (Aljedani 2017). Ingestion of abamectin may cause changes in bees midgut cells,
293 leading to severe digestive disturbances (Aljedani 2017).

294 The non-lethal toxicity of novaluron to adult bees has been reported in several studies
295 (Malone et al. 2007; Scott-Dupree et al. 2009). In general, insecticides inhibiting chitin
296 synthesis are considered low risk for adult insects because they act on the immature phases,
297 interfering in the process of ecdysis (Mommaerts et al. 2006). For adults of *Bombus terrestris*,
298 this group of insecticides also did not provoke acute toxicity, however for the immature stages
299 harmful effects were observed, including egg mortality and larval deformation (Mommaerts et
300 al. 2006). Studies exploring the effects of these insecticides on early stages of development of
301 *M. quadrifasciata* and *T. fiebrigi* are required.

302 In topical application, *M. quadrifasciata* and *T. fiebrigi* presented high susceptibility to
303 thiamethoxam, being more sensitive when compared to *A. mellifera* (29.90 ng a.i./bee) (Iwasa
304 et al. 2004). The high lethality of this insecticide was also observed for *Nannotrigona*
305 *perilampoides* (LD_{50} = 4.0 ng a.i./bee) (Valdovinos-Núñez et al. 2009), which corroborates the
306 lethal dose obtained for *T. fiebrigi* (5.50 ng a.i./bee). Despite the lack of studies, Dorneles et al.
307 (2017) also reported high sensitivity of *T. fiebrigi* to the topical application of chlorpyrifos
308 (organophosphate). The greater susceptibility of this species compared to *M. quadrifasciata*
309 may be related to the structure of the cuticle, which possibly facilitates the absorption of
310 insecticides. According to Bacci et al. (2007), the penetration rate is related to the composition
311 and chemical thickness of the insect cuticle and to the physic-chemical characteristics of the
312 compounds.

313 The routes of exposure studied in this work, sought to simulate the possible forms of
314 contamination of bees with insecticides in the field. In general, the species *M. quadrifasciata*
315 and *T. fiebrigi* showed greater susceptibility to the insecticide thiamethoxam followed by
316 spinetoram and abamectin in the two routes evaluated. The differential susceptibility observed

317 may have occurred due to specific characteristics of insecticides and bee species. Life story
318 traits, body weight, detoxification capacity, and cuticle structure are factors that may change
319 the level of toxicity (Oliveira et al. 2002; Hardstone and Scott 2010; Brittain and Potts 2011).
320 The lower molecular weight of thiamethoxam (291.71), followed by spinetoram (754.00) and
321 abamectin (873.10) (Yu 2008) may have influenced the different degrees of toxicity of
322 insecticides. According to Stock and Holloway (1993) substances with smaller molecular
323 weights have greater penetration capacity in the cuticle of the insects.

324 The locomotor activity of bees was altered in the presence of sublethal doses of
325 insecticides. Sublethal responses should be considered, since the lethality is only a simplistic
326 indicator of environmental impact (Tomé et al. 2015). Thiamethoxam decreased the locomotor
327 activity of *T. fiebrigi* and *M. quadrifasciata* shortly after exposure (4 hours). Although the short-
328 term response to neurotoxic insecticides usually occurs through hyper excitation, in this study
329 the initial decrease in activity may have occurred due to the severe symptoms observed,
330 including spasms and disorientation, which made it difficult to move. The symptoms of
331 prostration and motor disturbance caused by thiamethoxam on honey bees are due to the effect
332 of the compound on the synapses of the insect central nervous system (Kagabu 1997).

333 After 24 hours, bees of the species *M. quadrifasciata* exposed to LC_{50} presented average
334 speed superior to the bees belonging to LC_{10} and control. This species exhibited similar
335 behavior when exposed to imidacloprid (Tomé et al. 2015). Contradictory results were observed
336 for *A. mellifera*, which, after 24-48 h after application of thiamethoxam, showed lower mobility
337 and flight activity (Charreton et al. 2015; Tosi et al. 2017). Alkassab and Kirchner (2018)
338 observed that clothianidin treated bees increased the distance traveled over time (up to 120
339 minutes) relative to control, while El Hassani et al. (2008) did not observe changes in the
340 locomotor activity of bees exposed to thiamethoxam.

Different behavioral responses may have occurred due to exposure time, bee species and doses used in each study. Lambin et al. (2001) points out that sublethal effects are highly dependent on the dose of the insecticide. Moreover, several nicotinic receptor subtypes are involved in complex behaviors and memory processes, and may be differentially altered by sublethal doses of neonicotinoids (Gauthier 2010). In *A. mellifera*, exposure to sublethal doses of thiamethoxam (10 ppb) increased the expression of two subunits nAChRs, nAChRα9 and nAChRβ2, indicating a compensatory reaction to the functional loss of nAChRs due to the neonicotinoid (Christen et al. 2016; Shi et al. 2017). This insecticide seems to induce changes in the regulation of the gene responsible for memory formation in *A. mellifera* (NMDA receptor 1 (NR1)) (Shi et al. 2017), which may explain in part the adverse behavioral effects observed.

Spinetoram and abamectin also interfered in the locomotor activity of both species. Disorientation was the typical symptom of the first. Spinosad, although it did not alter the locomotion, affected the flight activity of *M. quadrifasciata* (Tomé et al. 2015). The sublethal toxicity of spinosyns has been reported as negative in bumblebees (Morandin et al. 2005).

Tremors and spasms, followed by disorientation and paralysis were caused when insects were exposed to abamectin. Avermectins, as well as neonicotinoids and spinosyns act in the transmission of the nerve impulse, however the first group acts like agonist of the gamma-aminobutyric acid (GABA), causing immobilization and paralysis of the insects, due to neuromuscular action (Sánchez-Bayo 2011). This fact may explain the locomotor difficulty observed in bees exposed to abamectin. In *A. mellifera*, this insecticide reduced the longevity of forage bees (Aljedani and Almehmadi 2017). These effects may cause impacts on the survival of the all colony, which require active and healthy bees for feeding, cleaning, cell building and various tasks (Winston 1987).

The susceptibility of *T. fiebrigi* to thiamethoxam was increased when the activity of the esterase enzyme was inhibited, which indicates that it may participate in the detoxification

mechanism of this insecticide. However, more research is needed to validate this conclusion and understanding the molecular mechanisms involved between neonicotinoid insecticides and different species of native bees. In *A. mellifera*, esterases appear to play some role in acetamiprid detoxification, although monooxigenages (P450s) are the most important (Iwasa et al. 2004). A P450 gene, *CYP6as5* was induced in *A. mellifera* treated whit sublethal dose of thiamethoxam, showing its central importance in the resistance to neonicotinoid insecticides in this species (Shi et al. 2017).

In conclusion, the insecticides thiamethoxam, spinetoram and abamectin presented high lethality for *M. quadrifasciat*a and *T. fiebrigi* under the conditions (oral and topical) evaluated. Novaluron was not harmful at highest tested dose. The toxicity sublethal identified using behavioral tests suggests that abamectin and spinetoram can be as toxic as thiamethoxam neonicotinoid towards native bees, and therefore implies that the molecules to be used would need to be carefully selected. These results confirm the importance of considering other species of bees in the risk assessments, not only using *A. mellifera* as reference (Decourtye et al. 2013). Further studies evaluating sublethal effects in semi-field and field experiments are necessary in order to investigate the impacts these products under more realistic conditions and the possibility of integrated pest and pollinator management.

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Figures and Tables

Figure 1. Experimental set up. A silicone tube (50 cm) coupled to an apparatus consisting of a wooden plate (angle 0 °).

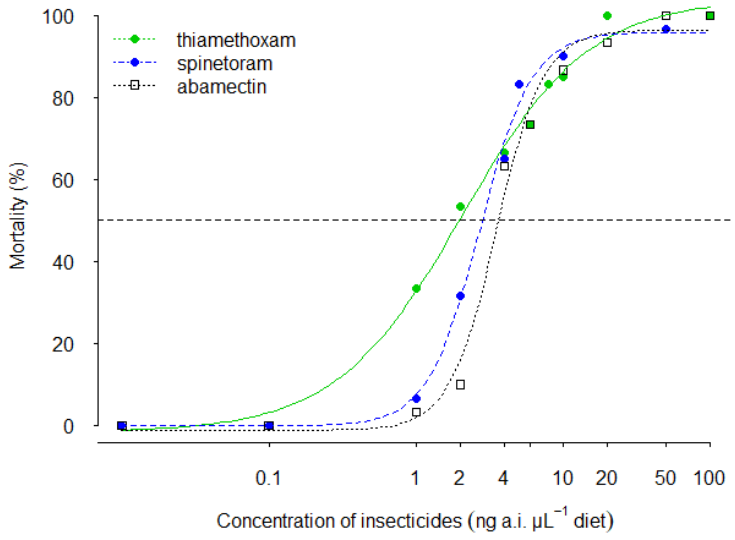


Figure 2. Acute oral toxicity (48 hours) of the insecticides thiamethoxam, spinetoram and abamectin for forage bees of *T. fiebrigi*. (The dashed line represents the LC_{50}).

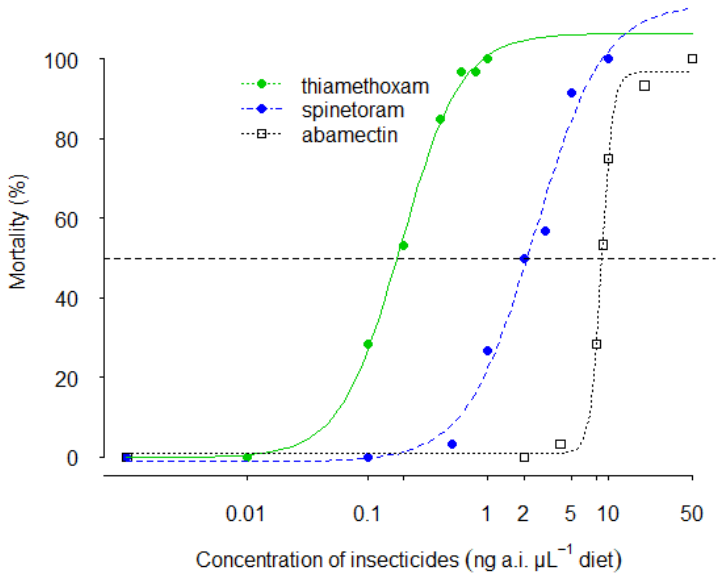


Figure 3. Acute oral toxicity (48 hours) of the insecticides thiamethoxam, spinetoram and abamectin for forage bees of *M. quadrifasciata*. (The dashed line represents the LC_{50}).

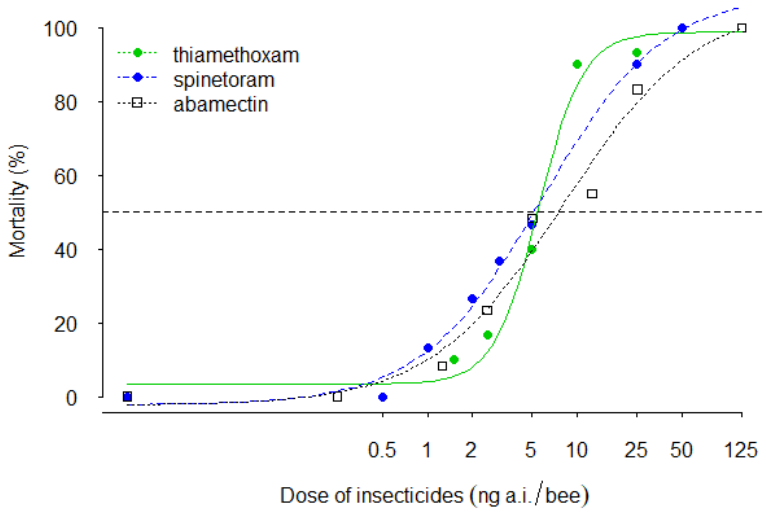


Figure 4. Acute topical toxicity (48 hours) of the insecticides thiamethoxam, spinetoram and abamectin for forage bees of *T. fiebrigi*. (The dashed line represents the LD_{50}).

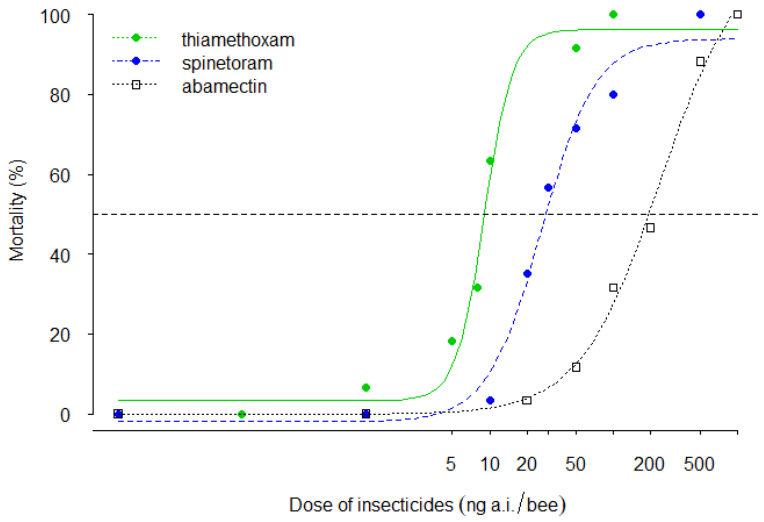
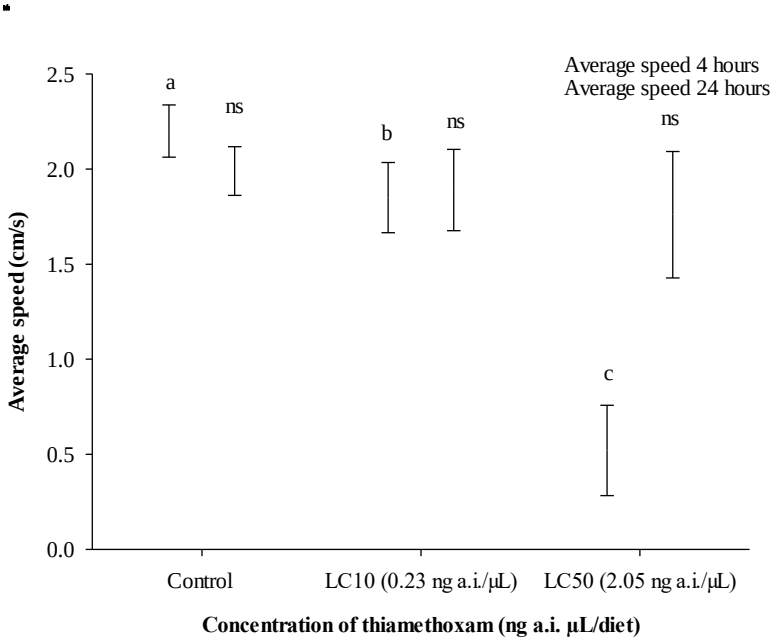
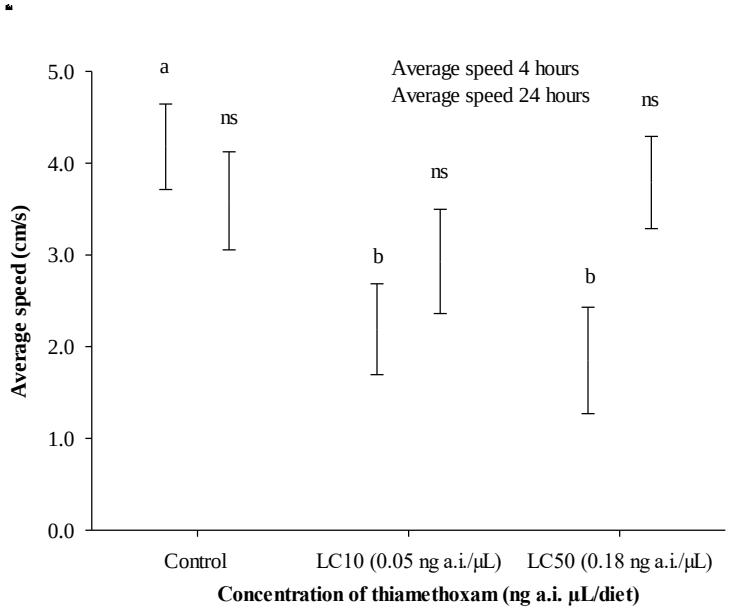


Figure 5. Acute topical toxicity (48 hours) of the insecticides thiamethoxam, spinetoram and abamectin for forage bees of *M. quadrifasciata*. (The dashed line represents the LD_{50}).



(A)



(B)

Figure 6. Locomotor activity of adult *T. fiebrigi* (A) and *M. quadrifasciata* (B) 4 and 24 hours after oral exposure to thiamethoxam.

* Averages followed by the same letter do not differ statistically from one another by the Dunn Test at 5% probability. White bars indicate the average speed (cm/s) of bees 4 hours after oral exposure to insecticide; Dashed bars indicate the average speed (cm/s) 24 hours after exposure of the bees to the insecticide.

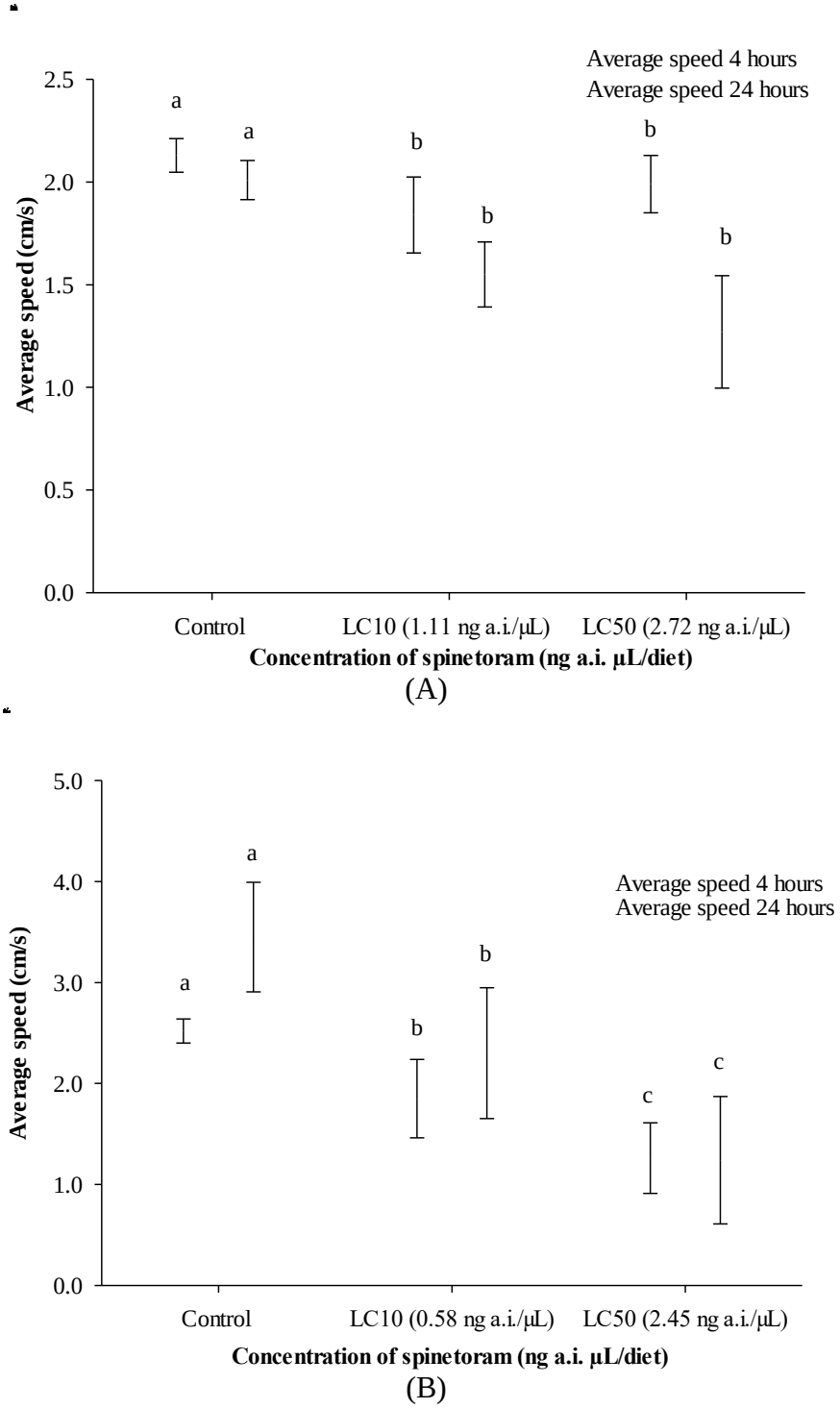


Figure 7. Locomotor activity of adult *T. fiebrigi* (A) and *M. quadrifasciata* (B) 4 and 24 hours after oral exposure to spinetoram.

* Averages followed by the same letter do not differ statistically from one another by the Dunn Test at 5% probability. White bars indicate the average speed (cm/s) of bees 4 hours after oral exposure to insecticide; Dashed bars indicate the average speed (cm/s) 24 hours after exposure of the bees to the insecticide.

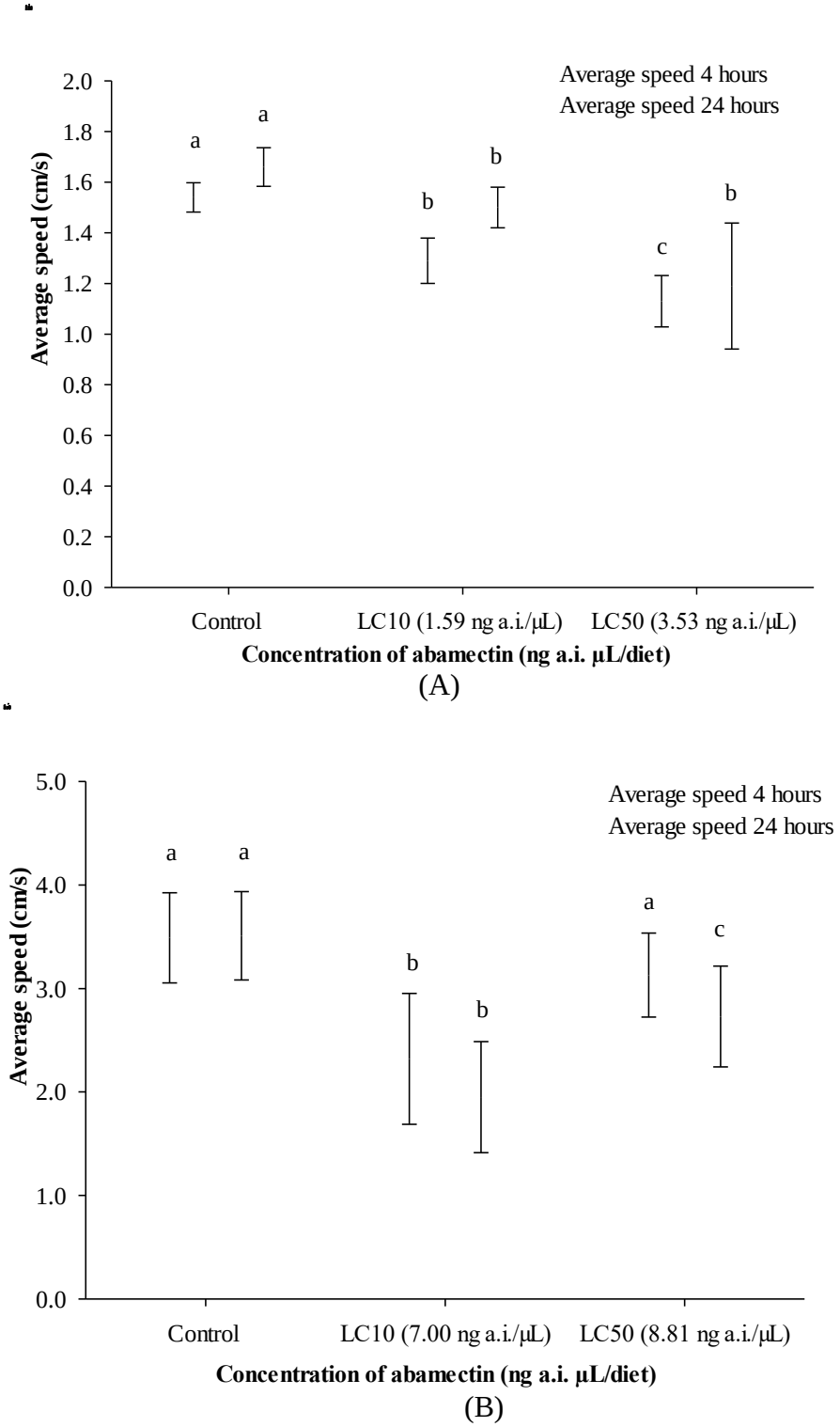


Figure 8. Locomotor activity of adult *T. fiebrigi* (A) and *M. quadrifasciata* (B) 4 and 24 hours after oral exposure to abamectin.

* Averages followed by the same letter do not differ statistically from one another by the Dunn Test at 5% probability. White bars indicate the average speed (cm/s) of bees 4 hours after oral exposure to insecticide; Dashed bars indicate the average speed (cm/s) 24 hours after exposure of the bees to the insecticide.

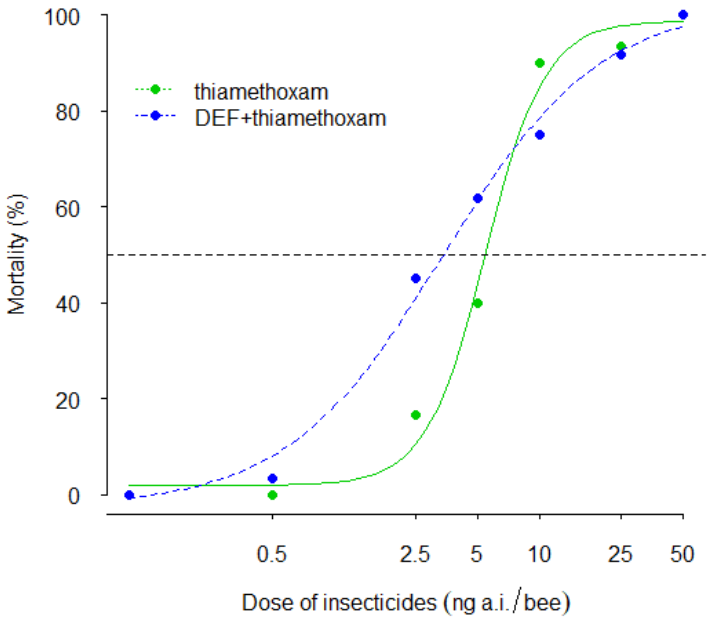


Figure 9. Dose-mortality curves of adult workers of the stingless bee *T. fiebrigi* topically exposed to thiamethoxam and DEF + thiamethoxam.

Table 1. Relative toxicity of orally exposed insecticides to *T. fiebrigi* and *M. quadrifasciata*.

Insecticide	**Specie**	**Slope (± SE)**	**LC50 (95 % FL) (ng a.i./µL)**	**t**	**p value**
Thiamethoxam	*T. fiebrigi*	1.0047 (±0.11)	2.05 (1.5217- 2.5858)	7.73	<0.0001
	M. quadrifasciata	1.7364 (±0.32)	0.18 (0.1453- 0.2311)	8.84	<0.0001
Spinetoram	*T. fiebrigi*	2.4631 (±0.38)	2.72 (2.2599- 3.1792)	11.92	<0.0001
	M. quadrifasciata	1.5342 (±0.30)	2.45 (1.6001-3.3013)	5.80	<0.0001
Abamectin	*T. fiebrigi*	2.7584 (±0.44)	3.53 (3.0996- 3.9732)	16.22	<0.0001
	M. quadrifasciata	9.5683 (±1.96)	8.81 (8.4575-9.1725)	49.69	<0.0001

Table 2. Relative toxicity of topically exposed insecticides to *T. fiebrigi* and *M. quadrifasciata*.

Insecticide	**Specie**	**Slope (± SE)**	**LD50 (95 % FL) (ng a.i./bee)**	**t**	**p value**
Thiamethoxam	*T. fiebrigi*	2.9547 (±0.65)	5.50 (4.8082- 6.2068)	15.87	<0.0001
	M. quadrifasciata	3.7765 (±1.32)	9.06 (8.1888-9.9387)	20.88	<0.0001
Spinetoram	*T. fiebrigi*	1.0743 (±0.14)	5.79 (3.7172- 7.8718)	5.62	<0.0001
	M. quadrifasciata	2.0000 (±0.59)	26.27 (18.2274-34.3170)	6.58	<0.0001
Abamectin	*T. fiebrigi*	0.9860 (±0.15)	8.07 (4.4483- 11.6968)	4.48	<0.0001
	M. quadrifasciata	1.3457 (±0.21)	237.74 (152.9804-322.4947)	5.65	<0.0001

Table 3. Relative toxicity of topically exposed insecticide thiamethoxam and DEF + thiamethoxam to *T. fiebrigi.*

Insecticide	**Slope (± SE)**	**LD_{50} (95 % FL) (ng a.i./bee)**	**t**	**p value**
Thiamethoxam	2.9547 (±0.65)	5.50 (4.8082- 6.2068)	15.87	<0.0001
DEF + thiamethoxam	1.1358 (±0.27)	3.37 (0.8006-1.7514)	4.21	<0.0001

Artigo 3- Associação Brasileira de Normas Técnicas (ABNT)

Systematic review and global meta-analysis of the effects of insecticides on bees

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Systematic review and global meta-analysis of the effects of insecticides on bees

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Abstract: Insecticides are applied worldwide to control pests in a variety of agricultural crops. However, they may be targeted not only to insect pests, but also on beneficial insects such as pollinators bees. There are evidences that the widespread use of insecticides represents a threat to bees but many controversies and gaps still remain on the subject. In order to get further insights about the effects of insecticides on bees a systematic review followed by meta-analysis was performed. The sublethal effect was emphasized, seeking to know those most studied and their relationships with the other variables investigated. There were investigated studies that evaluated the effects of the following chemical groups on bees: organophosphates, pyrethroids, neonicotinoids, inhibitors of chitin synthesis and spinosyns. The search was performed in four databases (Web of Science, Scopus, Pub Med and Science Direct). A total of 440 articles were selected from 35 countries. The largest number of studies were conducted in Europe (43,2%) and North America (32,5%). In the other regions such as in Africa and South America few studies have addressed the effects of insecticides on bees, with the exception of Brazil, which in recent years has presented an increasing number of articles. Neonicotinoids insecticides are the most studied group worldwide and are the most commonly reported to cause sublethal effects on bees. There was a growing number of studies investigating these effects, of which mostly have showed toxic effect. *Apis mellifera* was the most studied species, which suggests that other species are also part of future research. The laboratory evaluations were the most frequent, which emphasizes that more studies in field conditions should be carried out, considering the different stages of insect development and the specific characteristics of each species, factors that may interfere with toxicity.

Keywords: Toxicity. Pesticides. Pollinators. Environmental risk. Global research

Introduction

Pollinators are an essential component of global biodiversity, providing a vital ecosystem services to crops and wild plants (AHSMAN et al., 2004; AGUILAR et al., 2006; KLEIN et al., 2007; RICKETTS et al., 2008). About 87 of the leading global food crops is dependent upon animal pollination, being the bees the most important

pollinating agents, represented by several species distributed in various regions of the planet (POTTS et al., 2010a).

Recently, the decline of bee populations has grown on a global scale and worried researchers, farmers, governments and society about the possible causes of this phenomenon. Climate changes, pathogens, habitat fragmentation and the use of pesticides are some of the factors (MEMMOTT et al., 2007; KERR et al., 2015; SÁNCHES-BAYO et al., 2016). Insecticides have been considered one of the biggest threats to bees, especially since they are widely used in agriculture for pest control worldwide (VANBERGEN et al., 2013; WOODCOCK et al., 2016). Neonicotinoids, organophosphates, pyrethroids, inhibitors of chitin synthesis and spinosyns are among the most important chemical groups, as they comprise a large part of the insecticides marketed for pest control in agriculture (JESCHKE et al., 2011).

Studies on the impacts of insecticides on bees have revealed serious effects on several species, including exotic and native bees (SCOTT-DUPREE et al., 2009; TOMÉ et al., 2015; BARON et al., 2017; TOSI et al., 2017). A disruption in pollination services could have important negative ecological and economic consequences, because the cessation of these services could reduce wild plant diversity, narrow ecosystem stability, reduce crop production, and decrease food security and human welfare (AIZEN; HARDER, 2009; GALLAI et al., 2009; GARIBALDI et al., 2011). Concern over these issues has led some countries to temporarily restrict and reduce the use of certain products considered to be harmful to bees, such as neonicotinoids in the European Union and the United States (BARON et al., 2017). However, many controversies and gaps still remain about such restrictions and about the real causes of bees decline.

Reviews so far available in the literature on the effects of insecticides on bees have addressed only one or two chemical groups, based on few databases (WINFREE et al., 2009; ARENA; SGOLASTRA, 2014; LUNDIN et al., 2015). In this sense, a systematic review was carried out followed by meta-analysis with the objective of detecting more evidence about the effects of insecticides on bees and to know the step that approaches the research worldwide on this topic. For each study, the following parameters were analyzed: country, year of publication, bee species, insecticides, methodological approaches, observed effects, biological level, development stage and level of toxicity. Herein, we sought to identify and characterize general patterns on how

and on which extent these parameters may influence the toxicity of insecticides on bees. The sublethal effects from exposure to low dose levels and/or long-term concentrations are poorly understood and have not been considered in risk studies for discussion purposes (THOMPSON, 2003) although several papers have recently depicted serious disturbances for several species, both at the individual and at the colony level (SANTOS et al., 2016; PITTS-SINGER; BARBOUR, 2016; FRIOL et al., 2017). Therefore, the sublethal effects were also investigated.

Methods

To perform the systematic review, we investigated studies that evaluated the effects of the following chemical groups of insecticides on bees: organophosphates, pyrethroids, neonicotinoids, inhibitors of chitin synthesis and spinosyns. The search was performed in four databases as following: Web of Science, Scopus, Pub Med and Science Direct. The following keywords were used for each of the five chemical groups considered:

(organoph* OR chlorpyrifos OR malathion OR dimethoate OR fenitrothion OR acephate OR cadusafos OR diazinon OR disulfoton OR ethion OR ethoprophos OR fenamiphos) AND (*bee OR *bees);

(pyreth* OR cypermethrin OR deltamethrin OR alfa-cypermethrin OR beta-cypermethrin OR bifenthrin OR acrinathrin OR allethrin OR beta-cyfluthrin OR cyfluthrin OR d-allethrin) AND (*bee OR *bees);

(neonic* OR imidacloprid OR thiamethoxam OR acetamiprid OR clothianidin OR thiacloprid OR dinotefuran) AND (*bee OR *bees);

(chitin synthesis inhibitors OR diflubenzuron OR novaluron OR lufenuron OR teflubenzuron OR triflumuron OR bistrifluron OR chlorfluazuron OR flufenoxuron) AND (*bee OR *bees);

(spinosyn OR spinetoram OR spinosad) AND (*bee OR *bees).

Publications were considered in English, Portuguese and Spanish (last access date: January 2017). The systematic review followed the guidelines proposed by Prisma 2009 (Figure 1). The initial database consisted of 5212 publications, from which duplicates were removed. Review articles, meta-analyzes, and book chapters were excluded. Only primary research studies surveying the insecticide toxicity were

considered, excluding remaining articles such as those that evaluated insecticide residues in bee sub-products or in dead bees. After the initial search, the publications according our criteria were included in the study. The data mining for each publication followed a standard protocol previously established for all chemical groups. This protocol was designed dynamically based on the contents of selected articles. Therefore, we decide to extract the following for further analysis: country, year, bee species, insecticides, methodological approaches, observed effects (lethal, sublethal or both), biological level, development stage and level of toxicity. For the studies that we did not verify clearly the country where the research was carried out, we considered the country of the first author. Acaricides are massively used in the control of several mites inside the hives, then these were also considered.

Four methodological approaches were established: **laboratory**- if performed under controlled conditions; **semi-field-** if carried out in greenhouse, cages or tunnels in the field; **field**- only in the field; **combined**- when more than one methodological approach was used. The biological levels evaluated were as following: **individual** - when only a certain group of individuals or caste was evaluated; **colony** - when features related to colony were evaluated; **both** - when survey was carried out taking into considering individual and colony. The development phase of bees was also considered as an important parameter in the determination of toxicity and therefore in each study was considered the phase evaluated: **immature**, **adult** or **both**. Finally, information was collected regarding the level of toxicity, based on the conclusions of each study and classified as: **toxic**, **moderately toxic** and **innocuous**. The data obtained were analyzed through the Statistical Program R and MATLAB. A checklist for the systematic review is available in Anexs (Prisma Checklist).

Results

A total of 440 publications matched our criteria (Figure 1). The studies were conducted in 35 countries. The largest number was articles were from five countries: United States (107), France (63), United Kingdom (53), Brazil (35) and Canada (33) (Figure 2). About 43.2% of the studies were conducted in Europe (n=190), 32.5% in North America (n=143), 10.2% in Asia (n = 45), 9.8% in South America (n = 43), and less than 2% in Africa, Australia, Oceania and Central America (Figure 2). The first studies evaluating the effects of insecticides on bees started around 1952, with the

groups of pyrethroids and organophosphates (Figure 3), but only since 2006 there has been a steady increase and with large number of article mainly from 2010 to the present. On the last seven years the most studied chemical groups are as following: neonicotinoids (n=197), organophosphates (n=110), pyrethroids (n=99), chitin synthesis inhibitors (19) and spinosyns (15).

Neonicotinoids and inhibitors of chitin synthesis were the chemical groups with the greater number of studies considering the sublethal effects on bees (Figure 4 A). Most studies with spinosyns evaluated both effects (lethal and sublethal), while in the organophosphate group the majority addressed lethal effects only. In the pyrethroid group, a similar proportion of studies investigated the lethal, sublethal or both. Often, insecticides are considered innocuous when both effects were evaluated (lethal and sublethal) (Figure 4B). For insecticides considered moderately toxic, no apparent difference was observed between the types of effects evaluated (lethal and sublethal). When sub-lethal effects were evaluated, the toxic insecticides were mostly observed. In general, studies that evaluated all the stages of bee development (immature and adult) investigated both lethal and sublethal effects (Figure 4C). On the other hand, studies that evaluated only the immature phase or only the adult phase relied on sublethal effects. Approaches at colony level mostly involved sub-lethal effects while at the individual level involved both effects (Figure 5A). In the studies evaluating both levels, there were predominant sublethal evaluations, although the lethal ones also occurred. Analysis of sublethal effects prevailed in studies with bees with social behavior and lethal and sublethal effects of joint form in solitary bees (Figure 5B). Field studies, semi-field or those involving more than one approach investigated mainly both effects (lethal and sublethal). In laboratory conditions, there was a majority of studies evaluating lethal effects only (Figure 6).

PRISMA 2009 Flow Diagram

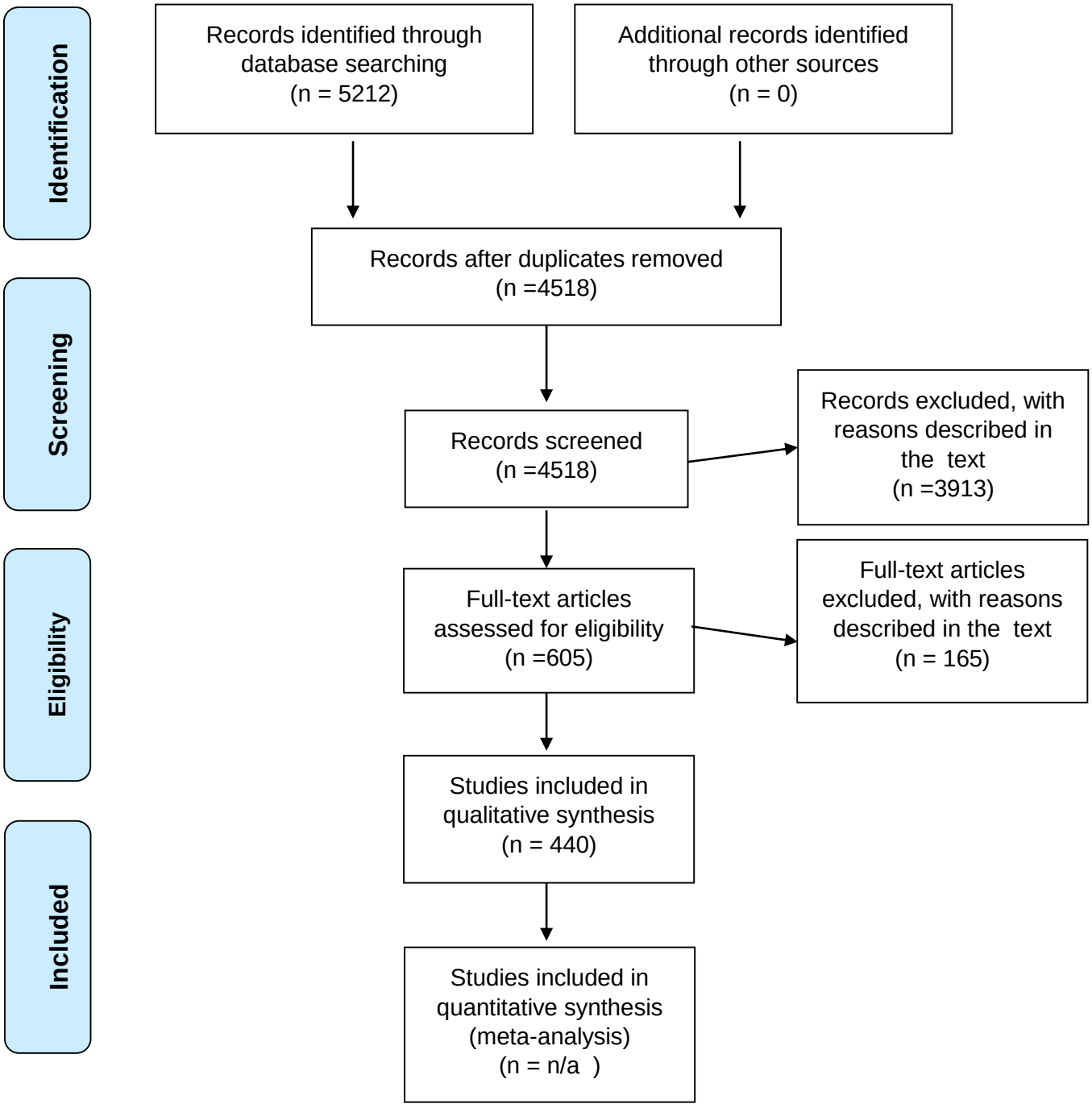


Figure 1. Flow diagram for the systematic review.

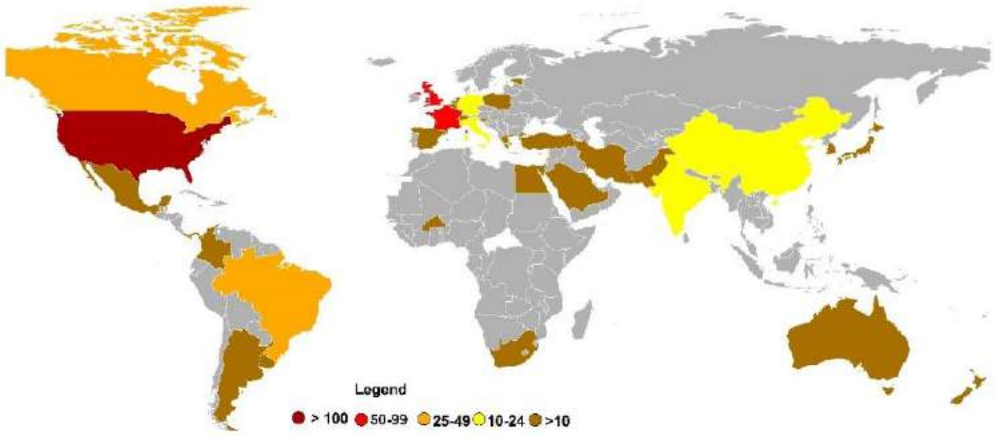


Figure 2. Geographical distribution of research with insecticides impacts on bees.

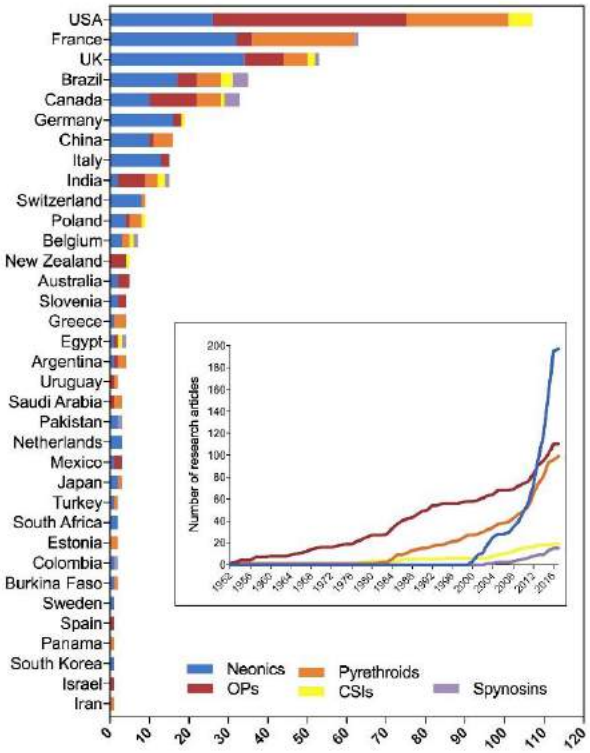
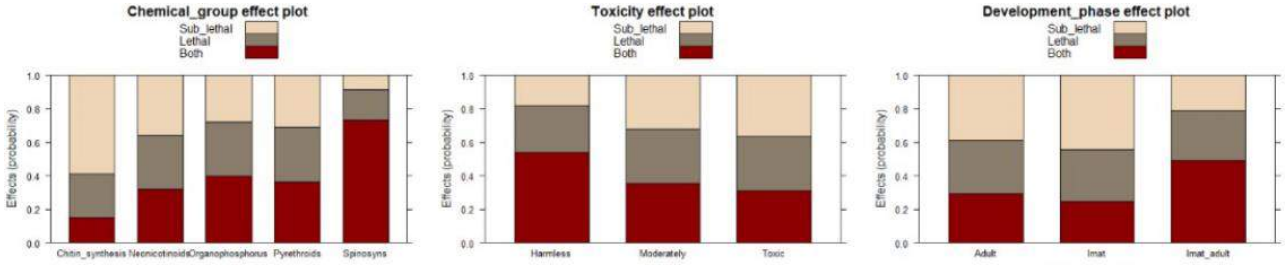
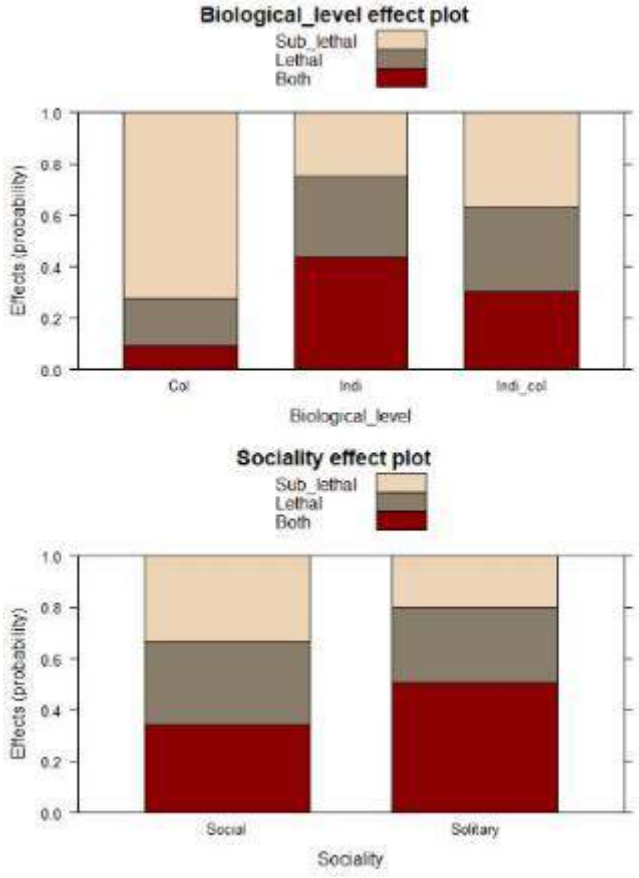


Figure 3. Number of publications by chemical group and country and the evolution of research on the effect of insecticides on bees over time. (OPS= Organophosphates, CSis= Inhibitors of Chitin Synthesis).



(A) (B) (C)

Figure 4. Effects of ordered logistic regression. Three variables related to bee poisoning. Colors are linked to *Effects* (sublethal, lethal, both).



(A)

(B)

Figure 5. Effects of ordered logistic regression. Two variables related to bee poisoning. Colors are linked to *Effects* (sublethal, lethal, both).

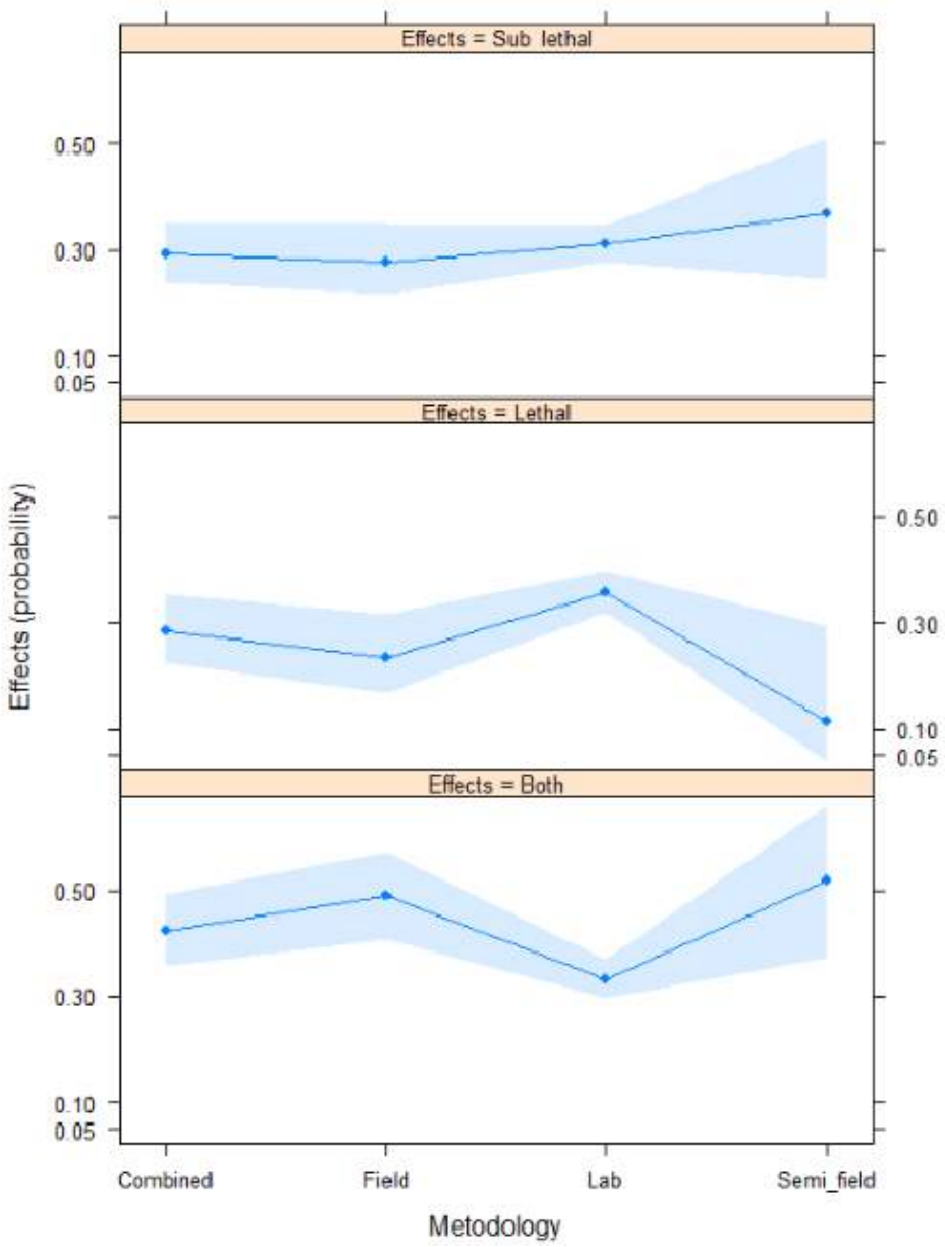


Figure 6. Multinomial effect. Percentage of studies according to the methodology.

Regarding sublethal effects, the majority of publications investigated aspects related to: survival, sanity, reproduction, physiology, morphology, feeding communication, colony, and behavior (Figure 7). With the exception of the effects under morphology, where there was a predominance of studies with insecticides inhibiting chitin synthesis, the other effects were studied mostly (+ 50%) in bees exposed to neonicotinoid insecticides. The spinosyns group had a lower percentage of studies with sublethal effects. *Apis mellifera* was the most prevalent species, followed by *Bombus terrestris*, *Bombus impatiens*, *Apis cerana,* and *Megachile rotundata* (Figure 8). The neonicotinoids group was the

most prevailed with sublethal effects. Impacts of spinosyns were investigated mainly in *B. impatiens* and *Melipona quadrifasciata*. For most species, aspects related to the effects of insecticides on insect behavior were studied. Most toxic effects were observed when evaluating sublethal effects and both effects (lethal and sublethal) with neonicotinoid insecticides. While when only lethal effects were evaluated, most of the studies were with organophosphate insecticides (Figure 9).

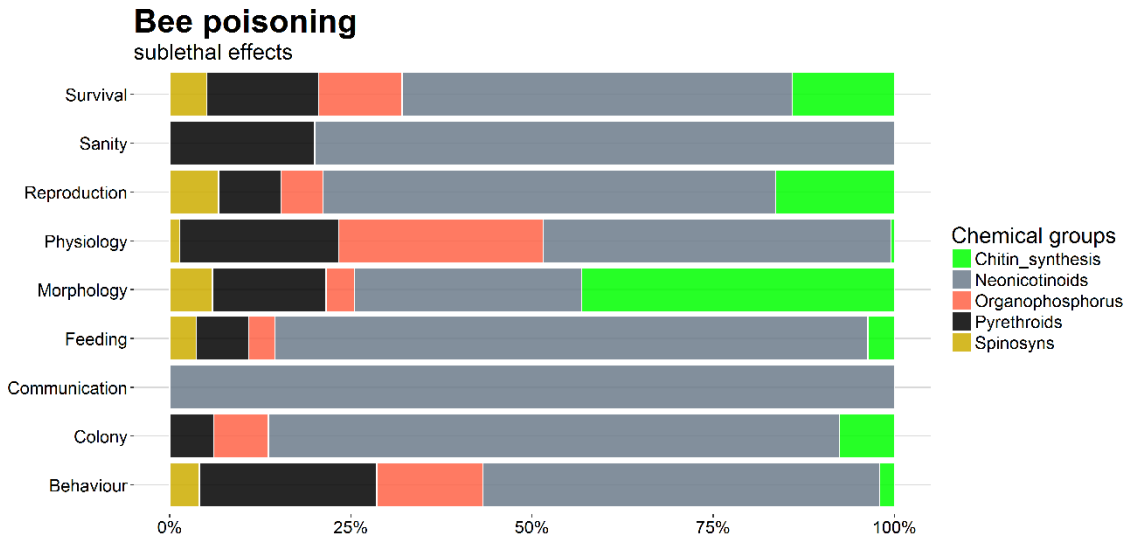


Figure 7. Percentage of poisoning of five major chemical groups concerning to sublethal effects on bees.

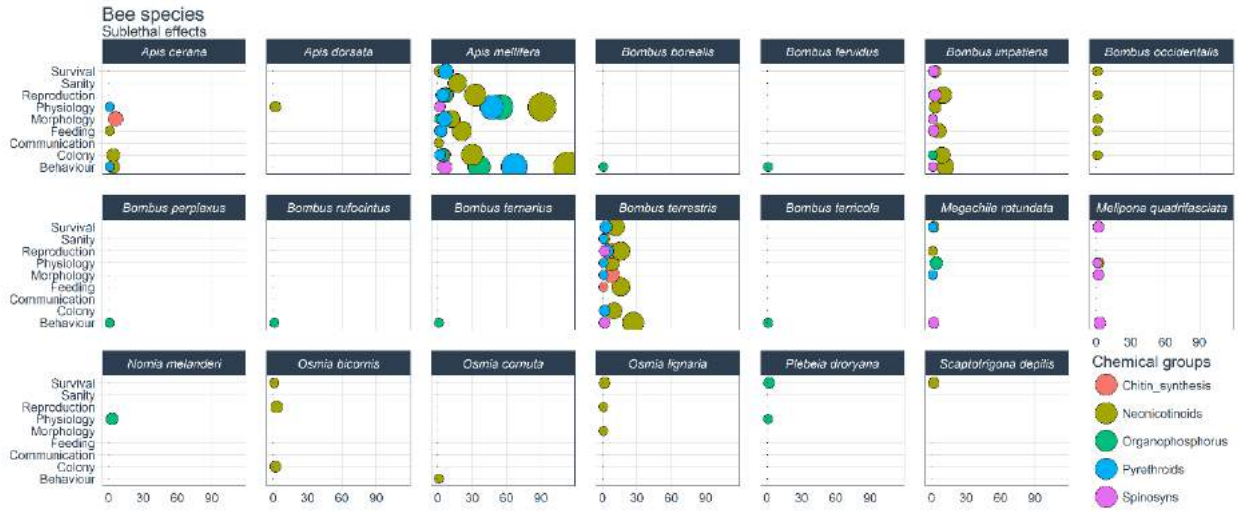


Figure 8. Relative values of five major chemical groups related to sublethal effects on bee species.

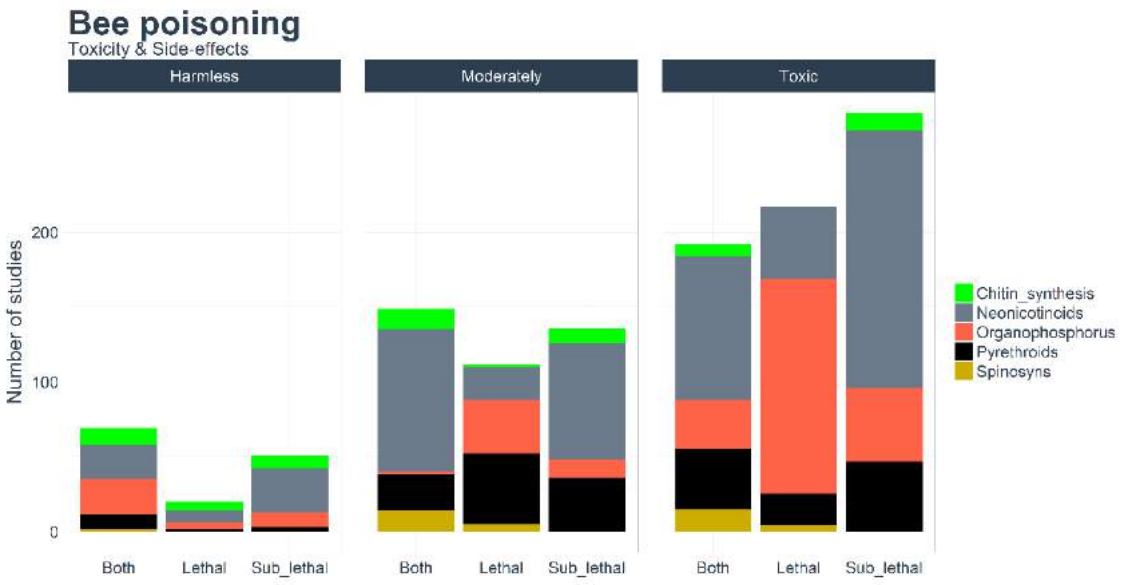


Figure 9. Number of studies related to chemical groups and according to level of toxicity and effects.

Discussion

From the results, it was observed that more studies are concentrated in few countries in Europe and North America. This may be associated with the fact that in the past these two continents have suffered severe bee declines and concern about the dependence of various agricultural crops on pollination has boosted the development of research into the possible causes of the phenomenon. In the United States alone, there was a 59% loss between 1947 and 2005 (VAN ENGELSDORP et al., 2008) whereas in central Europe there was a loss of 25% of the hives between 1985 and 2005 (POTTS et al., 2010b). Lundin et al. (2015) in a systematic review on the impacts of neonicotinoid insecticides on bees found similar geographical distribution. The unequivocal distribution of the studies still remains, which represents a serious problem, since most of the insecticides of the chemical groups discussed here are registered in several countries (JESCHKE et al., 2011). Brazil is currently the only country in South America with a considerable number of publications (n=35) behind the United States, France and the United Kingdom. The great diversity of stingless bees, about 240 species (PEDRO, 2014) and the importance of agriculture to this country, raised concern about the possible impacts of insecticides used on crops, which probably contributed to the development of research on this theme.

Until the mid-1990s, organophosphates and pyrethroids predominated in toxicity studies, probably due to the lack of other chemical groups, which were discovered only later. Neonicotinoids, for example, were marketed in 1990, and spinosyns around 1997 (SALGADO, 1998; LUNDIN et al., 2015).

The large number of studies conducted since 2006 may be related to the fact that consecutive evaluations in managed hives between 2006 and 2010 recorded successive mortalities of *A. mellifera* populations in the United States (about 30 %) and in some European countries (VAN ENGELSDORP et al., 2011). The record of the highest sales of neonicotinoid insecticides coincides with this period, which made this group one of the main factors involved in bee mortality and therefore the target of research. Since its introduction in 1990, sales of these insecticides have only increased, from 2005 to 2008, from 16% to 24% (JESCHKE et al., 2011). The systemic and residual action made this group one of the most used in agriculture, since they present efficiency in the control of several pest insects (BLACQUIÈRE et al., 2012). From 2013, with the establishment of restrictions on the use of three neonicotinoids (clothianidin, thiamethoxam and imidacloprid) for seed treatment in the European Union and policies for reducing use in Ontario, Canada, the number of researches with bees and insecticides mainly of this chemical group, grew rapidly. Systematic review by Lundin et al. (2015) found 216 primary research articles with the theme neonicotinoids and bees, which demonstrates the interest of studies with this group. In this review, however, 197 papers were found, which may seem contradictory, but it is not, since Lundin et al. (2015) considered works measuring the contamination not only of bees, but also of plants, products of the beehive and other materials that they had come in contact or ingested. In addition, they included publications related to the development of methods of analysis of neonicotinoids in natural environment. While in this review, only publications were evaluated evaluating the effect of neonicotinoid insecticides on the insect, seeking to find more specific responses to the insecticide and bee relationship.

The groups with the highest percentage of studies investigating sublethal effects were neonicotinoids and inhibitors of chitin synthesis. The residual and systemic action in plants may explain the large amount of insecticide research in the first group investigating long-term effects. In addition to spraying on crops, they are used in the treatment of seeds in crops such as maize and canola. In

the latter case, flowers quite attractive to bees may contain residues of insecticides, which are able to translocate up to pollen and nectar, the main food sources for these insects (DELAPLANE; MAYER, 2000). Bonmatin et al. (2003) verified that samples of sunflower pollen that received seed treatment had on average 3 µg kg^{-1} of imidacloprid residues. Disorientation, localization difficulty, and damage to flight and locomotion ability are some of the sublethal effects observed during exposure to some insecticides belonging to this group, such as thiamethoxam and imidacloprid (TOMÉ et al., 2015; TOSI et al., 2017). A large number of sublethal studies with inhibitors of chitin synthesis probably occurred due to the non-immediate action of these insecticides on the insects, which can be considered a sublethal effect, since they cause morphological alterations in the individual body, such as appendix deformations and interference in the metamorphosis, especially during the immature phases (SCOTT-DUPREE et al., 2009).

In this study, the highest percentage of works with insecticides considered to be toxic were those that evaluated sublethal effects, which were mostly related to the neonicotinoids group. This result is worrisome, since they are more in-depth studies, which generally involve longer periods of testing, and specific analyzes, such as at behavioral level, organs, cells and genes. Effects on bees communication and behavior are the most frequently addressed, including changes in memory, ability to travel, flight and food collection, which are mostly studied in insects under the effect of neurotoxic insecticides (LAMBIN et al., 2001; BORTOLOTTI et al., 2003; TOSI et al., 2017). This is related to the fact that the behavior of insects as well as of most animals is governed by neurons and their nervous systems and neurotoxic insecticides have precisely these sites of action (HAYNES, 1988). According to Haynes (1988) insecticides that act on the nervous system can negatively affect all elements of insect behavior at doses much lower than the lethal dose.

It is important to point out that many factors may be involved in the different degrees of toxicity observed in the studies addressed in this review, among them: species of bee, development phase, methodology, insecticide and dose/ concentration considered, which may interfere with greater or lesser susceptibility found in each publication. According to Arena and Sgolastra (2014), for each sub-lethal effect evaluated, different species of bees may have varied sensitivity and

therefore the extrapolation of the results to different conditions should be performed with caution.

From the observed results, it is clear the need for toxicity assessments at different stages of development of the bees, to try to understand which other variables may be related to susceptibility. Wu et al. (2001) did not observe mortality of *A. mellifera* larvae reared on food containing neonicotinoid residues, but observed that these were delayed to reach adult phase. Inhibitors of chitin synthesis appear to cause changes in the eggs and early stages of the development of *Megachile rotundata*, however, in adults, they appear to be non-toxic (HODGSON et al., 2011; PITTS-SINGER; BARBOUR, 2016). In addition, hive-level studies are essential because the delay in the development of the workers will cause in the short or long term the decline of the entire population, since these are responsible for the collection of food and maintenance of the swarm (WINSTON, 1987).

Laboratory approaches are the focus of short-term studies, which generally assess lethal toxicity, that is, are preliminary evaluations, so we observed higher percentage publications. While evaluations in field conditions, semi-field or combining different approaches are generally more in-depth studies, representing more realistic conditions, and therefore require more time and are so far more scarce. According to Blacquière et al. (2012), there is a discrepancy between laboratory and field tests for sublethal effects, so it is necessary to investigate more than one approach, focusing on the most realistic conditions.

In the papers evaluated in this review, *Apis mellifera* was the most searched species. This bee is an important pollinator of various crops of commercial interest, being handled and found across the globe. *Bombus terrestris* and *Bombus impatiens* were also among the most studied. These species are used for the pollination of several crops, including those grown in protected such as tomatoes, strawberries and blueberries (VELTHUIS; DOORN, 2006). Since 1987 with bumblebees for commercial purposes, production has expanded rapidly, reaching an annual production of 1 million colonies in 2004 (VELTHUIS; DOORN, 2006). However, these species are more restricted to certain regions of the globe, such as Eurasia (*B. terrestris*) and North America (*B. impatiens*), because they prefer lower temperatures and light intensities (VELTHUIS; DOORN, 2006). Wild bees on the other hand, as they are adapted

to their places of origin may present advantage in relation to this aspect. These bees are also important pollinators, mainly because they exert complementary effect to other species in diverse cultures. In the strawberry, they can pollinate parts of the flowers inaccessible to other bees and thus improve the quality of the fruits (MALAGODI-BRAGA; KLEINERT, 2007).

Although these bees often live in landscapes around crops, they may also be exposed to pesticides. In this sense, it is necessary that future studies investigate the impacts of these compounds on wild bees hitherto little studied, as for example the solitary species. Monitoring of populations over several seasons in landscapes with different patterns of insecticide use and/or the use of approaches involving population modeling are examples of studies that can be carried out (LUNDIN et al., 2015). The effects of insecticide mixtures with other pesticides as well as metabolites formed with degradation in the environment are some unexplored points that deserve attention.

Conclusions

Based on the research efforts and the results obtained in this systematic review we can conclude that many gaps still remain about the impacts of insecticides on bees. Neonicotinoids insecticides are the most studied group of in the world, with emphasis in Europe and North America. In addition, are most commonly reported to cause sublethal effects on bees. For this reason, they should be studied in other regions of the world that also use them in the handling of insect pests, as for example developing countries, which have great diversity of species of bees, often still unknown. The scarcity of studies with insecticides from the spinosyns group and inhibitors of chitin synthesis reinforces the importance of future studies.

Although in recent years have been had advances in research with sublethal effects, more work in field conditions is essential to understand more about the susceptibility of different species to insecticides in conjunction with various environmental factors.

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CONCLUSÕES

O cultivo do morangueiro em sistema semi-hidropônico recebe grande diversidade de visitantes florais, incluíndo insetos das ordens Hymenoptera, Diptera, Coleoptera, Hemiptera e Lepidoptera, sendo Hymenoptera a mais frequente e abundante.

As 13 espécies de abelhas pertencentes as famílias Apidae (*Apis mellifera*, *Tetragonisca fiebrigi*, *Tetrapedia* sp., *Trigona spinipes*, *Schwarziana quadripunctata*, *Plebeia emerina*, *Plebeia remota* e *Bombus pauloensis*) e Halictidae (*Dialictus* sp.1, *Dialictus* sp.2, *Augochloropsis* sp.1, *Augochloropsis* sp.2, *Augochlora* sp.1) são potenciais polinizadoras na cultura do morangueiro. Nos municípios de Bento Gonçalves, RS e Farroupilha, RS, a introdução de colmeias de *Tetragonisca fiebrigi*, *Plebeia emerina* ou *Plebeia remota* no interior de cultivos protegidos de morangueiro pode ser uma alternativa ao déficit de polinização.

Em laboratório, os inseticidas tiametoxam e espinetoram, assim como o acaricida abamectina apresentam alta letalidade (oral e tópica) para abelhas adultas de *M. quadrifasciata* e *T. fiebrigi*, enquanto novalurom não é prejudicial.

A atividade locomotora de *M. quadrifasciata* e *T. fiebrigi* é alterada quando expostas a doses subletais de abamectina, espinetoram e tiametoxam.

Neonicotinoides são os inseticidas mais estudados no mundo, com ênfase na Europa e América do Norte, e mais comumente relatados por causar efeitos subletais considerados tóxicos às abelhas.

Ainda existem muitas lacunas sobre os impactos dos inseticidas nas abelhas, especialmente em relação as abelhas nativas sem ferrão e a susceptibilidade em condições de campo.

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ANEXOS

Normas publicação periódico *Horticultura Brasileira*- disponível em:

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	2-3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	2-3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	3-4
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	3-4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	3-4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	3
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	3-4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	3-4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	3-4
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	n/a

Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	3-4
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	3-4
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	n/a
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	n/a
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	3-5, S1 Table
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	5-10, S1 Table
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	n/a
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	n/a
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	n/a
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	n/a
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	n/a
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10-18
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	10-18
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	18
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Funding statement