RODRIGO LOPES DE OLIVEIRA

TOXICITY OF CHLORANTRANILIPROLE IN EXTRAFLORAL NECTAR OF COTTON PLANT FROM SEEDS TREATED FOR *Harmonia axyridis* (COLEOPTERA: COCCINELLIDAE) AND *Chrysoperla externa* (NEUROPTERA: CHRYSOPIDAE)

LAVRAS-MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Entomologia, área de concentração em Entomologia, para a obtenção do título de Doutor.

Prof. Dr. Geraldo Andrade Carvalho
Orientador

Dr. Pablo da Costa Gontijo
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TOXICIDADE DE CLORANTRANILIPROLE EM NECTAR EXTRAFLORAL DE PLANTA DE ALGODÃO PROVENIENTE DE SEMENTES TRATADAS PARA *Harmonia axyridis* (COLEOPTERA: COCCINELLIDAE) E *Chrysoperla externa* (NEUROPTERA: CHRYSOPIDAE)

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Entomologia, área de concentração em Entomologia, para a obtenção do título de Doutor.

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A Deus e a Nossa Senhora Aparecida, por tudo que já me concederam e por estarem sempre presentes em meu caminho

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RESUMO GERAL

O uso de inseticidas sistêmicos em tratamento de sementes é considerado mais seguro para organismos não alvos em comparação com outras formas de aplicação. Entretanto, por estes produtos translocarem no interior das plantas, podem estar presentes em néctar extrafloral que é um importante alimento para os inimigos naturais. Diante disso, os objetivos do presente estudo foram avaliar se o clorantraniliprole causa efeitos negativos no desenvolvimento, reprodução e comportamento de (1) *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) e (2) *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) quando larvas e adultos desses predadores foram expostos a plantas de algodão cultivadas a partir de sementes tratadas com este inseticida, bem como reunir informações dos principais estudos realizados em tratamento de sementes com clorantraniliprole para oito inimigos naturais em três culturas. Clorantraniliprole não causou efeito letal sobre *H. axyridis,* porém foram observados efeitos negativos transgeracionais. Quando larvas foram expostas ao néctar extrafloral contaminado houve redução no tempo de desenvolvimento de larvas e pupas, peso de machos e sobrevivência dos insetos da geração seguinte à exposta. As joaninhas provenientes de adultos que entraram em contato com néctar extrafloral contaminado também tiveram o tempo de desenvolvimento pupal, viabilidade dos ovos e sobrevivência reduzidos. O predador *C. externa* não apresentou efeitos transgeracionais negativos, sendo que somente quando larvas foram expostas ao produto, as pupas formadas apresentaram período de desenvolvimento menor. As demais características biológicas observadas não sofreram influência negativa do inseticida. Larvas de *H. axyridis* têm atração por plantas de algodão sem tratamento em comparação com plantas de algodão oriundas de sementes tratadas com clorantraniliprole; entretanto, adultos de *C. externa* foram atraídos por plantas de algodão provenientes de sementes tratadas ou não de forma semelhante. Para os predadores *Lysiphlebus testaceipes* (Cresson) (Hymenoptera, Braconidae, Aphidiinae), *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinelidae), *Orius insidiosus* (SAY, 1832) (Hemiptera: Anthocoridae) e *C. externa*, o inseticida foi classificado como levemente prejudicial; porém, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), *H. axyridis*, *Hippodamia convergens* (Guérin-méneville) (Coleoptera: Coccinellidae) e *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) clorantraniliprole foi inócuo, segundo classificação de toxicidade da IOBC.

Palavras-chave: Inimigo Natural. Predador. Inseticida Sistêmico. MIP. Diamida

GENERAL ABSTRACT

The use of systemic insecticides in seed treatment is considered safer for non-target organisms compared to other forms of application. For this reason, there is concern about possible effects that contaminated extrafloral nectar may cause on beneficial organisms. (1) *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) and (2) *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) were studied in order to evaluate the effect of chlorantraniliprole on the development, when larvae and adults of these predators were exposed to cotton plants grown from seeds treated with this insecticide, as well as to gather information from the main studies carried out with chlorantraniliprole seed treatment for eight natural enemies in three cultures. Chlorantraniliprole did not cause a lethal effect on *H. axyridis*, but transgenerational negative effects were observed. When larvae were exposed to the contaminated extrafloral nectar there was a reduction in larval and pupal development time, males weight and insect survival of the next generation to that exposed. Ladybugs from adults who came in contact with contaminated extrafloral nectar also had reduced pupal development time, egg viability and survival. The predator *C. externa* did not present negative transgenerational effects, and only when larvae were exposed to the product, the pupae formed had a minor development period. The other biological characteristics observed were not negatively influenced by the insecticide. Larvae of *H. axyridis* are attracted to untreated cotton plants compared to cotton seedlings treated with chlorantraniliprole; however, *C. externa* adults were attracted to cotton plants from treated or untreated seeds. For the predators *Lysiphlebus testaceipes* (Cresson) (Hymenoptera, Braconidae, Aphidiinae), *Coleomegilla maculata* (Coleoptera: Coccinelidae), *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) and *C. externa*, the insecticide was classified as slightly harmful; However, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), *H. axyridis*, *Hippodamia convergens* (Guérin-méneville) (Coleoptera: Coccinellidae) and *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) chlorantraniliprole were harmless according to IOBC toxicity classification.

Keywords: Natural Enemy. Predator. Systemic Insecticide. IPM. Diamide

SUMÁRIO

PRIMEIRA PARTE 9
INTRODUÇÃO GERAL 9
OBJETIVOS ESPECÍFICOS 11
REFERENCIAL TEÓRICO 12
A cultura do algodoeiro 12
O tratamento de sementes no Manejo Integrado de Pragas do algodoeiro 14
Modo de ação do clorantraniliprole 15
Aspectos bioecológicos de *H. axyridis* 16
Aspectos bioecológicos de *C. externa* 18
REFERÊNCIAS 20
SEGUNDA PARTE – ARTIGOS 29
ARTIGO 1 - LONG-TERM EFFECTS OF CHLORANTRANILIPROLE REDUCED RISK INSECTICIDE APPLIED AS SEED TREATMENT ON LADY BEETLE *Harmonia axyridis* 29
Abstract 30
1. Introduction 30
2. Materials and methods 32
3. Results 35
4. Discussion 35
References 38
ARTIGO 2 - TOXICITY OF CHLORANTRANILIPROLE SEED TREATMENT TO *Chrysoperla externa* AND SYNTHESIS OF LABORATORY STUDIES FOR RISK ASSESSMENT TO EIGHT NATURAL ENEMIES 47
Abstract 48
1. Introduction 48
2. Materials and methods 50
3. Results 52
4. Discussion 53
5. Conclusions 55
References 56

PRIMEIRA PARTE

INTRODUÇÃO GERAL

O algodão é um produto de importância socioeconômica para o Brasil. Além de ser a mais importante fonte natural de fibras e segunda maior fonte de óleo vegetal, garante ao país lugar privilegiado no cenário internacional, como um dos cinco maiores produtores mundiais, ao lado de China, Índia, Estados Unidos e Paquistão (FAO, 2016).

O algodoeiro apresenta diferentes tipos de nectários, estruturas responsáveis por produzir secreções açucaradas que atraem diversos artrópodes, incluindo organismos benéficos (MONNEART et al., 2000). Os nectários extraflorais (NEF) já são encontrados na planta de algodão desde o primeiro par de folhas verdadeiras, em superfície abaxial da folha próximo aos principais feixes de transporte de seiva (FREE, 1970; STEWART et al., 2010).

O algodoeiro é acometido em todo o seu ciclo de desenvolvimento pelo ataque de artrópodes pragas. Dentre estes, destacam-se espécies de ocorrência inicial ao estabelecimento da cultura como pulgões e tripes. Há também pragas de ocorrência geral, que ocorrem no algodoeiro principalmente durante o florescimento e frutificação, e as pragas ocasionais que são de ocorrência menos frequente. É evidente que todas essas pragas causam danos à produção; contudo, as que ocorrem no período reprodutivo da cultura são mais danosas, já que comprometem a produtividade e a qualidade das fibras (SANTOS, 2007).

Para o controle de insetos-praga de solo e sugadores na fase inicial da cultura do algodoeiro, o tratamento de sementes vem sendo muito utilizado. Dentre os inseticidas sistêmicos amplamente empregados pode-se citar o clorantraniliprole, do grupo químico das diamidas que são moduladores dos receptores de rianodina nas miofibrilas dos músculos de insetos. A utilização deste produto protege as sementes e raízes contra insetos de solo e devido à sua sistematicidade é também eficiente contra insetos sugadores. O tratamento de sementes é realizado com menor quantidade de produto quando comparado com a pulverização na parte aérea da planta, e isto reduz o impacto ao ambiente e diminui o custo de produção (ALTMANN, 1990; HEATHERINGTON e BOLTON, 1992).

No manejo integrado de pragas (MIP), os produtos empregados devem causar o mínimo de impacto sobre os insetos considerados benéficos. Estes produtos também devem ser menos agressivos ao ambiente, o que justifica o emprego do tratamento de sementes, o qual pode propiciar seletividade ecológica aos inimigos naturais (DEGRANDE et al., 2002).

Nos últimos anos, estudos de seletividade ecológica têm sido realizados com o objetivo de avaliar os efeitos do tratamento de sementes sobre inimigos naturais e polinizadores. Este interesse se deve à preocupação de que os inseticidas utilizados no tratamento de sementes possam translocar-se até o pólen e néctar das plantas, que são muito utilizados pelos inimigos naturais e polinizadores (JESCHKE et al., 2011; LAURENT e RATHAHAO, 2003; STONER; EITZER, 2012).

O conhecimento do impacto de inseticidas sobre inimigos naturais é importante para a integração dos métodos biológico e químico, sendo necessária a avaliação dos efeitos letal e subletal dos produtos fitossanitários sobre inimigos naturais (DESNEUX et al., 2007). Os efeitos subletais são definidos como aqueles que ocorrem com os espécimes que sobrevivem à exposição a produtos químicos. Estes efeitos podem se manifestar como reduções no tempo de vida, taxas de desenvolvimento, fecundidade, mudanças na razão sexual ou alterações no comportamento, os quais muitas vezes não são avaliados (BAYRAM et al., 2010; DESNEUX et al., 2004; STARK; BANKS, 2003).

Dentre os inimigos naturais presentes na cultura algodoeira, as joaninhas apresentam grande destaque, pois a maioria das espécies é entomófaga e suas larvas e adultos consomem grande diversidade de presas, alimentando-se de pulgões, ácaros, larvas de coleópteros e lagartas desfolhadoras (CLAUSEN, 1972; HODEK, 1973). As principais espécies de joaninhas predadoras (Coleoptera: Coccinellidae) presentes na cultura do algodoeiro são *Harmonia axyridis* (Pallas, 1773), *Cycloneda sanguinea* (Linnaeus, 1763), *Eriopis connexa* (Germar, 1824), *Hippodamia convergens* (Guérin Méneville, 1842), *Coleomegilla maculata* (De Geer, 1775), *Olla v nigrum* (Mulsant, 1866), *Hyperaspis festiva* (Mulsant, 1850) e *Scymnus* sp. (IPERTI, 1999; SILVIE et al., 2001). Em particular, *H. axyridis* é uma espécie abundante presente na cultura do algodoeiro e desempenha papel importante no controle biológico dos insetos-praga desta cultura no Brasil e, além dessas presas, este predador pode alimentar-se de fungos, pólen, seiva e néctar (LUNDGREN et al., 2004; LUNDGREN et al., 2011).

Dentro da ordem Neuroptera, destaca-se principalmente a família Chrysopidae, que tem papel importante no controle de insetos-praga. No Brasil, *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae) é a espécie com maior distribuição nos agroecossistemas e com maior volume de pesquisas já realizadas (RIBEIRO, 1991). Estes insetos são encontrados constantemente na cultura do algodoeiro alimentando-se tanto de presas quanto de recursos vegetais como pólen, seiva e néctar (LIMBURG e ROSENHEIM, 2001).

O néctar é uma importante fonte de alimento para insetos predadores e o seu consumo é frequentemente observado em diversos cultivos (PEMBERTON e VANDENBERG, 1993; RICCI et al., 2005; STEPHENSON, 1982). Seagraves et al. (2011) constataram que o consumo de açúcar é muito importante na dieta desses insetos, e Lundgren e Seagraves (2011) concluíram que o néctar aumenta a sobrevivência, a reserva de nutrientes e a capacidade reprodutiva de predadores durante períodos de ausência de presas.

Devido a grande diversidade e quantidade de nectários no algodoeiro, em situações onde é realizado o tratamento de sementes com inseticidas sistêmicos, as plantas de algodão são capazes de absorver essas moléculas por meio de suas raízes e transportá-las via sistema vascular e assim contaminar néctar floral e extrafloral, tornando-se um risco eminente aos insetos benéficos (DIVELY e KAMEL, 2012; EASTON e GOULSON, 2013; KRUPKE et al., 2012).

Inimigos naturais tais como *Orius insidiosus* (Say, 1832) (Hemiptera: Anthocoridae), *Lysiphlebus testaceipes* (Cresson, 1880) (Hymenoptera: Braconidae, Aphidiinae), *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) e as joaninhas *C. maculata* e *H. convergens* sofrem efeitos subletais quando entram em contato com néctar extrafloral de girassol proveniente de tratamento de sementes (GONTIJO et al., 2014; GONTIJO et al., 2015; MOSCARDINI et al., 2014; MOSCARDINI et al., 2015). Diante do exposto, objetivou-se avaliar os efeitos do tratamento de sementes de algodoeiro com clorantraniliprole sobre características biológicas e comportamentais dos predadores *H. axyridis* e *C. externa*.

OBJETIVOS ESPECÍFICOS

- Avaliar os efeitos do tratamento de sementes de algodoeiro com clorantraniliprole sobre *H. axyridis* quando larvas e adultos (geração maternal) foram expostos às plantas de algodoeiro provenientes de sementes tratadas e tendo como fonte de alimento complementar o néctar extrafloral das plantas.

- Avaliar os efeitos do tratamento de sementes de algodoeiro com clorantraniliprole no desenvolvimento e reprodução de espécimes da geração F1 de *H. axyridis* que foram expostos às plantas de algodoeiro provenientes de sementes tratadas e tiveram como fonte de alimento complementar o néctar extrafloral dessas plantas.

- Avaliar os efeitos do tratamento de sementes de algodoeiro com clorantraniliprole sobre o comportamento de larvas de terceiro instar, fêmeas virgens, machos e fêmeas acasalados de *H. axyridis*.

- Avaliar os efeitos do tratamento de sementes de algodoeiro com clorantraniliprole sobre *C. externa* quando larvas e adultos (geração maternal) foram expostos às plantas de algodoeiro provenientes de sementes tratadas e tendo como fonte de alimento complementar o néctar extrafloral das plantas.

- Avaliar os efeitos do tratamento de sementes de algodoeiro com clorantraniliprole no desenvolvimento e reprodução dos espécimes da geração F1 de *C. externa* que foram expostos às plantas de algodoeiro provenientes de sementes tratadas e tiveram como fonte de alimento complementar o néctar extrafloral dessas plantas.

- Avaliar os efeitos do tratamento de sementes de algodoeiro com clorantraniliprole sobre o comportamento de larvas de terceiro instar e adultos de *C. externa*.

- Reunir informações dos principais estudos realizados em tratamento de sementes com clorantraniliprole para oito inimigos naturais em três culturas.

REFERENCIAL TEÓRICO

A cultura do algodoeiro

O algodão tem sua origem relatada na pré-história do Velho e do Novo Mundo, com as primeiras referências da cultura encontradas no Código de Manu, séculos antes de Cristo. Vestígios do algodoeiro mais antigo foram encontrados em escavações arqueológicas nas ruínas de Mohenjo Daro, em Sina, no Vale do rio Indo (Paquistão Ocidental) em um pequeno fragmento de tela e um cordão, com aproximadamente 3000 anos a.C. Na América, o algodoeiro mais antigo foi encontrado nas escavações de Huaca Prieta no Peru, com aproximadamente 2500 anos a.C. Estes dois algodoeiros encontrados, possuíam fibras grossas e ásperas, assemelhando-se às produzidas pelas espécies *Gossypium arboreum* e *Gossypium barbadense*, levando a concluir que a cerca de 5000 anos atrás tais espécies já se achavam diferenciadas. Evidências citológicas e genéticas fundamentam a hipótese de que algodoeiros do Novo Mundo teriam se originados da hibridação entre uma das espécies de algodoeiro do Velho Mundo e uma das espécies silvestres de *Gossypium* da América (NEVES, 1965).

O algodoeiro (*Gossypium hirsutum* Linnaeus) é uma malvácea pertencente ao grupo das dicotiledôneas. É uma das culturas mais importantes do mundo, considerando aspectos econômico e social, sendo cultivado em mais de 80 países, com produção anual de aproximadamente 20 milhões de toneladas de fibra (DUTRA; MEDEIROS FILHO, 2009; SEAGRI/BA, 2012).

A cultura algodoeira apresenta grande importância econômica, uma vez que, mundialmente, o algodão é responsável por mais de 40% da matéria prima para vestuário, o que representa mais de 60% dos insumos têxteis no Brasil e 65% nos Estados Unidos. No Brasil, durante a safra de 2016/2017, foram cultivados 939,1 mil hectares de algodoeiro, obtendo-se produção estimada de 1,53 milhão de toneladas e média de colheita de 1.628 quilos de algodão por hectare (CONAB, 2017).

Áreas cultivadas com algodoeiro em vários países vêm aumentando, com médias anuais de 2%. O comércio mundial do algodão tem movimentado cerca de US$ 12 bilhões anualmente, envolvendo mais de 350 milhões de pessoas em sua linha de produção. Este produto é produzido em todos os continentes, sendo que os principais países produtores de fibra de algodão são China, Índia, Estados Unidos, Paquistão e Brasil (FAO, 2016). O Brasil se destaca como o terceiro maior exportador de algodão do mundo (MAPA, 2017). Os maiores estados brasileiros produtores desta malvácea são o Mato Grosso, Bahia, Goiás, Mato Grosso do Sul e Minas Gerais (CONAB, 2017).

As plantas de algodoeiro apresentam porte subarbustivo, crescimento indeterminado e produzem um número variável de capulhos, com três a cinco lóculos que contêm, em média, 32 a 40 sementes. Suas sementes são cobertas por dois tipos de células diferenciadas que constituem as fibras longas e fiáveis e as curtas ou o “linter”, as quais proporcionam à cultura grande valor comercial (FARIAS, 2005; PENNA, 1999). Ainda apresentam cinco diferentes tipos de nectários que produzem secreções açucaradas, sendo estas, responsáveis pela atração de diversos organismos que as utilizam como fonte de alimento (MONNEART et al., 2000). Segundo Free (1970) e Stewart et al. (2010), o algodoeiro possui 1tipo de nectário floral localizado na base interna do cálice da flor e 4 tipos de nectários extraflorais, sendo que estão localizados na base externa do cálice da flor, na base do pedicelo logo abaixo das brácteas, na página abaxial da folha próximo aos principais feixes de transporte de seiva. Além disso, possui nectários diminutos localizados sobre os pedúnculos florais e em pecíolos foliares jovens.

Esta planta é considerada parcialmente autógama, possuindo sistema reprodutivo considerado misto, com flores hermafroditas. Desta forma, parte de suas sementes são provenientes da autofecundação, sendo a taxa de cruzamento natural variável, dependendo das condições ambientais e da presença de agentes polinizadores das cultivares (CARVALHO, 1993).

Na cultura do algodoeiro ocorrem diversos insetos-praga que podem causar prejuízos econômicos, exigindo a intervenção do produtor para promover a redução de suas populações a fim de se evitar prejuízos. Dentre as principais pragas que atacam essa cultura, destacam-se bicudo do algodoeiro *Anthomonus grandis* (Boheman, 1843) (Coleoptera: Curculionidae), considerado praga chave da cultura; pulgões *Aphis gossypii* (Glover, 1877) e *Myzus persicae* (Sulzer 1778) (Hemiptera: Aphididae); curuquerê *Alabama argillacea* (Hübner, 1818) (Lepidoptera: Noctuidae), lagarta rosada *Pectinophora gossypiella* (Saunders, 1843) (Lepidoptera: Gelechiidae) e complexo de lagartas das maçãs composto pelas espécies *Chloridea virescens* (Fabricius, 1781), *Helicoverpa zea* (Boddie, 1850) e *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) (TORRES, 2008). Além dessas pragas, nos últimos anos o algodoeiro vem sofrendo com ataque de outro importante lepidóptero, a *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae).

O tratamento de sementes no Manejo Integrado de Pragas do algodoeiro

No contexto do controle químico de pragas, uma das maneiras de utilização de produtos fitossanitários é por meio do tratamento de sementes (XAVIER, 2011), onde os compostos geralmente matam e defendem a planta contra ataque de pragas (CASTRO et al., 2008) que danificam sementes e plântulas (MARTINS et al., 2009). Amplamente empregado, o tratamento de semente mostrou-se ser efetivo na prevenção de danos causados pelo ataque de pragas, protegendo diversas culturas de importância econômica (NUYTTENS et al., 2013; STRAUSBAUGH et al., 2010). O tratamento de sementes é um processo em que a semente recebe um revestimento protetor de inseticida, criando assim uma película de ingrediente ativo que atua contra insetos no solo, e quando o tratamento é feito com inseticidas sistêmicos o produto passa a circular pelo interior da planta recém-emergida protegendo-a contra insetos pragas na fase inicial do seu desenvolvimento (NUYTTENS et al., 2013).

Cada ingrediente ativo tem suas características diferenciadas de modo de ação e eficácia no controle de grupos diferentes de pragas (XAVIER, 2011). Dentre os inseticidas sistêmicos amplamente empregados pode-se citar o tiametoxam que pertence ao grupo químico dos neonicotinoides. Este inseticida atua como agonista dos receptores nicotínicos da acetilcolina, causando a morte de artrópodes por hiperexcitação. Outro inseticida muito utilizado no tratamento de sementes é o clorantraniliprole, do grupo das diamidas, que são moduladores dos receptores de rianodina nas miofibrilas dos músculos dos insetos. Estas moléculas provocam a saída descontrolada de cálcio das células causando paralisia muscular e consequente morte do inseto (ALTMANN, 1990; HEATHERINGTON e BOLTON, 1992).

No tratamento de sementes utiliza-se quantidade muito menor de inseticida quando comparada à pulverização na parte aérea das plantas. Além de reduzir o impacto negativo ao ambiente, custo de produção e exposição do aplicador, este tipo de aplicação causa o mínimo de impacto sobre os insetos benéficos, uma vez que não há exposição direta desses organismos ao inseticida, como ocorre nos métodos convencionais de aplicação (HULL e BEERS, 1985; XAVIER, 2011). Em função dessas características, o tratamento de sementes torna-se compatível com programas de Manejo Integrado de Pragas (MIP), propiciando seletividade ecológica aos inimigos naturais (ALTMANN, 1990; DEGRANDE et al., 2002; HEATHERINGTON e BOLTON, 1992). Porém, poucos estudos têm sido realizados para avaliar o efeito do tratamento de sementes com neonicotinoides sobre os organismos não alvos via exposição indireta, haja visto que esses compostos translocam-se pela planta, contaminando os recursos vegetais que são utilizados como fontes de alimento por muitos desses organismos (SAEED, RAZAQ e HARDY, 2016; SIMON-DELSO et al., 2015).

Modo de ação do clorantraniliprole

Clorantraniliprole (CAP) é um inseticida da classe das diamidas antranílicas que está sendo utilizado em todo o mundo em várias culturas para controle de importantes pragas pertencentes à ordem Lepidoptera e alguns insetos das ordens Coleoptera, Diptera e Isoptera. As contrações dos músculos dos insetos dependem da liberação controlada de cálcio intracelular, pela ativação dos receptores de rianodina (RyR) (LAHM et al., 2007). As moléculas inseticidas das diamidas atuam na liberação irregular dos estoques de cálcio nos retículos sarcoplasmáticos, ocasionando uma contração irregular das células musculares dos insetos, levando posteriormente à cessação de alimentação, letargia, paralisia e por fim a morte (RIBEIRO, 2014).

O receptor de rianodina deriva seu nome do metabólito da planta sul-americana *Ryania speciosa* (Salicaceae), que contém naturalmente um composto inseticida conhecido por modificar os canais de cálcio (CORONADO et al., 1994). A atividade inseticida dos extratos de *Ryania* spp. foram descritos para uma série de lepidópteros e hemípteros-praga, sendo que a família dos ryanoides consiste em uma variedade diversificada de produtos naturais com a ryanodina como principal constituinte ativo (ROGERS et al., 1948).

A toxicidade de clorantraniliprole para mamíferos é relativamente baixa em relação aos insetos, apresentando DL50 > 5000 mg kg^{-1} em ratos (LAHM et al., 2007). A baixa toxicidade para mamíferos ocorre devido à sua especificidade para RyR nos insetos ser distinta sobre as formas de RyR encontrados em mamíferos (CORDOVA et al., 2006). Clorantraniliprole também apresenta segurança para insetos polinizadores, predadores, parasitoides e também para minhocas e microorganismos de solo. Esse produto demonstrou-se pouco tóxico para abelhas (DINTER et al., 2008). Sua baixa toxicidade é uma importante característica de diferenciação desta molécula em comparação com a maioria dos inseticidas sintéticos utilizados atualmente.

Clorantraniliprole apresenta mobilidade via xilema na planta, isto é, permite que o inseticida se mova de maneira ascendente, por esta razão, é utilizado também na forma de tratamento de sementes (BASSI et al., 2007). Desta maneira, esse é absorvido pelas raízes da planta e proporciona um controle efetivo de lepidópteros e outras pragas que danificam folhas e tecidos. Vale ressaltar que, pelo alto efeito residual destes inseticidas nos tecidos vegetais, a probabilidade de seleção de populações de insetos resistentes se torna alta, pois múltiplas gerações de pragas provavelmente estarão expostas a concentrações letais de uma única aplicação, aumentando assim a pressão de seleção (ADAMS et al., 2016).

Aspectos bioecológicos de *H. axyridis*

A joaninha *H. axyridis* pertence à família Coccinellidae, ordem Coleoptera e é geralmente predadora de outros artrópodes (LU e MONTGOMERY, 2001). As larvas e os adultos dessa joaninha apresentam grande atividade de busca pela presa e elevada voracidade, podendo ser encontrados em praticamente todos os hábitats explorados por suas presas (BOIÇA JUNIOR et al., 2004). Essa espécie é predadora de pulgões, ácaros fitófagos, cochonilhas, moscas-branca, psilídeos, ovos e larvas neonatas de Coleoptera e Lepidoptera (SARMENTO et al., 2007).

Entre os coccinelídeos predadores de pulgões na cultura do algodoeiro na Ásia, destaca-se *H. axyridis* (ALMEIDA e SILVA, 2002). Esse predador foi introduzido diversas vezes na América do Norte como agente de controle biológico clássico, obtendo sucesso no controle de pulgões nas culturas de alfafa, tabaco, noz-pecã, pimentão e em roseiras (FERRAN et al., 1996; GORDON, 1985; LAROCK et al., 2003; TEDDERS e SCHAEFER, 1994). Nos EUA, *H. axyridis* contribui para o controle de *Aphis glycines* Matsumura, na cultura da soja e em milho-doce reduz a população das pragas *Ostrinina nubilalis* (Hübner, 1976) (Lepidoptera: Pyralidae) e *Rhopalosiphum maidis* (Fitch, 1856) (Hemiptera: Aphididae) (KOCH, 2003).

O predador *H. axyridis* foi encontrado pela primeira vez no Brasil em 2002 em cultivos de pinus, sendo que posteriormente foi relatado predando afídeos em brassicásseas e em frutíferas (ALMEIDA e SILVA, 2002; MILLÉO et al., 2008; RESENDE et al., 2010; RESENDE et al., 2011). No Sul do país, este predador foi observado alimentando-se de cochonilhas, psilídeos e 15 espécies de pulgões associados a 19 espécies de plantas (MARTINS et al., 2006). Além de alimentar de outros insetos, *H. axyridis* alimenta-se também de tecidos e exsudatos de plantas (MOSER, HARWOOD e OBRYCKI, 2008), sendo este comportamento muito importante, pois aumenta significativamente a fecundidade, reduz seu tempo de desenvolvimento e o canibalismo entre os insetos (MOSER e OBRYCKI, 2009).

Harmonia axyridis é um inseto holometábolo, que passa pela fase de ovo, estágio larval com quatro instares, pupa e adulto (HODEK, 1973). A larva possui coloração negra e é do tipo campodeiforme; possui o corpo formado por protuberâncias providas de setas chamadas de lobos e cápsula cefálica mais larga. O adulto possui o corpo oval e convexo com cabeça de coloração amarela palha sem mancha. São insetos que caminham e voam bem, geralmente encontrados sobre plantas onde depositam seus ovos. O tamanho é variável, mas geralmente fêmeas são maiores que os machos. O comprimento pode variar de 4,7-6,6 mm por 3,7- 5,0 mm de largura, para as fêmeas, enquanto que os machos têm 4,2-5,2 mm de comprimento por 3,3-4,3 mm de largura (ARRUDA FILHO, 2005).

Esta espécie é altamente polimórfica, com élitros que variam desde coloração amarelo alaranjado até vermelho intenso, com até dezenove máculas, caráter morfológico relacionado à adaptação da espécie a diferentes condições climáticas (KOCH, 2003; KOCH et al., 2006; SOARES et al., 2003). Segundo Seo et al. (2008), o padrão de variação de cores de *H.*

axyridis se deve não apenas à temperatura, mas também devido a diferenças na constituição genética e nos ambientes em que as joaninhas são expostas. A variação no padrão de cores de *H. axyridis* é a maior entre os coccinelídeos. Essa característica utilizada pela espécie constitui-se em estratégia de sobrevivência, sinal de advertência aos seus predadores, que evitam ingeri-la ao associarem o padrão de coloração dos élitros à toxicidade e ao sabor desagradável da presa (DOLENSKÁ et al., 2009; MAJERUS, 2006).

Harmonia axyridis possui algumas características que podem explicar o sucesso do seu estabelecimento como espécie invasora. Uma das características é sua proteção química contra predação (SATO e DIXON, 2004), além de possuir alta taxa de fecundidade (IABLOKOFF-KHNZORIAN, 1982) e rápido desenvolvimento dos imaturos em relação às espécies nativas (LANZONI et al., 2004). Além disso, apresenta comportamento agressivo, o qual lhe dá vantagens sobre seus inimigos naturais (YASUDA e OHNUMA, 1999), e alta mobilidade que lhe permite refugiar-se em situações desfavoráveis e favorecer a sua procura por alimentos (OSAWA, 2000), além de apresentar baixa susceptibilidade a patógenos (HOOGENDOORN e HEIMPEL, 2002). Este predador possui alta capacidade de localizar populações de afídeos no espaço e tempo (OSAWA, 2000), sendo que um adulto é capaz de consumir de 90 a 270 afídeos por dia; enquanto que cada larva, de 600 a 1200 durante todo o seu desenvolvimento (OSAWA, 1993).

Aspectos bioecológicos de *C. externa*

Os crisopídeos são insetos que estão regularmente presentes em diversos agroecossistemas, sendo considerados importantes, pois suas larvas são capazes de predar diferentes espécies de pragas (CANARD, 2007). Dentro da família Chrysopidae, destacam-se os gêneros *Ceraeochrysa* e *Chrysoperla*, sendo esses os mais abundantes em espécies. As espécies do gênero *Chrysoperla* são as mais utilizadas em programas de controle biológico na região Paleártica (HENRY e WELLS, 2007) e Neotropical (FREITAS, 2001). A espécie *C. externa* é a que mais se destaca dentro do controle biológico, devido à sua ampla distribuição geográfica, alta capacidade predatória e tolerância a diversos produtos fitossanitários (ADAMS, 1983; ADAMS e PENNY, 1985; BATTEL, 2011; BRETTEL, 1982; FREITAS, 2002).

Os crisopídeos ocorrem em diferentes agroecossistemas (CARDOSO et al., 2003; COSTA, 2006; MONTES et al., 2007), demonstrando adaptação a diversos habitats, estando presentes em cultivos de frutíferas (MONTES et al., 2007), em hortaliças (RESENDE et al., 2007), áreas agropastoris e sistemas naturais (COSTA, 2006). São importantes predadores encontrados em muitas culturas de interesse econômico como algodoeiro, citros, milho, soja, alfafa, fumo, videira, macieira, seringueira e outras. Podem alimentar-se de ovos, lagartas neonatas, pulgões, cochonilhas, ácaros e vários outros artrópodes de pequeno tamanho e de tegumento facilmente perfurável (CARVALHO e SOUZA, 2000). Assim, são predadores que devem ser conservados no ambiente por possuírem importante papel no controle biológico natural de artrópodes pragas.

Os ovos de *C. externa* possuem pedicelos, com comprimento variando de 4 a 8 mm e composto de uma substância gelatinosa exsudada na ocasião da postura e que endurece em contato com o ar; possuem forma elipsoidal, com a cor variando de verde-clara até amarelo-esverdeada, tornando-se mais escuros, próximos à eclosão das larvas (LIRA et al., 2003). A postura pode ser realizada de maneira isolada ou em grupo. Em geral, as fêmeas ovipositam nas plantas infestadas com presas, embora alguns ovos sejam encontrados em locais sem sua presença (GEPP, 1984). Segundo Ribeiro (1991), os ovos de *C. externa* mantidos em condições de 25 ± 2ºC, com apresentam período embrionário médio de 4 a 5 dias e viabilidade média de 87,6%.

As larvas dos crisopídeos são do tipo campodeiforme, com cabeça triangular, prognata, aparelho bucal sugador mandibular, pernas ambulatórias e corpo com várias cerdas (SHELDON e MACLEOD, 1974). O primeiro instar dura de 3 a 6 dias ou até 11 dias em temperaturas mais baixas; o segundo pode durar de 2 a 7 dias, sendo que na maioria dura de 3 a 4 dias. A duração do terceiro instar pode variar muito, especialmente se o alimento for escasso, evidenciando que a qualidade do alimento e a temperatura são fatores determinantes para o sucesso ou fracasso do desenvolvimento deste predador (RIBEIRO, 1991).

A fase de pré-pupa ocorre após o completo desenvolvimento larval, quando a larva pára de se alimentar e procura abrigo para desenvolver seu casulo de forma esférica, constituído de fios de seda branca, onde permanece até a pupa (RIBEIRO, 1991). A fase de pupa caracteriza-se pela presença de um disco preto na extremidade do casulo, o qual indica a liberação do mecônio, correspondente à última ecdise larval. Adultos de crisopídeos normalmente emergem 12-20 dias após a fase de pré-pupa (RIBEIRO, 1991).

Após todo o desenvolvimento, as pupas se libertam dos casulos por uma abertura circular feita com as mandíbulas (ADAMS e PENNY, 1985). Fora do casulo, a pupa inicia a fase farata, equivalente à pupa móvel que, após se fixar a um substrato, realiza a última ecdise com a consequente emergência do crisopídeo adulto. Os insetos adultos são de coloração esverdeada, com asas membranosas reticuladas e asas anteriores com nervuras transversais costais simples (BORROR e DELONG, 1988). Os adultos de *C. externa* não são predadores e utilizam recursos vegetais como pólen, néctar e néctar extrafloral como fontes de alimento (SHELDON e MCLEOD, 1971); apresentam hábitos matutino e noturno e, durante o dia, são encontrados em repouso, sob as folhas de árvores e arbustos. Porém, segundo Philippe (1971) a oviposição se inicia antes do crepúsculo e coincide com sua hora de vôo.

Nesse contexto, a espécie Neotropical *C. externa* destaca-se como um excelente candidato para a utilização em programas de controle biológico de pragas na cultura algodoeira, e estudos acerca da exposição desses organismos a produtos químicos utilizados nesta cultura são de suma importância para um bom funcionamento destas táticas de controle dentro do manejo integrado de pragas.

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SEGUNDA PARTE – ARTIGOS

ARTIGO 1

LONG-TERM EFFECTS OF CHLORANTRANILIPROLE REDUCED RISK INSECTICIDE APPLIED AS SEED TREATMENT ON LADY BEETLE *Harmonia axyridis*

Este artigo foi escrito de acordo com os padrões da revista Journal of Pest Science.

Long-term effects of chlorantraniliprole reduced risk insecticide applied as seed treatment on lady beetle *Harmonia axyridis*

Abstract

Chlorantraniliprole (CAP) is a reduced risk insecticide commonly used as seed treatment in many crops. However, CAP residues can contaminate pollen, floral and extrafloral nectar, becoming a potential risk to beneficial arthropods. The aims of this study were to (1) determine the non-target effects of CAP seed treatment of cotton on *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) over two generations, and (2) assess the ability of the lady beetles to recognize plants grown from treated seeds. The exposure of *H. axyridis* larvae and adults to cotton seedlings grown from CAP treated seeds did not significantly affect any life history parameters of the lady beetles directly exposed (F0). However, CAP caused significant transgenerational effects in both the larval and adult stages of *H. axyridis*. The larvae exposure to CAP treatment reduced the larval and pupal development time and the male body weight of F1 generation, as well as the survival of the lady beetles over the developmental stages. In the adult bioassay, CAP seed treatment reduced both pupal development time and egg viability of F1 generation and decrease the *H. axyridis* survival over the F1 generation. In olfactometer test, only the *H. axyridis* larvae were able to recognize cotton seedlings grown from CAP treated seeds. The results of this study confirm the hypothesis that systemic insecticides, when applied as seed treatments, can cause negative effects on non-target organisms. In addition, the study emphasizes the importance of long-term assessments of the pesticides side-effects on beneficial organisms.

Keywords: Cotton, systemic insecticides, extrafloral nectar, diamide, side-effects, transgenerational effects

1. Introduction

The cotton agroecosystem has a high diversity of arthropod natural enemies, such as lady beetles, green lacewings, spiders and aphid parasitoids (Ali et al., 2016; Assimwe et al., 2016). Natural enemies are important biological control agents that contribute to regulating pest populations at low economic threshold levels (Symondson et al., 2002). Among the natural enemies of cotton pests, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) stands out as being a polyphagous predators native to central and eastern Asia, where it is considered the main predator of aphids in cotton fields (Kuznetsov, 1997). The lady beetle *H. axyridis* has been used as a biological control agent around the world. Since 1982, *H. axyridis* has been commercially available in Europe for both classical and inundative biological control strategies and has had a much longer history in North America (Roy et al., 2006). In Brazil, the first record of *H. axyridis* was in 2002, feeding on *Tinocallis kahawalnokani* (Kirkaldy) (Hemiptera, Aphididae) and on *Lagerstroemia indica* (Lythraceae) (Almeida and Silva, 2002). Both larvae and adults of *H. axyridis* have high search capacities for prey and contribute to the biological control of many agriculture pests, e.g. mite, whitefly, aphids, thrips and neonate larvae of lepidopterans (Hodek, 1996; Koch, 2003; Landis et al., 2004).

Although larvae and adults of lady beetles are predators, both stages consume pollen and floral and extrafloral nectar (EFN) as food resources to complement diet, especially during periods of shortage or absence of preys (Pemberton and Vandenberg, 1993; Coll and Guershon, 2002; Choate and Lundgren, 2013). Lundgren and Seagraves (2011) found that *Coleomegilla maculata* (DeGeer) that fed on EFN of *Vicia faba* (Fabaceae) had physiological benefits and increased survival and fecundity rates. In cotton plants (*Gossypium hirsutum* L., Malvaceae), EFNs are present since the early development stages (Wackers et al., 2001), being located in largest midribs under the leaves, on the squares between bracts, and at the bases of bracts (Eleftheriou and Hall, 1983; Hagen, 1986). Natural enemies have easy access to the cotton EFN (Stapel et al., 1997), which contributes to plant defense against arthropods pests by increasing the attractiveness of natural enemies (Marazzi et al., 2013).

Despite the EFN having an important ecological role as the mediator of tritrophic interactions, recent studies have shown that EFN is a potential route of natural enemies' exposure to systemic insecticides applied as seed treatment (Gontijo et al., 2015; 2018; Moscardini et al., 2014; 2015), since systemic insecticides can contaminate the EFN as they are translocated to all plants tissues (Cloyd and Bethke, 2011). The exposure of non-target organisms to insecticides can cause lethal and sublethal effects that compromise life history and behavioral parameters (Desneux et al., 2007), resulting in stress to communities and a potential risk to natural enemies' ecological services (Guedes et al., 2016; 2017).

In cotton fields, the main groups of insecticides used in seed treatment are the neonicotinoids to control early-season sucking insect pests, such as thrips, whitefly, aphids and leafhoppers (Lentz and Austin, 1994; Zhang et al., 2011; 2016) and the anthranilic diamide chlorantraniliprole (CAP) to control leaf-feeding *insects* such as cotton bollworms (Lepidoptera: Noctuidae) (Gordy et al., 2015). CAP acts as a ryanodine receptor modulator to block insect muscle contraction (Lahm et al., 2007; Isaacs et al., 2012) and presents high insecticidal efficiency and mammalian safety, therefore being widely used for pest control in agriculture and considered compatible with integrated pest management programs (Lahm et al., 2009).

Although negative effects of CAP seed treatment have already been reported for some natural enemies, these studies usually focus on short-term assessments, disregarding possible transgenerational effects and, underestimating the real impacts of the insecticides on non-target organisms (Guedes et al., 2016). Given the potential risk of CAP seed treatment to natural enemies and the lack of information about the impacts of long-term through EFN, the aims of this study were to (1) determine the non-target effects of cotton seed-applied CAP on life history parameters of *H. axyridis* over two generations and (2) assess the ability of the lady beetles to recognize cotton seedlings grown from treated seeds.

2. Materials and methods

2.1. Insects and CAP seed treatment

The insects used in all the bioassays were obtained from laboratory mass-rearing at the Department of Entomology, Federal University of Lavras (Lavras, MG, Brazil), without a history of pesticide use. The lady beetles were maintained in plastic containers (15 cm diam x 20 cm ht) covered with a film plastic. The inner walls of the containers were coated with filter paper to serve as a substrate for oviposition. Both larvae and adults of lady beetles were fed with frozen eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) ad libitum and water was provided on a sponge piece, both refreshed every 48 h. The *H. axyridis* colony as well as the bioassays were performed in a climate-controlled room held at 25 ± 2ºC, 70 ± 10% RH and a 12:12 (L:D) photoperiod.

The bioassays prepared with cotton seedlings var. Bollgard® (Monsanto, São Paulo, SP, Brazil) grown from seeds treated industrially with CAP at rate 60 mL 100 kg^{-1} seeds (Dermacor®, suspension concentrate, DuPont do Brasil, Barueri, SP, Brazil). Similar plants grown from untreated seeds were used as controls. Both treated and untreated seeds were planted in plastic pots (200 mL) filled with a mixture of soil and commercial substrate

(Plantmax®, 1:1) and germinated in a greenhouse at 25 ± 3ºC, 70 ± 10% RH under natural light.

2.2. Non-target effects bioassays

Both larvae and adults of *H. axyridis* were exposed to cotton seedlings grown from CAP treated seeds at 15 days post-emergence, sufficient time for the plant to present the first extrafloral nectars. To assess the non-target impact of CAP seed treatment on *H. axyridis* larvae, 50 neonate larvae (2 h old) were caged, per treatment, on cotton seedlings treated or untreated seeds until they became a pupa. The lady beetles larvae were fed with frozen eggs of *E. kuehniella* ad libitum refreshed every 48 h. However, during the exposure period to cotton seedlings, no water source was provided to the insects to induce the use of EFN as source of hydration. After adult emergence, the males and females were kept isolated for sexual maturation (8 days). Posteriorly, males and females were paired in Petri dishes (10 cm diam) for five days to mate and then separated in new Petri dishes (5.5 cm diam) to assess survival and reproduction. Male were separated to avoid egg cannibalism.

Lady beetle adults were fed with frozen eggs of *E. kuehniella* ad libitum and water was provided on a sponge piece, both refreshed every 48 h. The insects were monitored daily and the biological parameters recorded according to Gontijo et al. (in press) and Moscardini et al. (2015). Survival of pairs was monitored until the 13th day of oviposition. Eggs of the fifth clutch were used to assess the egg viability and the transgenerational effects on the F1 generation. For this, 60 neonate larvae, per treatment, were individualized in Petri dishes (5.5 cm diam) and reared (as above) until adult stage, when new pairs were established to evaluate reproduction.

The exposure of *H. axyridis* adults was performed similarly to larvae exposure. However, the lady beetle pairs (n = 23 per treatment) were exposed to cotton seedlings grown from CAP treated seeds for only eight days. After the exposure period, the lady beetle pairs were held in a Petri dish (10 cm diam), and reproductive and developmental parameters of F0 and F1 generations were evaluated as described above.

2.3. Olfactometer bioassay

A glass Y-tube olfactometer (main arm: 9 cm long; side arms: 9 cm long; 1 cm internal diameter; arms angle 90º) was used to investigate the choice of both third instar larvae and adults (72 h old , ♂ and ♀) of *H. axyridis* toward cotton seedling volatiles grown from CAP treated seed. Cotton plants grown from CAP treated or untreated seed (as above) were individualized in two glass chambers (12 cm diam x 23 cm ht) connected to the arms of the Y-tube from the top of the chambers. The clear air was pushed inside the glass chambers and the olfactometer arms by a pump system (Millipore® 60Hz) with a charcoal filter. Airflow was adjusted to 0.45 L/min/arm for both larvae and adults test.

The experiments were conducted in the laboratory under controlled conditions at 25 ± 2ºC, 70 ± 10% RH and during the day. For both lady beetle larvae and adults were tested the odors combinations: (1) clear air *vs.* cotton plant grown from untreated seed and (2) cotton plant grown from untreated seed *vs.* cotton plant grown from CAP treated seed. A single insect (n = 40 per experiment) was introduced into the olfactometer basis and the choice was reported when the insect reached at least 4.5 cm along the arm, remaining for at least 30 s. In the adults test, the Y-tube olfactometer was placed at 45º because of the positive phototropism of the insects, while in the larvae test it was kept horizontally. Lady beetles (larvae and adults) that did not make a choice within 5 min were excluded from analysis. Insects were used only once and, after each trial, the olfactometer was inverted to prevent directional bias. Before experiments, the insects were starved for 24 hours. Every five insects tested, a new pair of cotton plants was used, and the Y-tube olfactometer and glass chambers were washed with water, soap and ethanol (70%) and dried in an oven at 100ºC.

2.4. Statistical analyses

Developmental and reproductive data from larval and adult bioassays were subjected to independent Student's t-test or Mann-Whitney test, when the data violated the assumptions of normality and homoscedasticity confirmed by Shapiro-Wilk and Bartlett tests, respectively. Sex ratio (∑♀/∑ (♀ + ♂)) and choice data in the olfactometer test were analyzed using the Chi-square goodness-of-fit test. Lady beetles survival data were submitted to survival analysis using log-rank test, and survival curves were estimated using the Kaplan-Meier estimator and compared by Bonferroni test. Data from lady beetles survivorship on the final day of observation were censored. All analyses were performed using SigmaPlot 12.5 software (α = 0.05) (Systat, 2013).

3. Results

3.1. Non-target effects

The exposure of larvae and adults of *H. axyridis* to cotton seedlings grown from CAP treated seeds did not significantly affect any life history parameters of lady beetles directly exposed (F0). However, CAP seed treatment caused significant transgenerational effects in both larval and adult stages of *H. axyridis*. The larvae exposure to CAP seed treatment reduced the larval and pupal development time and the body weight of F1 generation males (Table 1). The lady beetle survival over the developmental stages of F1 generation was also reduced by exposure of *H. axyridis* larvae to CAP seed treatment (F0 generation: log-rank test, χ^2 = 2.4, df = 1, P = 0.120; F1 generation: log-rank test, χ^2 = 7.5, df = 1, P = 0.006) (Figure 1A). In the adult bioassay, CAP seed treatment reduced both pupal development time and egg viability of F1 generation (Table 2) as well as the lady beetle survival over the F1 generation development (log-rank test, χ^2 = 6.9, df = 1, P = 0.009) (Figure 1B).

3.2. Olfactometry

The olfactometry results showed that only the *H. axyridis* larvae (L3) present a significant choice to cotton plants (larvae: χ^2 = 6.1, df = 1, P = 0.014; ♂: χ^2 = 3.3, df = 1, P = 0.070; mated ♀: χ^2 = 2.7, df = 1, P = 0.099; unmated ♀: χ^2 = 2.2, df = 1, P = 0.140) and were able to recognize plants grown from CAP treated seeds (larvae: χ^2 = 9.0, df = 1, P = 0.003; ♂: χ^2 = 0.1, df = 1, P = 0.814; mated ♀: χ^2 = 1.4, df = 1, P = 0.239; unmated ♀: χ^2 = 0.0, df = 1, P = 1.000) (Figure 2). However, *H. axyridis* adults were more responsive to the odors tested than larvae (ca. 50% did not respond).

4. Discussion

Although CAP is considered a reduced risk insecticide, compatible with integrated pest management programs (Lahm et al., 2009), the results of this study showed significant negative effects of CAP seed treatment on *H. axyridis* exposed to cotton seedlings grown from treated seeds. Curiously, CAP caused only transgenerational effects in both larvae and adults of *H. axyridis*. In the F1 generations, the survival and developmental time of immature stages were apparently the parameters most affected by CAP treatment. However, CAP also reduced the males' fresh body weight and the egg viability of F1 generations, when *H. axyridis* were exposure as larvae and adults, respectively.

The negative effects observed on *H. axyridis* confirm that CAP applied as seed treatment can contaminate the cotton EFN, poisoning *H. axyridis* with the CAP sublethal concentrations. Since EFN was the only possible exposure route of lady beetles to CAP and although not quantified in this study, CAP residues can be found in various plant tissues (Singh et al., 2012; Zhang et al., 2012; Teló et al., 2015). Moreover, the bioassays were prepared with cotton seedlings at 15 days post-emergence when EFN is present, and systemic insecticides applied in the seeds are in high concentrations in the plant tissues. Although EFN was available to both larvae and adults of beetles, *H. axyridis* adults directly exposed to plants did not obtain sufficient hydration from the cotton EFN, compromising the survival over the F1 generation in comparison with the larvae exposure where adults received supplemental water (controls: Figure 1A *vs.* 1B). Michaud and Grant (2005) reported that larvae and adults of lady beetles have high water demand, and limited access to water can compromise their life history parameters (e.g. survival and reproduction).

Negative effects of CAP seed treatment on lady beetles were also reported by Moscardini et al. (2015), who found that CAP seed treatment delayed adults emergence and reduced the clutches number of *C. maculata* and *Hippodamia convergens* Guérin-Méneville, when larvae and adults were directly exposed to sunflower plants grown from treated seeds, respectively. However, unlike our study, the authors did not evaluate the development and reproduction of F1 generation, neglecting possible transgenerational effects, which may have underestimated the CAP non-target effects on *H. axyridis*. In a similar study to ours, Gontijo et al. (in press) also observed significant transgenerational effects of neonicotinoid thiamethoxam seed treatment on larvae and adults of *H. axyridis* fed on cotton EFN. However, these authors concluded that *H. axyridis* is apparently less sensitive to cotton seed-applied thiamethoxam than green lacewings *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae), although thiamethoxam was harmful for both predators. Thiamethoxam and clothianidin also caused lethal and sublethal effects with neurotoxic symptoms (e.g. trembling, paralysis, and loss of coordination) on *H. axyridis* larvae exposed to corn plants grown from treated seeds (Moser and Obrycki, 2009).

Besides lady beetles, other natural enemy groups may also suffer non-target effects of CAP seed treatment. Gontijo et al. (2014) found significant decrease in the survival and fecundity of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) adults fed on sunflower EFN of plants grown from CAP treated seeds. Similarly, the consumption of CAP-contaminated sunflower EFN caused no lethal effect, but reduced the number of aphids attacked and parasitized by *Lysiphlebus testaceipes* Cresson (Hymenoptera: Braconidae) (Moscardini et al., 2014). In contrast, CAP caused hormesis effect (positive stimulus) on *Podisus nigrispinus* Dallas (Hemiptera: Pentatomidae) life table parameters when adult stink bugs fed on soybean plants grown from treated seeds (Gontijo et al., 2018).

In contrast to this study, the topical application of CAP sublethal concentrations in second instar *H. axyridis* larvae prolonged significantly the larval and pupal development time and preoviposition period, while adult longevity and fecundity were both significantly reduced (Nawaz et al., 2017). Barbosa et al. (2017) also found that the *H. convergens* adults exposed to CAP freshly dried residues and ingestion of treated prey resulted in similar toxicity to lady beetle, whereas topical application was a less toxic route of exposure. These authors also reported that CAP recommended field concentration (200 mg a.i. L^{-1}) for the control of tobacco budworm (Lepidoptera: Noctuidae) is not safe for *H. convergens* populations. Opposite results were found by Cabrera et al. (2014), who considered CAP tested at rate 50.75 g a.i. ha^{-1} harmless to larvae of *H. axyridis* and *C. maculata* via ingestion to treated aphids, but was toxic to both lady beetle species when in contact with freshly dried residues. These results indicate the CAP toxicity can be influenced by multiple factors such as the species and developmental stage of natural enemy and the exposure route. The developmental stage of plants can also influence the toxicity of insecticidal seed treatments to natural enemies. Gontijo et al. (2015) concluded that the nymphal stage of pirate bug *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) was the most susceptible to CAP applied as sunflower seed treatment and the toxicity of CAP decreased with plant development.

The olfactometer trials indicate that, unlike larvae, *H. axyridis* adults (mated or unmated) are not able to recognize cotton seedling volatiles as well as do not differentiate plants grown from CAP treated or untreated seeds. Thus, increasing the risk of exposure of lady beetles to EFN contaminated, leading to adverse effects as previously reported. Based on the preference of *H. axyridis* larvae for cotton plants without CAP seed treatment, we hypothesized that besides direct effects, CAP can have caused indirect effects on the behavior of larvae, which likely reduced EFN consumption by larvae from the CAP treatment. Therefore, the negative effects observed in F1 generation of CAP treatment may also be related to food deficit of maternal generation (Table 1), since EFN is a rich source of sugars essential to complement the diet of coccinellids (Lundgren, 2009; Hodek and Evans, 2012).

Similar to this study, Vargas et al. (2014) found that maternal effects can modify progeny phenotypes and the restricted diet for larvae reduced the developmental time and adult body size of *C. maculata*. Vargas et al. (2013) also reported that the resources acquired during larval feeding of *H. convergens* can influence life history parameters of lady beetles via effects on adult body size and other traits. During the bioassays, we performed visual evaluations that confirmed that both larvae and adults of *H. axyridis* visited and fed on cotton extrafloral nectaries. However, the visitation time and frequency were not evaluated, which could help to clarify the occurrence of CAP indirect effects on larval behavior.

In summary, the results of our study confirm that CAP seed treatment can cause negative impacts, especially transgenerational effects, on beneficial insects such as *H. axyridis,* through the contamination of EFN. Furthermore, we observed that unlike larvae, the lady beetle adults are not able to recognize cotton seedlings grown from treated seeds, which may contribute to the greater exposure of these insects to EFN contaminated. Based on the results of this study and previous research (as cited above), we conclude that further long-term studies are needed to estimate real impacts of insecticidal seed treatments on non-target organisms. Further studies should also incorporate the assess of the community of natural enemies in the field crops with continuous use of systemic insecticides applied as seed or soil treatments, since these insecticides can be persistent in the soil (Jones et al., 2014; Sharma et al., 2014) and can contaminate the non-target plants in the margins of fields of seeds treated with insecticides residue, sometimes in lethal concentrations for beneficial arthropods (Botías et al., 2016).

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Table 1. Developmental and reproductive parameters of F0 and F1 generations of *Harmonia axyridis* larvae exposed to cotton plants grown from chlorantraniliprole (CAP) treated seeds.

Parameter	F0 generation					F1 generation				
	Control	CAP	Stats	df	*P*	Control	CAP	Stats	df	*P*
Developmental time (days)	*n* = 47	*n* = 42				*n* = 56	*n* = 43			
Larval	12.1 ± 0.2	11.6 ± 0.2	1.8[a]	87	0.08	11.7 ± 0.2 a	11.0 ± 0.2 b	836.5[b]	43,56	0.01
Pupal	4.7 ± 0.1	4.9 ± 0.1	-0.9[a]	87	0.35	5.4 ± 0.1 a	4.9 ± 0.1 b	2.9[a]	97	0.00
No. adults emerged	45	40				53	42			
Sex ratio[1]	0.56 ± 0.1	0.58 ± 0.1	0.0[c]	1	0.96	0.42 ± 0.1	0.57 ± 0.1	1.7[c]	1	0.19
Fresh body weight (mg)										
Male	37.4 ± 0.8	38.1 ± 0.7	-0.6[a]	30	0.55	35.2 ± 0.8 a	31.8 ± 1.5 b	2.2[a]	47	0.03
Female	42.1 ± 1.3	44.5 ± 0.7	170.0[b]	20,22	0.21	45.1 ± 0.8	42.5 ± 1.7	214.0[b]	21,24	0.39
Reproductive parameters										
No. pairs	19	14				21	18			
Preoviposition period (days)	7.1 ± 0.5	6.0 ± 0.7	1.4[a]	23	0.18	4.8 ± 0.4	5.4 ± 0.4	-0.9[a]	21	0.37
Fecundity[2] (eggs female^{-1})	68.9 ± 21.5	104.3 ± 38.9	61.5[b]	10,15	0.47	63.1 ± 6.3	54.8 ± 7.0	53.5[b]	8,15	0.69
Egg viability (% eclosing)	91.0 ± 5.4	88.7 ± 6.1	0.3[a]	8	0.78	73.2 ± 3.8	70.7 ± 5.1	0.39[a]	19	0.70

[1]Female proportion = [∑♀/∑ (♀ + ♂)].
[2]Fecundity = F0: 13-days and F1: 10-days.
Means (± SE) followed by different letters were significantly different within rows by (a) *t*-test, (b) Mann-Whitney or (c) Chi-square test (α =the 0.05).

Table 2. Reproductive and developmental parameters of F0 and F1 generations of *Harmonia axyridis* exposed as adults to cotton plants grown from chlorantraniliprole (CAP) treated seeds.

Parameter	Control	CAP	Stats	df	*P*
F0 generation					
No. pairs	23	23			
Preoviposition period (days)	11.4 ± 0.6	10.5 ± 0.2	234.5[b]	23,23	0.50
13-days fecundity (eggs female^{-1})	152.3 ± 21.9	179.3 ± 20.4	-0.9[a]	44	0.37
Egg viability (% eclosing)	78.2 ± 6.5	62.9 ± 7.0	1.5	25	0.14
Fresh body weight (mg)					
Male	37.0 ± 0.8	35.6 ± 0.8	183.0[b]	22,23	0.11
Female	48.3 ± 0.9	45.8 ± 1.2	1.6[a]	42	0.10
F1 generation					
Developmental time (days)	*n* = 37	*n* = 20			
Larval	13.4 ± 0.3	12.8 ± 0.3	1.3[a]	55	0.19
Pupal	4.7 ± 0.1 a	4.3 ± 0.1 b	243.5[b]	20,37	0.02
No. adults emerged	34	20			
Sex ratio[1]	0.47 ± 0.08	0.52 ± 0.11	0.0[c]	1	0.92
Fresh body weight (mg)					
Male	29.0 ± 0.7	31.0 ± 1.5	-1.4[a]	24	0.17
Female	36.9 ± 1.4	38.3 ± 1.9	70.5[b]	10,16	0.63
Reproductive parameters					
No. pairs	15	10			
Preoviposition period (days)	2.9 ± 0.4	2.8 ± 0.4	24[b]	6,8	1.0
10-days fecundity (eggs female^{-1})	61.0 ± 4.8	64.0 ± 10.1	-0.3[a]	12	0.78
Egg viability (% eclosing)	95.3 ± 3.4 a	61.6 ± 15.6 b	2.6[a]	11	0.02

[1]Female proportion = [∑♀/∑ (♀ + ♂)].

Means (± SE) followed by different letters were significantly different within rows by (a) *t*-test, (b) Mann-Whitney or (c) Chi-square test (α = 0.05).

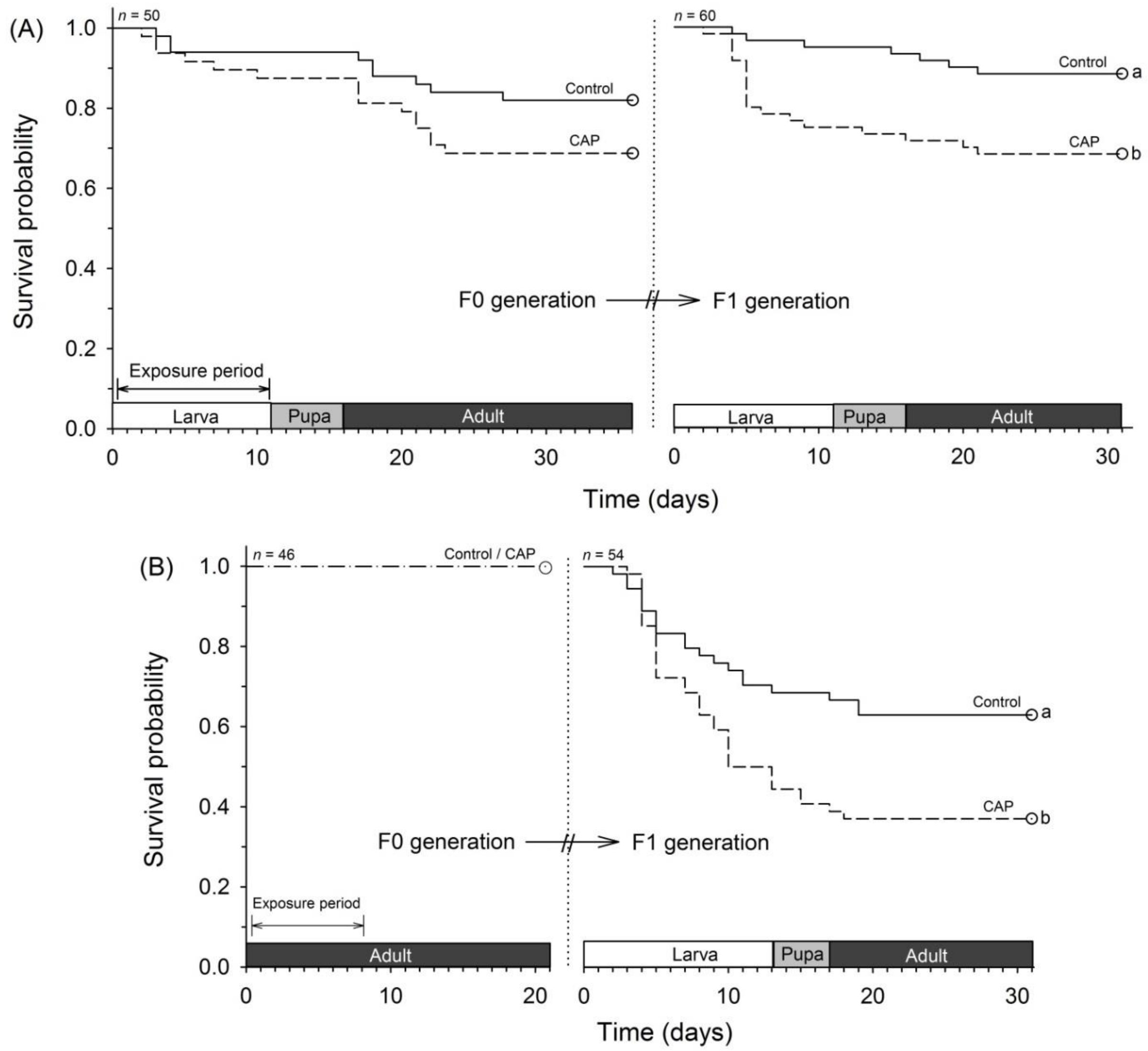

Figure 1. Survival curves for F0 and F1 generations of *Harmonia axyridis* exposed as larvae (A) and adults (B) to cotton plants grown from chlorantraniliprole (CAP) treated seeds. Curves bearing different letters were significantly different (log-rank test, α = 0.05). "o" censored data on the final day of observation.

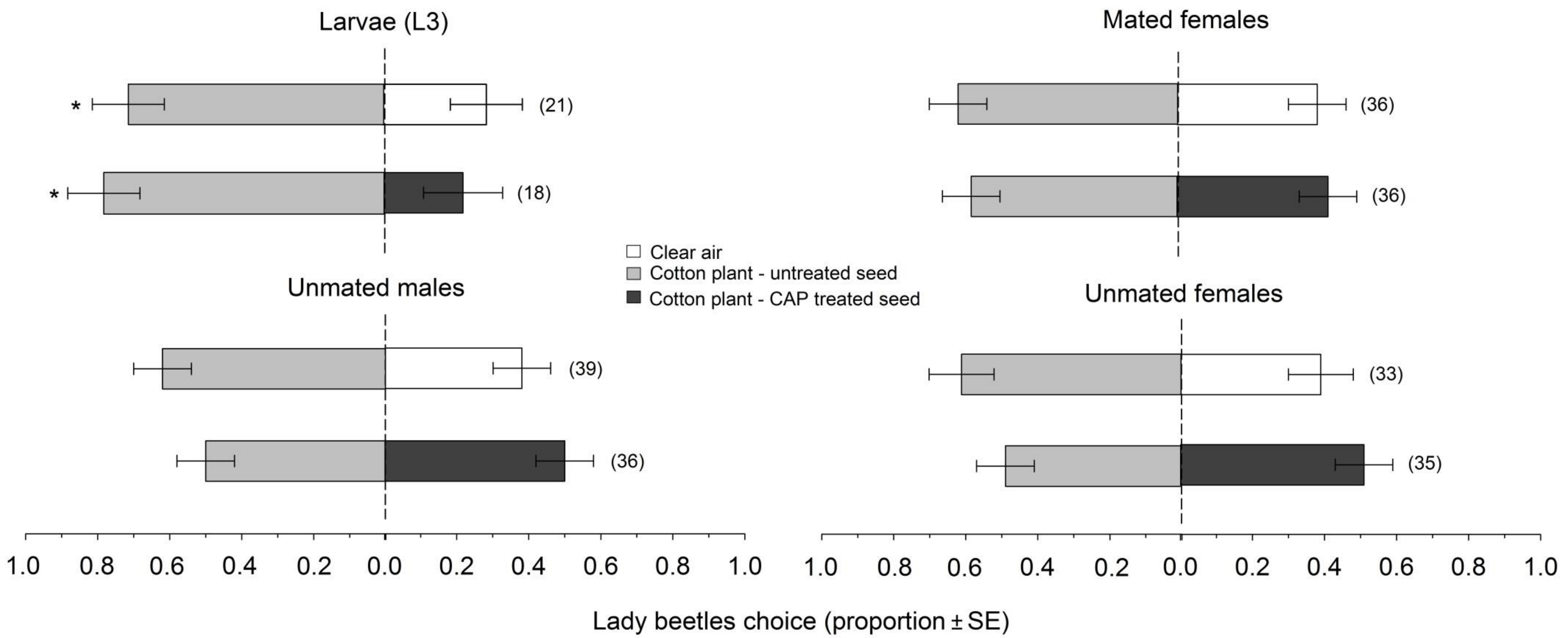

Figure 2. Olfactory response of *Harmonia axyridis* to cotton plant volatiles grown from chlorantraniliprole (CAP) treated seeds. The combinations of odors tested were: (1) clear air *vs.* cotton plant grown from untreated seed and (2) cotton plant grown from untreated seed *vs.* cotton plant grown from CAP treated seed. Forty lady beetles were used in each test and the numbers of insects that responded are indicated within parentheses. Insects that did not make a choice within 10 min were excluded from analysis. Asterisks indicate significant difference between responses within a trial (Chi-square test, $\alpha = 0.05$).

ARTIGO 2

TOXICITY OF CHLORANTRANILIPROLE SEED TREATMENT TO *Chrysoperla externa* AND SYNTHESIS OF LABORATORY STUDIES FOR RISK ASSESSMENT TO EIGHT NATURAL ENEMIES

Este artigo foi escrito de acordo com os padrões da revista Biological Control.

Toxicity of chlorantraniliprole seed treatment to *Chrysoperla externa* and synthesis of laboratory studies for risk assessment to eight natural enemies

Abstract

Chlorantraniliprole is an insecticide belonging to the chemical group of diamidas and has been used in seed treatment of several crops to control soil and shoot pests in their early stages of development. This compound presents high systemicity, being able to translocate throughout the plant and contaminate the pollen, nectar floral and extrafloral, and cause damage to non-target organisms that feed on these resources. The objectives of this work were (1) to evaluate the effects of the seed treatment of cotton (*Gossypium hirsutum*) with chlorantraniliprole on biological and behavior characteristics of the predator *Chrysoperla externa* (Hagen, 1861) (Neuroptera, Chrysopidae) and (2) synthesize the main studies performed with chlorantraniliprole seed treatment on eight natural enemies. The insecticide did not cause transgenerational effect in the parameters of the life table of *C. externa* larvae or adults. Only the duration of the pupal period was reduced when larvae were exposed to contaminated extrafloral nectar; entertaining when adults were exposed had their eggs viability reduced. Larvae of *C. externa* are attracted to cotton plants without treatment similar to cotton plants treated with chlorantraniliprole; however adults of *C. externa* are attracted to cotton plants, without, however, identifying whether the plant originated from treated or untreated seeds. For the *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae), and *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinelidae), *C. externa* and *Orius insidiosus* (Say, 1832) (Hemiptera: Anthocoridae), the insecticide was classified as slightly harmful, and for *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), *Hippodamia convergens* (Guerin-Meneville) (Coleoptera: Coccinellidae), and *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) chlorantraniliprole was harmless, according to IOBC toxicity classification.

Keywords: Diamide, non-target organisms, IOBC, sublethal effects, biological control, IPM

1. Introduction

Generalist predators are known worldwide as regulators of phytophagous arthropod populations (Symondson et al., 2002). Among them, *Crysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae), a polyphagous insect, is considered a potential biological control agent of different agricultural pests such as mites, whiteflies, aphids and thrips (Rimoldi, 2009; Rodrigues Barbosa et al., 2008). The larvae of *C. externa* are very voracious and preferentially feed on soft body insects and are commonly found in several crops such as cotton, citrus, corn, soybeans, among others (Carvalho and Souza, 2000; Soto and Iannacone, 2008).

In the absence of prey, *C. externa* uses pollen and plant nectar as an alternative source of food. Extrafloral nectar is an important resource for these predators, being consumed to supplement the diet, or even as the only source of food in the absence of its prey in the field (Limburg, Rosenheim 2001, Moser and Obrycki, 2009). The cotton tree presents the first extrafloral nectaries at the beginning of its development, on the abaxial face of the first pair of true leaves, which makes it a great attraction for this predator (Stewart et al., 2010).

For the control of soil pest insects and suckers in the initial phase of the cotton crop, seed treatment with neonicotinoids has been the most used (Gordy et al., 2015). A widely used insecticide is chlorantraniliprole (CAP) from the chemical group of diamides that are modulators of the rianodine receptors on the myofibrils of insect muscles (Lahm et al., 2007; Isaacs et al., 2012). These products protect the seeds and roots against soil insects and because of their systematicity are also efficient against sucking insects. In addition, when the seed treatment is carried out, the amount of product used is lower when compared to the spray in the aerial part of the plant, and thus reduces the impact to the environment and decreases the cost of production (Altmann, 1990; Heatherington and Bolton, 1992).

However, with the use of the treatment of cotton seeds with insecticides of high systematicity, the products can be translocated to the nectar, being able to contaminate the natural enemies that feed there, contradicting reports about the benefit of the ecological selectivity that the seed treatment can provide beneficial organisms (Jeschke et al., 2011; Monneart et al., 2000; Laurent and Rathahao, 2003; Stoner and Eitzer, 2012). Thus, many predators and parasitoids are exposed to insecticides even indirectly through the feeding of contaminated exudates, and may be negatively affected by the action of these compounds (Gontijo et al., 2014; 2015; Moscardini et al., 2014; 2015).

Knowledge about the impact of insecticides on natural enemies is important for the integration of biological and chemical methods, and it is necessary to evaluate the lethal and sublethal effects of insecticides on natural enemies to know the total effect of insecticide application (Desneux et al., 2007). Sublethal effects are defined as those occurring with specimens that survive insecticide exposure. These effects can manifest themselves in the biology, reproduction and even in the behavior of these insects, which are often not evaluated (Bayram et al., 2010; Desneux et al., 2004; Stark and Banks, 2003). Therefore, the objective of this work was to evaluate the effects of the cotton seed treatment with CAP on biological characteristics of *C. externa* in different generations and its influence on the recognition of the predator by plants contaminated with this compound, as well as to synthesize the main studies carried out with treatment of seeds with chlorantraniliprole on eight natural enemies in three agricultural crops.

2. Materials and methods

2.1. Toxicity of CAP seed treatment to C. externa

A series of bioassays were conducted according to Gontijo et al. (2014) to evaluate the toxicity of CAP seed treatment to larvae and adults of *C. externa*. The bioassays were performed in a climate-controlled room held at 25 ± 2 ºC, 70 ± 10% RH and a 12:12 (L:D) photoperiod and the insects used in the bioassays were obtained from laboratory mass rearing. Both larvae (*n* = 40 per treatment) and adult pairs (*n* = 24 per treatment) of *C. externa* were exposure to cotton seedlings (ca. 15 days post-emergence) grown from treated or untreated (control) seeds with CAP (Dermacor®, 60 mL 100 kg^{-1} seeds, DuPont do Brasil, Barueri, SP, Brazil) (MAPA, 2017). Lacewings larvae and adults were exposure to cotton seedlings until pupation or for 8-days, respectively. During the exposure period, larvae were fed with frozen eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) ad libitum, while adults were fed with artificial diet of honey and brewer's yeast (1:1), both refreshed each 48 h. However, no water source was provided to the lacewings to induce the use of cotton EFN as source of hydration. After the exposure period, lacewings (larvae and adults) were similarly kept to mass rearing (Soares and Macêdo, 2000), with availability of food ad libitum and water, refreshed every 48 h. The insects were monitored daily and the following parameters were recorded: survival, developmental stages duration, female proportion as well as reproductive parameters of the bioassays with larvae and adults. To analysis the immature survival, the lacewings were grouped in eight replicates (*n* = 5 insects per replicate). The fecundity was evaluated until 10-days and eggs viability was assessed by harvesting eggs on the fifth day oviposition of each female. Transgenerational effects in the developmental parameters of F1 generations were evaluated using 40 neonate larvae per treatment.

We also assessed the olfactory response of *C. externa* toward cotton seedling volatiles grown from CAP treated seed in a glass Y-tube olfactometer according to Oliveira et al. (unpublished data). A total of 40 third instar larvae and mated females (72 h old) of *C. externa* were tested only once to the odors combinations: (1) clear air *vs.* cotton plant grown from untreated seed and (2) cotton plant grown from untreated seed *vs.* cotton plant grown from CAP treated seed. Airflow was adjusted to 0.45 and 1.5 L/min/arm for larvae and females test, respectively. The lacewings choice was reported when the insect reached at least 4.5 cm along the *olfactometer* arm, remaining for at least 30 s. Insects that did not make a choice within 10 min were excluded from analysis. After each trial, the olfactometer was inverted to prevent directional bias and each five insects tested a new pair of cotton plants was used and the Y-tube olfactometer and glass chambers were sterilized. Before bioassays, the insects were starved for 24 hours.

2.2. Synthesis of laboratory bioassays

We performed a synthesis of laboratory studies to compare the non-target effects of CAP seed treatments to eight natural enemies. The studies considered for use in the synthesis analysis are part of the research project "Non-target impacts of insecticide seed treatments on natural enemies” started in 2013 by Department of Entomology at Federal University of Lavras (Lavras, MG, Brazil), in collaboration with the Agricultural Research Center-Hays of Kansas State University (Hays, KS, USA). All reviewed studies present similar experimental designs and evaluated the exposure of natural enemies to CAP seed treatment through consumption of plant resource such as the extra floral nectar.

The natural enemies evaluated in the studies represent important biological control agents of sunflower, soybean and cotton pests. A summary of the reviewed studies with description of natural enemy species as well as the main findings of the studies are given in the Table 1.

2.3. Data analysis

2.3.1. Toxicity of CAP to C. externa

All data were subjected to Shapiro-Wilk and Bartlett tests to confirm normal distribution and homoscedasticity, respectively. Subsequently, data were analyzed using an independent Student's t-test or non-parametric Mann-Whitney test, when did not meet the assumptions of normality and homoscedasticity. Sex ratio (∑♀/∑ (♀ + ♂)), proportion of pharate adults and choice data in the olfactometer test were analyzed using the Chi-square test. All analyses were performed using SigmaPlot 12.5 software ($\alpha = 0.05$) (Systat, 2013).

2.3.2. Synthesis of laboratory bioassays

To synthesize the results of the reviewed studies, the life history parameters of the natural enemies (adults and immature stages) exposed to plants grown from seeds treated with CAP were standardized by the controls. Lethal effect of CAP was estimated by the survival reduction of developmental stage in which the insects were exposed to treatment and sublethal effects were estimated in the fecundity, fertility and sex ratio (female proportion) parameters. Reduction coefficient (R) of the life history parameters was estimated as R (%) = 100 – [(seed treatment/control) x 100], according to Hassan (1998). The total effect of CAP (E), across life history parameters and developmental stages exposed, was estimated according to van de Veire et al. (1996) using the formula: E (%) = 100 – (100 – Mc) x ERn, where Mc is the corrected mortality (Abbott, 1925) and ER_n is the effect in the *n* parameters: ER = seed treatment/control. Response variables to estimate the reduction coefficients and total effect of CAP were extracted from the text and tables for reviewed studies. Based on the total effects, CAP seed treatment was classified as harmless (< 30% effect), slightly harmful (≥ 30 and ≤ 80%), and moderately harmful (> 80% effect) according to criteria established by the *International Organization for Biological Control* (IOBC) for pesticide risk assessments conducted in the laboratory (Sterk et al., 1999).

3. Results

3.1. Toxicity of CAP to C. externa

CAP caused sublethal effects, but significant in larvae and adults of *C. externa* exposed to cotton plants grown from treated seeds. The pupal period and egg viability were reduced by exposure of larvae and adults to CAP treatment, respectively. However, CAP did not affected no other parameter of the exposed generation as well as of the F1 generation in both bioassays (Tables 2 and 3).

Both larvae and mated females of *C. externa* were responsive to olfactometer test. However, *C. externa* larvae did not present any significant choice for the odors tested (clear air *vs.* cotton plant - untreated seed: $\chi^2 = 1.2$, df = 1, $P = 0.276$; cotton plant - untreated seed *vs.* cotton plant - CAP treated seed: $\chi^2 = 0.9$, df = 1, $P = 0.325$) (Fig. 1A). In contrast, *C. externa* females present significant choice by cotton plants grown from untreated seed compared to clear air ($\chi^2 = 4.1$, df = 1, $P = 0.042$), but did not show significant choice between the cotton plants grown from seeds treated or untreated with CAP ($\chi^2 = 0.5$, df = 1, $P = 0.467$) (Fig. 1B).

3.2. Synthesis of laboratory bioassays

The synthesis analysis showed differences in CAP toxicity among the groups and natural enemies' species. CAP was classified as slightly harmful for the lacewings *C. carnea* and *C. externa*, lady beetle *C. maculata*, parasitoid *L. testaceipes* and the predator bug *O. insidiosus*. In contrast, CAP was classified as harmless for the *H. axyridis*, *H. convergens* and *P. nigrispinus* (Table 4).

4. Discussion

The present study confirms that CAP can cause sublethal effects in *C. externa* when this predator uses EFN of plants from seeds treated with this product as an alternative food. Effects such as reduction of pupal development time and low viability of eggs from exposed adults can be observed for the specimens of the genus that fed on contaminated nectar, and did not pass these effects to the next generation. What probably occurred was the contamination of extrafloral nectar with CAP, since these insects did not feed on plant tissues, but were observed feeding on extrafloral nectar in most of the time they were in contact with it. EFN is an important resource for larvae and adults of *C. externa*, which use it to supply their water requirements and supplement their diet (Limburg and Rosenheim, 2001; Moser and Obrycki, 2009).

Negative effects on reproductive parameters were found only when adults of *C. externa* were exposed to contaminated EFN. This may have occurred due to the fact that adults require a greater amount of water than larvae, and that only the EFN present in the plant is insufficient to meet their water requirements. According to Gontijo et al. (2014), dehydration during the exposure period is the probable cause of infertility after adult exposure compared to larval exposure, and that the high water demand of adults causes a higher consumption of EFN in relation to larvae, mainly due to to its high sugar content. Similar results were found by Gontijo et al. (2017; unpublished data), where they observed a reduction in the number of viable eggs when adults of *C. externa* were exposed to EFN from cotton contaminated with thiametoxam, a neonicotinoid that like CAP, translocates through the plant and generally reaches the EFN.

The duration of the pupal period of *C. externa* from lavae that were exposed to CAP was reduced. When natural enemies are exposed to low doses of insecticides that are not sufficient to cause their death, they may have negative effects on their development, reduced feeding and consequently longevity and fecundity will be impaired (Desneux; Decourtye; Delpuech, 2007), and may also extend to subsequent generations (Liang et al., 2012; Wang et al., 2017; Xiao et al., 2015). CAP belongs to the group of diamides that are modulators of the rianodine receptors in the myofibrils of the insect muscles. These molecules cause uncontrolled calcium output from the cells causing muscle paralysis (Altmann, 1990).

Negative effects of CAP on green lacewing exposed to contaminated EFN have been previously reported by Gontijo et al. (2014), who observed a reduction in the survival and fecundity of *C. carnea* adults when larvae and adults fed on EFN contaminated with this insecticide. Predator intoxication will depend on how it will come into contact with the insecticide, Amarasekare and Shearer (2013) found that when adults and larvae of *C. carnea* and *C. johnsoni* (Henry, Wells and Pupedis, 1993) (Neuroptera: Chrysopidae) fed diets or preys contaminated with CAP had a high mortality rate. However, topical application of CAP on larvae of *C. externa* did not cause negative effects (Zotti et al., 2013).

In the olfactometry tests, it could be observed that *C. externa* larvae do not identify cotton plants in relation to clean air. However, adults are attracted to cotton plants regardless of whether they come from seed treatment or not. In the context of biological control, this characteristic becomes a disadvantage for the predator, since not knowing if the plant is or is not contaminated with CAP, the predator can become infected and suffer all the negative effects mentioned above, resulting in a reduction of its population in the crop and consequently in its efficiency as a biological control agent.

It has been demonstrated that volatiles emitted by plants before being attacked by herbivores are not attractive for adults of green lacewing, since they are not indicative of the presence of food resources that can guarantee the development of their progeny (Da Silva et al., 2016). However, the results of the present study evidenced the search of *C. externa* by cotton plants in comparison to pure air. This can be explained by the fact that the EFN contained in the cotton plant is a food resource for the adult phase of this predator. According to Drukker et al. (2000), these predators depend on experience and learning to forage. It is possible that they use flexible foraging strategies that depend on experience and can be learned during adult life (Steidle and van Loon, 2003; Vet and Dicke, 1992).

Regarding the effects of CAP, each species of natural enemy presents a differentiated behavior when feeding on EFN contaminated with this product. These differences can be explained by the ability of each species to degrade the compound in their organism and/or may also be related to their particular habit. In addition, parasitoids are more sensitive than predators, and this sensitivity may be related to the fact that parasitoids in the adult stage perform their hydration and nutrition by feeding on EFN, pollen and nectar (Heimpel et al., 1996; Heimpel et al., 1997). The variations occur even among insects of the same family, as is the case of the green lacewing; but both species have greater tolerance to the product in the young phase than in the adult phase. According to Gontijo et al. (2014), the high water demand of adults causes a higher consumption of extrafloral nectar in relation to larvae, and that dehydration during the exposure period is the probable cause of greater female infertility, longer oviposition and smaller fecundities after adult exposure compared to larval exposure.

Predatory bedbugs and ladybugs also show great variation as to the total effect, and for some groups of insects CAP was innocuous and for outrs it was considered slightly harmful. The species that presented low total effect value in some evaluated parameters were even benefited by the consumption of EFN contaminated with CAP, perhaps due to the effect of hormone, where the insecticide ingested by the insect at a low dosage may be responsible for triggering beneficial reactions at the same time (Guedes and Cutler, 2014).

5. Conclusions

Our results suggest that chlorantraniliprole used in seed treatment causes significant negative effects on the biology and reproduction of these predators due to translocation within plants and the potential contamination of pollen and nectar and should not be considered an IPM compatible method in cotton crops, sunflower and soybean. Further studies are needed to determine exactly how seed treatment may be compatible with IPM programs that depend on conservative biological control, since the subtle effects caused by this insecticide have the potential to disrupt the population dynamics of beneficial organisms and consequently compromise the biological control in culture.

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Table 1. Description and results of the reviewed studies for risk assessment of chlorantraniliprole (CAP) seed treatment to eight natural enemies.

Natural enemy group	Species	Life stage exposed	Plant	Main findings	Reference
Green lacewings (Neuroptera: Chrysopidae)	*Chrysoperla carnea*	Larvae Adults	Sunflower	CAP reduced survival and fecundity of lacewings adults exposed to sunflower plants grown from treated seeds.	Gontijo et al., 2014
	Chrysoperla externa	Larvae Adults	Cotton	CAP reduced the pupal period and egg viability, when *C. externa* was exposed as larva and adults, respectively to cotton plants grown from treated seeds.	Oliveira et al., (present study)
Lady beetles (Coleoptera: Coccinellidae)	*Coleomegilla maculata*	Larvae Adults	Sunflower	CAP delayed adult emergence by prolonging the pupal, when *C. maculate* larvae were exposed to sunflower plants grown from treated seeds.	Moscardini et al., 2015
	Harmonia axyridis	Larvae Adults	Cotton	The exposure of *H. axyridis* larvae and adults to cotton plants grown from seed treated with CAP did not significantly affect any parameters of generations exposed. However, CAP caused significant transgenerational effects in the both larval and adult stages.	Oliveira et al., unpublished data
	Hippodamia convergens	Larvae Adults	Sunflower	CAP reduced the egg viability and increased the eclosion time of *H. convergens*, when lady beetle was exposed as larvae to sunflower plants grown from treated seeds. CAP also reduced the pupation time of next generation after adult exposure to treatment.	Moscardini et al., 2015
Aphid parasitoid (Hymenoptera: Braconidae)	*Lysiphlebus testaceipes*	Adults	Sunflower	CAP reduced the numbers of aphids attacked and parasitized by *L. testaceipes*. However, the offspring developmental time and parasitoid emergence were unaffected by CAP.	Moscardini et al., 2014
Minute pirate bug (Hemiptera: Anthocoridae)	*Orius insidiosus*	Eggs Nymphs Adults	Sunflower	CAP was not lethal to any predator life stage, but sublethal effects were evident.	Gontijo et al., 2015
Stink bug (Hemiptera: Pentatomidae)	*Podisus nigrispinus*	Adults	Soybean	CAP increased intrinsic rate of population growth of stink bugs. However, CAP did not significantly affect bug walking.	Gontijo et al., 2018

Table 2. Developmental and reproductive parameters of F0 and F1 generations of green lacewing *Chrysoperla externa* exposed as larvae to cotton plants grown from chlorantraniliprole (CAP) treated seeds.

Parameter	Control	CAP	Stats	df	*P*
		F0 generation			
<u>Larval period</u>					
Survival (%)	90.0 ± 3.8	97.5 ± 2.5	20.0[b]	8, 8	0.234
Duration (d)	8.9 ± 0.1	8.7 ± 0.1	316.5[b]	25, 28	0.522
<u>Pupal period</u>					
Survival (%)	100	100			
Duration (d)	10.0 ± 0.0 a	9.7 ± 0.1 b	266.0[b]	25, 28	0.029
Pharate adults (proportion)	0.19 ± 0.01	0.36 ± 0.01	1.8[c]	1	0.184
Sex ratio (female proportion)	0.50 ± 0.02	0.32 ± 0.02	1.1[c]	1	0.294
<u>Reproductive parameters</u>	*n* = 13	*n* = 7			
Preoviposition period (d)	4.0 ± 0.0	4.3 ± 0.6	32.5[b]	7, 13	0.156
10-days fecundity (eggs $female^{-1}$)	193.1 ± 12.8	180.3 ± 14.4	0.63[a]	18	0.539
Egg viability (% eclosing)	92.1 ± 2.5	83.9 ± 6.5	1.34[a]	16	0.198
		F1 generation			
<u>Larval period</u>					
Survival (%)	95.0 ± 5.0	92.5 ± 3.7	25.5[b]	8, 8	0.505
Duration (d)	10.0 ± 0.0	10.0 ± 0.0			
<u>Pupal period</u>					
Survival (%)	95.0 ± 5.0	100	28.0[b]	8, 8	0.721
Duration (d)	10.2 ± 0.1	10.4 ± 0.1	17.5[b]	8, 8	0.130
Pharate adults (proportion)	0.25 ± 0.01	0.19 ± 0.01	0.12[c]	1	0.730
Sex ratio (female proportion)	0.56 ± 0.02	0.50 ± 0.02	0.02[c]	1	0.878

Means (± SE) followed by different letters were significantly different within rows by (a) t-test, (b) Mann-Whitney or (c) Chi-square test (α = 0.05).

Table 3. Reproductive and developmental parameters of F0 and F1 generations of green lacewing *Chrysoperla externa* exposed as adults to cotton plants grown from chlorantraniliprole (CAP) treated seeds.

Parameter	Control	CAP	Stats	df	*P*
	F0 generation				
Reproductive parameters					
Preoviposition period (d)	5.0 ± 0.5	4.2 ± 0.1	171.5[b]	21, 21	0.125
10-days fecundity (eggs female^{-1})	98.1 ± 10.1	87.3 ± 6.8	0.9[a]	34	0.367
Egg viability (% eclosing)	76.8 ± 6.5 a	53.7 ± 7.7 b	2.2[a]	29	0.033
	F1 generation				
Larval period					
Survival (%)	95.0 ± 3.3	85.0 ± 6.3	22.0[b]	8, 8	0.328
Duration (d)	10.2 ± 0.1	10.3 ± 0.1	30.5[b]	8, 8	0.878
Pupal period					
Survival (%)	100	100			
Duration (d)	10.5 ± 0.1	10.3 ± 0.2	17.0[b]	8, 8	0.130
Pharate adults (proportion)	0.22 ± 0.01	0.29 ± 0.01	0.18[c]	1	0.673
Sex ratio (female proportion)	0.48 ± 0.02	0.36 ± 0.02	0.32[c]	1	0.572

Means (± SE) followed by different letters were significantly different within rows by (a) t-test, (b) Mann-Whitney or (c) Chi-square test (α = 0.05).

Table 4. Reduction coefficients of life history parameters of eight natural enemies exposed to plants grown from chlorantraniliprole treated seeds and the IOBC toxicity class for the total effect.

Group	Natural enemy	Parameter	Immature stage exposure	Adults exposure	Total effect[2]
Green lacewings (Chrysopidae)	*Chrysoperla carnea*	Survival			**36.4**
		Fecundity			
		Fertility			
		Sex ratio[1]			
	Chrysoperla externa	Survival			**35.5**
		Fecundity			
		Fertility			
		Sex ratio			
Lady beetles (Coccinellidae)	*Coleomegilla maculata*	Survival			**33.9**
		Fecundity			
		Fertility			
		Sex ratio			
	Harmonia axyridis	Survival			0.0
		Fecundity			
		Fertility			
		Sex ratio			
	Hippodamia convergens	Survival			10.9
		Fecundity			
		Fertility			
		Sex ratio			
Parasitoid (Braconidae)	*Lysiphlebus testaceipes*	Parasitism	-		**64.2**
		Aphids mummified	-		
		Adult emergence	-		
		Sex ratio	-		
Predators bugs (Anthocoridae/Pentatomidae)	*Orius insidiosus*	Survival			**32.2**
		Fecundity			
		Fertility			
		Sex ratio			
	Podisus nigrispinus	Longevity	-		0.0
		Fecundity	-		
		Fertility	-		
		Sex ratio	-		

The histograms represent the reduction coefficient: R (%) = 100 – [(seed treatment/control) x 100], according to Hassan (1998). Bar length is proportion the reduction value (green negative and red positive). 1Female proportion. 2Total effect of insecticides, across life history parameters and developmental stage of natural enemy exposed, according to van de Veire et al. (1996): E (%) = 100 – (100 – Mc) x ERn, where Mc is the corrected mortality (Abbott, 1925) and ERn is the effect of the n parameters: ER = seed treatment/control. IOBC toxicity class to total effect: value not highlighted = harmless (< 30% effect); highlighted in bold = slightly harmful (≥ 30 and ≤ 80%), according to Sterk et al. (1999).

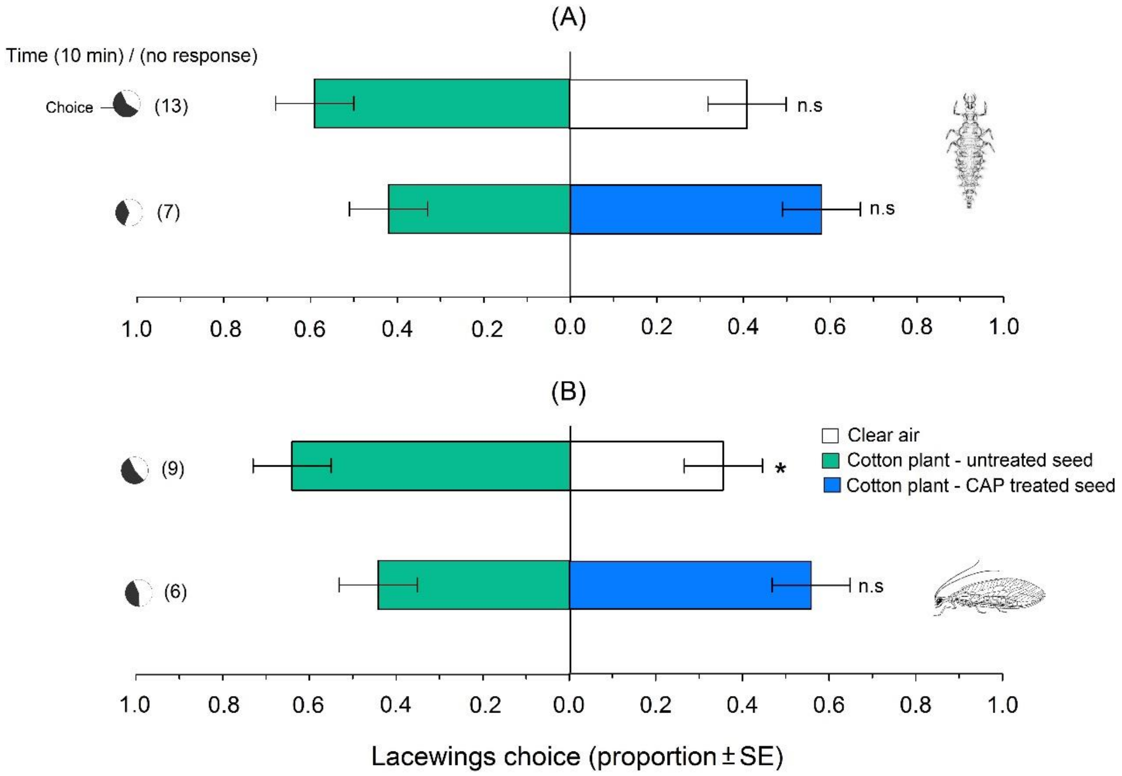

Figure 1. Response in a Y-tube olfactometer of larvae (A) and mated females (B) of *Chrysoperla externa* to cotton plant volatiles grown from seeds treated or untreated with chlorantraniliprole (CAP). The combinations of odors tested were: (1) clear air *vs.* cotton plant grown from untreated seed and (2) cotton plant grown from untreated seed *vs.* cotton plant grown from CAP treated seed to both larvae and mated females. Pie charts represent the time for choice (black). Forty lacewings were used each test and insects that did not make a choice within 10 min (given in parentheses) were excluded from analysis. *Significant difference between responses within a trial and *n.s* not significant (Chi-square test, $\alpha = 0.05$).