

TOXICIDADE RELATIVA DE INSETICIDAS SINTÉTICOS À JOANINHA *Hippodamia  
convergens* (GUÉRIN-MÉNEVILLE) (COLEOPTERA: COCCINELLIDAE)

por

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RESUMO

Considerando a necessidade de se harmonizar métodos de controle biológico e químico no manejo de pragas agrícolas, o presente trabalho avaliou a toxicidade dos inseticidas clorantraniliprole, lambda-cialotrina e dicrotofós à joaninha *Hippodamia convergens* (Guérin-Méneville) (Coleoptera: Coccinellidae). Mediante bioensaios de concentração-mortalidade, a toxicidade de clorantraniliprole foi determinada para *H. convergens*, *Alabama argillacea* (Hübner) e *Chloridea virescens* (Fabr.) (Lepidoptera: Noctuidae), enquanto a lambda-cialotrina e dicrotofós foram testados em três populações de *H. convergens* coletadas em regiões com diferentes históricos de uso desses inseticidas. A resposta da população resistente de *H. convergens* à mistura da lambda-cialotrina e dicrotofós, bem como as características biológicas das três populações na ausência de inseticidas também foram avaliadas. A toxicidade do clorantraniliprole para *H. convergens* foi em ordem crescente na aplicação tópica, ingestão e contato com resíduo seco. As dosagens de campo contra *A. argillacea* e *C. virescens* revelaram-se 383 e 37 vezes superiores às suas respectivas  $CL_{90}$ s. Uma população de *H. convergens* apresentou altos níveis de resistência tanto à lambda-cialotrina quanto ao dicrotofós, enquanto as outras populações foram suscetíveis a ambos os produtos. A mortalidade da população resistente tratada com a mistura de inseticidas foi similar à observada no controle usando apenas dicrotofós e duas

vezes superior àquela causada pela lambda-cialotrina isoladamente. A viabilidade de ovos foi a única característica biológica diferente, sendo maior na população resistente e similar entre as outras duas populações. Concluimos que a via de exposição e a concentração aplicada influenciam na toxicidade do clorantraniliprole a *H. convergens* e sugerimos que as atuais dosagens recomendadas para o controle *A. argillacea* e *C. virescens* podem promover adequado controle dessas pragas, porém representando riscos a *H. convergens*. Finalmente, sugerimos que a resistência de *H. convergens* à lambda-cialotrina e ao dicrotofós refletem seu histórico de exposição a esses produtos.

**PALAVRAS-CHAVE:** Controle integrado de pragas, seletividade de inseticidas, diamidas, organofosforados, piretroides, resistência a inseticidas.

RELATIVE TOXICITY OF SYNTHETIC INSECTICIDES TO THE CONVERGENT LADY

BEETLE *Hippodamia convergens* (GUÉRIN-MÉNEVILLE) (COLEOPTERA:  
COCCINELLIDAE)

by

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ABSTRACT

Aiming to offer harmonization of biological and chemical controls, we evaluated the toxicity of chlorantraniliprole, lambda-cyhalothrin and dicotophos to the lady beetle *Hippodamia convergens* (Guérin-Méneville) (Coleoptera: Coccinellidae). Chlorantraniliprole toxicity was determined through concentration-mortality bioassays to *H. convergens*, *Alabama argillacea* (Hübner) and *Chloridea virescens* (Fabr.) (Lepidoptera: Noctuidae), whereas lambda-cyhalothrin and dicotophos were tested on three populations of *H. convergens* collected from localities with different historical usage of insecticide. Furthermore, we applied a mixture of lambda-cyhalothrin and dicotophos on a resistant population of *H. convergens*, and evaluated the biological traits of three populations in absence of insecticide exposure. Contact with dried residues and ingestion of contaminated moth eggs showed similar toxicity to *H. convergens*, being topical application a less toxic route. The field rates (FRs) currently recommended against *A. argillacea* e *C. virescens* were 383- and 37-fold greater than their respective CL<sub>90S</sub>. Lady beetles from one population were highly resistant to both lambda-cyhalothrin and dicotophos, but the other two populations were susceptible to both insecticides. Beetles from the resistant population treated with these insecticides in mixture exhibited similar mortality to those receiving only dicotophos, but it was

twice greater than those exposed to lambda-cyhalothrin alone. Egg fertility was superior for resistant *H. convergens* population, the only different biological trait across all populations evaluated. We concluded that both route of exposure and concentrations may affect toxicity of chlorantraniliprole to *H. convergens*, and suggested that current FRs against *A. argillacea* and *C. virescens* may provide adequate control of these pest species, although showing risks to *H. convergens*. Finally, we suggested that resistance to lambda-cyhalothrin and dicotophos in *H. convergens* reflect their exposure history to these materials.

**KEY WORDS:** Integrated pest control, insecticide selectivity, diamides, organophosphates, pyrethroid, insecticide resistance.

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## CAPÍTULO 1

### INTRODUÇÃO

A busca por métodos práticos e eficientes de controle de artrópodes praga, que ao mesmo tempo representem baixo risco aos organismos não alvo, é objetivo comum entre entomologistas em todo o mundo. Essa necessidade tem instigado pesquisas relacionadas à biodiversidade e dinâmica populacional da entomofauna benéfica nos mais variados agroecossistemas (Barros *et al.* 2006, Crowder & Jabbour 2014, Werling *et al.* 2014), enquanto outros estudos buscam medidas que garantam a sobrevivência e, quando possível, incrementem a efetividade dos agentes de biocontrole em campo (Araj *et al.* 2009, Simpson *et al.* 2011), quer sejam parasitoides, predadores ou entomopatógenos (bactérias, fungos, vírus e nematoides). Também temas de investigação, a especificidade de pesticidas<sup>1</sup> contra determinadas pragas e seus efeitos sobre inimigos naturais (Tohnishi *et al.* 2005, Gogi *et al.* 2006, Srivastava *et al.* 2014), se somam aos esforços direcionados ao aperfeiçoamento de metodologias que reduzam o impacto negativo desses compostos sobre os artrópodes benéficos (Naranjo *et al.* 2003, Roubos *et al.* 2014).

Por compartilharem o mesmo hábitat com espécies fitófagas, os inimigos naturais podem ser negativamente afetados pela aplicação de pesticidas tanto de forma direta quanto indireta. O impacto direto reflete a toxicidade aguda do(s) produto(s) causando a morte dos organismos atingidos, enquanto os efeitos subletais podem incluir mudanças comportamentais; redução da longevidade, capacidade de predação e desempenho reprodutivo; alteração no período de

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<sup>1</sup>Qualquer substância ou mistura de substâncias usada para repelir, erradicar ou mitigar qualquer praga (FAO & WHO 2016). Incluem inseticidas, nematicidas, rodenticidas, herbicidas e fungicidas (Yu 2015).

desenvolvimento; estivação pela redução na disponibilidade de presas; e abandono das áreas tratadas, dentre outros (Croft & Brown 1975, Theiling & Croft 1988, Desneux *et al.* 2007).

Todavia, existem inseticidas sintéticos de ação seletiva capazes de efetivamente controlar as pragas sem infligir tais efeitos negativos a espécies não-alvo (Croft 1990), destacando-se aqueles sem atividade neurotóxica (Cloyd 2012). Apesar da dificuldade em se definir a essência da seletividade, didaticamente ela é categorizada como ecológica ou fisiológica. A primeira é representada por alguma característica inerente ao produto ou da forma de aplicação pela qual os inimigos naturais não são atingidos ou são menos afetados (Hull & Beers 1985), enquanto a seletividade fisiológica diz respeito a alguma característica intrínseca do inimigo natural que o faz menos sensível que a praga alvo quando expostos à mesma concentração/dose de determinado inseticida (Mullin & Croft 1985, Croft 1990). Em ambos os casos, inimigos naturais e inseticidas seletivos poderiam ser combinados sem grande prejuízo aos primeiros, assim viabilizando a integração desses importantes métodos (biológico e químico) de controle de pragas.

Primeiramente proposto por Stern *et al.* (1959), o controle integrado de pragas considera os agentes de biocontrole e inseticidas sintéticos como complementares, preconizando-se ainda que, seguindo adequado monitoramento, estes sejam aplicados apenas quando e onde os fatores naturais de mortalidade (bióticos e abióticos) não forem suficientes para prevenir que a população de pragas atinja níveis de dano econômico<sup>2</sup>. Os mesmos autores também sugerem que o sucesso da integração dos controles biológico e químico exige uma compreensão holística dos sistemas agrícolas, onde ambiente, planta, entomofauna e inseticidas, quando aplicados, interagem podendo afetar um ao outro.

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<sup>2</sup>Menor densidade populacional da praga que causa perda econômica, a qual é definida como sendo o nível de injúria que justifique o custo com medidas artificiais de controle, podendo variar de uma região para outra, de um ano/safra para outro, ou mesmo em função do valor econômico da cultura (Stern *et al.* 1959).

Sobre tais princípios forjou-se o manejo integrado de pragas (MIP), um conceito multidisciplinar amplamente difundido em que populações de espécies fitófagas são mantidas em equilíbrio através da utilização harmônica de múltiplos métodos de controle, incluindo o biológico, cultural, legislativo, resistência de plantas e, em condições excepcionais, a aplicação de pesticidas (Bottrell 1979, Bottrell & Smith 1982, Barzman *et al.* 2015). De acordo com Bottrell (1979), todo programa de MIP deve ser norteado pelas seguintes diretrizes: determinar as pragas chave e seu nível de dano econômico (*i*); adotar medidas preventivas que mantenham a população de pragas abaixo do nível de controle<sup>3</sup> (*ii*); em situações de emergência, optar por medidas curativas de baixo impacto ecológico (*iii*); e estabelecer técnicas eficientes de monitoramento tanto para espécies fitófagas quanto para seus inimigos naturais (*iv*).

Apesar do amplo conhecimento (pelo menos da comunidade científica) dos procedimentos necessários à implementação do MIP, sua adoção na prática segue tímida, particularmente em países em desenvolvimento (Mancini *et al.* 2008, Panizzi 2013, Parsa *et al.* 2014). Justificativas para tal são das mais diversas, incluindo a complexidade de fatores e interações a serem considerados ou parcerias a serem firmadas entre vários colaboradores; ausência de políticas públicas de incentivo; carência de treinamento e suporte técnico aos produtores; insuficiente quadro de extensionistas que promovam a interlocução entre pesquisadores e produtores através da transferência de conhecimentos; baixo nível de instrução dos agentes envolvidos; influência das empresas de agrotóxicos; e ainda alterações no sistema de cultivo que alteram a diversidade e dinâmica populacional de pragas em culturas outrora bem manejadas. Assim, muitos sistemas agrícolas seguem focando as aplicações de pesticidas como a principal medida de controle de pragas, ao invés de incluí-los de forma equilibrada e racional no manejo das mesmas.

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<sup>3</sup> Densidade populacional da praga em que medidas de controle devem ser adotadas para evitar que o NDE seja atingido (Stern *et al.* 1959).



Não como regra, agroecossistemas com programas de MIP bem estabelecidos tendem a se tornar menos dependentes do uso de pesticidas, o que pode reduzir os custos de produção e os riscos à saúde humana e ao meio ambiente (Ehler 2006, Cameron *et al.* 2009, Weddle *et al.* 2009). No entanto, existem culturas onde o complexo de pragas é por si tão diverso e dinâmico que o controle químico geralmente se faz necessário, como é o caso do algodoeiro, *Gossypium hirsutum* L. (Malvaceae). Apesar da redução significativa no número de aplicações de inseticidas na cotonicultura a partir de meados da década de 1990, devido à ampla adoção das variedades resistentes a pragas da ordem Lepidoptera (algodão *Bt*<sup>4</sup>) (Brookes & Barfoot 2010, Naranjo *et al.* 2011), o algodoeiro continua no ranking das culturas agrícolas que mais consomem pesticidas em todo o mundo (Oerke 2006). Esta é, dentre as grandes culturas do agronegócio brasileiro, a que mais consome inseticidas por hectare cultivado (Silva *et al.* 2011).

Comparadas a cultivos de algodão convencional, áreas de algodão *Bt* na Austrália apresentaram redução média no número de pulverizações contra *Helicoverpa* spp. (Lepidoptera: Noctuidae) variando de 43% a 80% ao longo dos anos/safra de 1998/1999 e 2001/2002, respectivamente, realizando-se em média 9,9 aplicações na área não-*Bt* e 4,5 no cultivo resistente (Fitt 2003). Em países asiáticos, cotonicultores que implementaram o MIP reduziram de 11,7 para 5,5 o número médio de aplicações de inseticidas em suas lavouras, enquanto nenhuma diferença significativa foi observada em cultivos que não adotaram esse sistema de manejo (Walter-Echols & Soomro 2005). Na região sudeste dos Estados Unidos da América (EUA), a erradicação do bicudo-do-algodoeiro, *Anthonomus grandis* Boehman (Coleoptera: Curculionidae), possibilitou reduzir de 14,4 para 5,4 o número de pulverizações por ciclo de cultivo (Haney *et al.* 2009),

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<sup>4</sup>Variedades de algodão geneticamente modificadas para expressar toxina(s) derivada(s) da bactéria *Bacillus thuringiensis*, tendo como alvo principal artrópodes mastigadores (Sanahuja *et al.* 2011).

média que foi reduzida para aproximadamente 3,0 aplicações a partir da introdução das variedades *Bt* (Roberts 2015).

Como consequência da redução no número de pulverizações direcionadas a lagartas, algumas espécies antes simultaneamente controladas ganharam relevância na cotonicultura americana, como é o caso dos percevejos *Acrosternum hilare* (Say), *Euschistus servus* (Say), *Nezara viridula* (L.) (Hemiptera: Pentatomidae) (Snodgrass *et al.* 2005, Greene *et al.* 2006), e *Lygus hesperus* Knight (Hemiptera: Miridae) (Snodgrass *et al.* 2009). Percevejos e outros artrópodes, até então considerados como pragas secundárias, também têm emergido como pragas importantes na cultura do algodoeiro na China (Lu *et al.* 2010), na Austrália (McColl *et al.* 2011) e no Brasil (Soria *et al.* 2010, 2011). Além da adoção de variedades *Bt*, outro fator que pode contribuir para aumentar a incidência de percevejos em campos de algodão no Brasil é seu cultivo próximo a áreas cultivadas com soja, principal hospedeiro dos percevejos pentatomídeos (Panizzi 1997, Soria *et al.* 2009, Panizzi 2015).

Essa mudança no complexo de pragas do algodoeiro, somada a eventuais infestações simultâneas por múltiplas espécies de artrópodes, bem como a evolução de resistência<sup>5</sup> a variedades de algodão *Bt* em algumas populações de Lepidoptera e o menor custo se comparado a moléculas mais recentes, têm garantido a continuidade de uso de inseticidas de amplo espectro como organofosforados (OP's), carbamatos [ambos inibidores da acetilcolinesterase (Fukuto 1990)] e piretroides [moduladores dos canais de sódio (Vais *et al.* 2001)] em áreas produtoras de algodão (Greene *et al.* 2001, Roberts & Toews 2016), apesar dos vários registros de resistência de pragas a estes produtos. Robusta documentação de casos de resistência de artrópodes a pesticidas

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<sup>5</sup>Característica pré-adaptativa, genética e hereditária (Dobzhansky 1951), definida como a habilidade herdada de um organismo em tolerar ou evitar doses de um tóxico que seriam letais para a maioria dos indivíduos da mesma espécie (WHO 1957).

pode ser acessada no banco de dados online ‘Arthropod Pesticide Resistance Database - APRD’ (Whalon *et al.* 2016).

Em efeito cascata, a resistência de pragas a variedades de algodão *Bt* pode elevar a demanda de inseticidas sintéticos, sendo os de amplo espectro muitas vezes escolhidos apenas por serem mais baratos que outros mais seletivos. Entretanto, o uso intensivo de produtos como OP’s, piretroides e carbamatos também tem pressionado para resistência em populações de pragas (Elzen *et al.* 1992, Alvi *et al.* 2012), o que pode motivar aumento de doses ou mesmo aplicações de misturas de inseticidas com diferentes modos de ação (Gunning *et al.* 1999, Martin *et al.* 2003, Ahmed *et al.* 2009). Nocivos aos inimigos naturais, aplicações de produtos de amplo espectro podem ainda desencadear surtos de pragas secundárias (Tillman & Mulrooney 2000, Gross & Rosenheim 2011), levando ao uso de outros inseticidas que também pressionarão para resistência. Esse é o caso, por exemplo, de resistência a neonicotinoides [agonistas de receptores nicotínicos da acetilcolina (Jeschke *et al.* 2011)] em populações de *L. hesperus* (Dennehy & Russell 1996) e do pulgão-do-algodoeiro, *Aphis gossypii* Glover (Hemiptera: Aphididae) (Herron & Wilson 2011, Gore *et al.* 2013).

No Brasil, áreas integradas a um programa para supressão do bicudo-do-algodoeiro no estado de Goiás receberam em média 14,1 e 12,2 aplicações de inseticidas nos anos/safra de 2006/2007 e 2007/2008, respectivamente (Lima Jr. *et al.* 2013). Vale ressaltar que desde sua introdução, esta continua sendo a principal praga da cotonicultura nacional. Entretanto, a lista de artrópodes potencialmente pragas dessa cultura para os quais se têm inseticidas e acaricidas registrados no Brasil contempla mais de 50 espécies, com destaque para os pertencentes às ordens Lepidoptera e Hemiptera (AGROFIT 2016). Apenas citando algumas das pragas-chave e o número de produtos registrados nesta mesma fonte temos: *Helicoverpa armigera* (Hübner), *Spodoptera frugiperda* (Smith), *Chloridea* (= *Heliiothis*) *virescens* (Fabr.) e *Alabama argillacea*

(Hübner) (Lepidoptera: Noctuidae) com 20, 38, 92 e 161 produtos registrados, respectivamente; *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) aparece com 37; *A. grandis* com 86 e *A. gossypii* com 124 inseticidas registrados.

Majoritariamente, esses produtos pertencem aos grupos dos OP's e piretroides, mas também contemplam neonicotinoides e outros considerados como de baixo impacto a inimigos naturais, por exemplo, os reguladores de crescimento [inibidores da síntese de quitina, juvenoides, anti-juvenoides e agonistas de ecdisteroides (Insecticide Resistance Action Committee - IRAC 2016)], e as diamidas [ativadores de receptores rianodínicos dos canais de cálcio (Lahm *et al.* 2009, Fernandes *et al.* 2016)] (AGROFIT 2016).

Por não agirem no sistema nervoso dos insetos, as diamidas surgem como importantes componentes no manejo da resistência de artrópodes a inseticidas de ação neurotóxica como OP's, carbamatos, piretroides e neonicotinoides. Até o momento, três ingredientes ativos (i.a.) das diamidas estão disponíveis comercialmente: flubendiamida, ciantraniliprole e clorantraniliprole (IRAC 2016). Tanto o primeiro quanto o segundo contam com apenas um produto registrado no Brasil, sendo a flubendiamida recomendada contra lagartas em algodoeiro, milho, soja e tomate, e o ciantraniliprole indicado somente contra espécies sugadoras em 30 culturas (AGROFIT 2016). Por sua vez, o clorantraniliprole aparece registrado para 16 culturas e com 10 produtos formulados, sendo cinco deles apenas diamida e os outros cinco em mistura com avermectina [ativadores dos canais de cloro (IRAC 2016)], piretroide ou neonicotinoide, o que amplia sua gama de exposição a artrópodes alvo e não alvo (AGROFIT 2016).

Assumindo um volume médio de calda de 100 L/ha, a maior dosagem de clorantraniliprole recomendada contra *C. virescens* em algodoeiro no Brasil [150 mL de Premio<sup>®</sup>/ha  $\approx$  300 mg i.a./L (AGROFIT 2016)] fica abaixo da indicada para essa mesma espécie nos EUA [27 oz de Prevathon<sup>®</sup>/ac  $\approx$  798 mL/ha  $\approx$  411 mg i.a./L (Roberts & Toews 2016)], mas ambas são bem

superiores à recomendada contra *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) em brássicas [7,5 mL de Premio<sup>®</sup>/ha  $\approx$  15 mg i.a./L (AGROFIT 2016)]. Embora não pareça razoável qualquer comparação entre dosagens de campo recomendadas para diferentes espécies em diferentes culturas e países, vale aqui fazer essa ponderação, particularmente se considerarmos o número de casos de resistência a pesticidas registrados para a traça-das-crucíferas (782 casos e 93 i.a., que já inclui diamidas) e a lagarta-das-maçãs (120 registros e 35 i.a., ainda não inclui diamidas) (Whalon *et al.* 2016). Isso demonstra a grande dependência no uso de inseticidas e por conseguinte alta pressão de seleção em ambos os cultivos, o que pode impactar negativamente a entomofauna benéfica ali presente, importante para manutenção do equilíbrio nos agroecossistemas.

Com muitas espécies ainda não registradas na literatura, os principais grupos de inimigos naturais associados à cultura do algodoeiro e suas pragas no Brasil elencados por Bastos & Torres (2005) incluem 13 famílias de insetos predadores e nove de parasitoides, além da participação de pássaros, ácaros, aranhas e entomopatógenos. Visando a conservação desses organismos, estudos que investiguem o impacto de inseticidas sintéticos sobre parasitoides e predadores se mostram essenciais, o que pode contribuir para evitar que populações de espécies fitófagas naturalmente controladas atinjam níveis de praga.

Avaliando a diversidade e abundância da entomofauna em algodoeiro convencional e *Bt*, submetidos ou não à aplicação de inseticidas na China, Men *et al.* (2003) identificaram 36 diferentes espécies de artrópodes, sendo 20 de pragas e 16 de inimigos naturais. De acordo com esses autores, as aplicações de inseticidas reduziram a abundância tanto de pragas, quanto de inimigos naturais em ambas as áreas, além de afetar negativamente a riqueza de espécies no algodão *Bt*. Em estudos conduzidos no estado do Arizona, EUA, Sisterson *et al.* (2004) identificaram artrópodes pertencentes a 69 famílias sem qualquer efeito significativo da variedade de algodão cultivada, bem como a abundância dos predadores *Chrysoperla carnea* Stephens

(Neuroptera: Chrysopidae) e *Orius tristicolor* (White) (Hemiptera: Anthocoridae) em algodão *Bt* não diferiu do não-*Bt*, embora significativamente reduzida nesta última se considerado o fator aplicações de inseticidas (Sisterson *et al.* 2007).

Durante estudos para avaliar a comunidade de artrópodes predadores epigeais e no dossel do algodoeiro no estado da Geórgia, importante produtor de algodão na região sudeste dos EUA, algumas áreas de algodão *Bt* receberam aplicações de inseticidas contra percevejos, enquanto as de algodão convencional foram pulverizadas contra percevejos e lagartas Heliotinae (Torres & Ruberson 2005). Apesar de diferenças pontuais no tempo (três ciclos de cultivo) ou técnica de amostragem (três metodologias), a abundância de predadores nos diferentes cultivos não foi afetada e um total de 30 taxa diretamente relacionados à cultura do algodão foram coletados. Os autores destacam haver permuta de espécies de predadores entre o dossel e o nível do solo em resposta à aplicação de inseticidas, e dedicam atenção especial para a maior abundância da joaninha predadora *Hippodamia convergens* (Guérin-Méneville) (Coleoptera: Coccinellidae) em áreas de algodão convencional após aplicações do piretroide lambda-cialotrina, enquanto a densidade de outras espécies não diferiu ou até mesmo apresentou declínio.

*Hippodamia convergens* encontra-se amplamente distribuída pelo mundo, sendo uma das mais importantes espécies predadoras de pulgões em diversos agroecossistemas com diferentes regimes de utilização de inseticidas. Além do algodoeiro (Barros *et al.* 2006, Sujii *et al.* 2007), citam-se trigo (Michaud & Qureshi 2006), brássicas (Rodrigues *et al.* 2013a, Jessie *et al.* 2015) e sorgo (Colares *et al.* 2015), dentre outros. Logo, é possível que populações de *H. convergens* sejam expostas a inseticidas de diferentes grupos químicos em campo e, a depender da pressão de seleção à qual são submetidas, evoluam para resistência a esses compostos, o que poderia ser caracterizado como seletividade fisiológica.

Essa hipótese não fora aventada por Tillman & Mulrooney (2000) que, obtendo maior abundância de *H. convergens* em parcelas de algodão tratadas com lambda-cialotrina no estado do Mississippi, EUA, atribuíram tal resultado à coincidente maior densidade de pulgões. Por outro lado, Torres & Ruberson (2005) sugeriram uma possível resistência à lambda-cialotrina para explicar seus resultados, o que veio a ser confirmado posteriormente através de testes em laboratório direcionados à detecção e determinação dos níveis de resistência a pesticidas em populações de artrópodes (Ruberson *et al.* 2007, Rodrigues *et al.* 2013b). Além dos altos níveis de resistência ao piretroide, adultos de *H. convergens* provenientes de áreas produtoras de algodão na Geórgia apresentaram 100% de sobrevivência quando tratados com dicrotofós numa dose 10 vezes superior à aplicada em campo (Rodrigues *et al.* 2013b), indicando possível resistência também a OP's.

Assim, o presente trabalho objetivou avaliar a suscetibilidade da joaninha predadora *H. convergens* a inseticidas sintéticos comumente utilizados na cultura do algodoeiro. Inicialmente, a toxicidade do inseticida clorantraniliprole foi avaliada em três vias de exposição (tópica, oral e residual) a uma população de *H. convergens* coletada em cultivo orgânico de hortaliças no estado de Pernambuco, Brasil. Também foi verificada a efetividade dessa diamida no controle de lagartas do curuquerê-do-algodoeiro, *A. argillacea*, e da lagarta-das-maçãs, *C. virescens*, quando se determinou a razão de tolerância ( $RT_{50}$ ) de *H. convergens* ao clorantraniliprole comparando a concentração letal a 50% das joaninhas ( $CL_{50}$ ) às estimadas para matar 50% das pragas. Posteriormente, a dose de campo e a concentração correspondente ao limite superior da  $CL_{90}$  estimada para *C. virescens* foram aplicadas em plantas de algodão cultivadas em casa de vegetação para investigar a toxicidade do resíduo do clorantraniliprole após diferentes intervalos de aplicação, tanto para a lagarta-das-maçãs quanto para adultos da joaninha.

Em outra série de bioensaios avaliou-se a toxicidade dos inseticidas lambda-cialotrina e dicrotofós a três populações de *H. convergens* coletadas em diferentes regiões dos EUA. Também foram realizados cruzamentos recíprocos entre a população mais resistente com a mais sensível a ambos os inseticidas para verificar se tal característica seria autosomal ou ligada ao sexo. Tendo em vista que muitos produtores recorrem à combinação de inseticidas para manejar determinadas pragas, já havendo no mercado produtos formulados com mais de um ingrediente ativo, investigou-se ainda a toxicidade de uma mistura da lambda-cialotrina e dicrotofós para a população resistente de *H. convergens*, além de acompanhar o desenvolvimento e reprodução das três populações de joaninha na ausência de inseticidas para verificar possíveis custos adaptativos.

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## CHAPTER 2

### HIGH RATES OF CHLORANTRANILIPROLE REDUCE ITS COMPATIBILITY WITH A KEY PREDATOR<sup>1</sup>

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ABSTRACT – Diamides act by disrupting insect muscle contraction, and represent one of the newest insecticide groups. Recommended field rates (FRs) are highly variable among target pests and cropping systems, with concomitant risks for non-target organisms. We evaluated the toxicity of chlorantraniliprole to the key predator *Hippodamia convergens* (Guérin-Méneville) (Coleoptera: Coccinellidae) through topical, oral and residual exposure routes. In addition, we estimated its lethal concentrations (LCs) to two target pests, cotton leafworm, *Alabama argillacea* (Hübner), and tobacco budworm, *Chloridea virescens* (Fabr.) (Lepidoptera: Noctuidae) by exposing larvae to treated cotton leaves. We also assessed residual activity at different intervals after application to cotton plants. Exposure to dried residues and ingestion of treated moth eggs resulted in similar toxicity to *H. convergens*, whereas topical application was a less toxic route of exposure. All LCs obtained for *H. convergens* were higher than those calculated for the pests, but lower than the FRs. Residues at the upper limit of the LC<sub>90</sub> of chlorantraniliprole for *C. virescens* remained effective against this pest for up to 16 days, while remaining relatively harmless to *H. convergens*. However, the *C. virescens* FR caused significant lady beetle mortality, indicating likely negative impacts on natural populations. However, chlorantraniliprole is potentially harmless to *H. convergens*, depending on dosage and the route of exposure. The results indicate that current FRs against *A. argillacea* and *C. virescens* should provide effective control of these pests in Brazilian cotton fields.

KEY WORDS: Integrated pest control, physiological selectivity, ryanodine receptors, risk assessment, dose-mortality test

## ALTAS DOSAGENS DE CLORANTRANILIPROLE DIMINUEM SUA COMPATIBILIDADE COM UM PREDADOR CHAVE

RESUMO – As diamidas interferem no processo de contração muscular dos insetos e representam um dos mais recentes grupos de inseticidas. As dosagens de campo (FRs) variam muito de acordo com a praga alvo e o sistema de cultivo, assim concomitando em riscos para organismos não-alvo. No presente trabalho, avaliamos a toxicidade do clorantraniliprole à joaninha predadora *Hippodamia convergens* (Guérin-Méneville) (Coleoptera: Coccinellidae) pelas vias de contaminação tópica, oral e residual. Discos de folhas tratadas foram empregados para estimar as CLs desse inseticida para duas pragas alvo, curuquerê-do-algodoeiro, *Alabama argillacea* (Hübner) e lagarta-das-maçãs, *Chloridea virescens* (Fabr.) (Lepidoptera: Noctuidae). Também avaliamos o efeito residual após diferentes intervalos de aplicação em plantas de algodão. O contato com resíduo seco e ingestão de presa contaminada apresentaram toxicidade similar para *H. convergens*, enquanto a aplicação tópica resultou em menor toxicidade. Todas as CLs calculadas para *H. convergens* foram superiores àquelas determinadas para as espécies praga e inferiores às FRs. Resíduos do clorantraniliprole em concentração correspondente ao limite superior da CL<sub>90</sub> estimada para *C. virescens* permaneceram efetivos contra essa praga e com baixa toxicidade a *H. convergens* por até 16 dias. Entretanto, a FR para *C. virescens* causou significativa mortalidade de *H. convergens*, indicando possível impacto negativo em suas populações naturais. Clorantraniliprole pode ser pouco tóxico a *H. convergens* dependendo da concentração aplicada e da via de exposição. Os resultados sugerem que as atuais FRs contra *A. argillacea* e *C. virescens* no Brasil devem promover adequado controle dessas pragas em campos de algodão.

PALAVRAS CHAVE: Controle integrado de pragas, seletividade fisiológica, receptores de rianodina, avaliação de risco, teste dose-mortalidade

## Introduction

Pest management is a complex task, especially when agroecosystems contain diverse arthropod communities, mainly because measures adopted against one pest species may impact the natural enemies of others and lead to secondary pest outbreaks (Gross & Rosenheim 2011). Among natural enemies, predatory lady beetles make major contributions to biological control of sap sucking pests such as aphids, mealybugs, psyllids and whiteflies (Obrycki 1998, Weber & Lundgren 2009, Michaud 2012). The convergent lady beetle, *Hippodamia convergens* (Guérin-Méneville) (Coleoptera: Coccinellidae), is a widely distributed species that regularly preys on aphids in cotton fields (Whitcomb & Bell 1964, Torres & Ruberson 2005), cereal crops (Michaud & Qureshi 2006, Colares *et al.* 2015), vegetable gardens (Rodrigues *et al.* 2013a, Jessie *et al.* 2015), and a wide range of herbaceous plants. Given its importance in various agroecosystems, many studies have addressed the susceptibility of *H. convergens* to different insecticides (e.g., Rodrigues *et al.* 2013a, 2013b, Mills *et al.* 2015, Barbosa *et al.* 2016). These studies provide information useful in guiding the selection of insecticides for field application that will conserve this and other important natural enemy species.

Diamides are a recently discovered group of insecticides that includes, for the moment, three active ingredients: flubendiamide, chlorantraniliprole and cyantraniliprole (IRAC 2016). They are generally considered to pose reduced risk to natural enemies (Brugger *et al.* 2010, Mills *et al.* 2015), being more selective than older molecules (Rodriguez-Saona *et al.* 2016). Diamides bind to ryanodine receptors (RyRs) of the calcium channels, promoting uncontrolled Ca<sup>2+</sup> release from the sarcoplasmic reticulum that results in disruption of muscle contraction (Lahm *et al.* 2005, Cordova *et al.* 2006). Whereas mammals express three isoforms of RyRs associated with skeletal and cardiac muscle (Fill & Copello 2002), insects express only a single RyR that is notably different (Sattelle *et al.* 2008), resulting in a 500-fold greater activity of diamides in

insects (Cordova *et al.* 2006). This remarkable selectivity has motivated a search for new derivatives of this group (Lahm *et al.* 2009, Zhang *et al.* 2012).

The selective profile of diamides, combined with a lack of cross-resistance with older compounds, led to their emergence as important insecticides worldwide as early as 2012, when sales of chlorantraniliprole and flubendiamide represented 8% of the value of the world insecticide market (Casida 2015). In Brazil, chlorantraniliprole has been registered since 2009 for use against pests of 16 different crops including brassicas, corn, soybean and cotton (AGROFIT 2016), all agroecosystems where *H. convergens* and other coccinellid species are regularly present. The field rates (FRs) of chlorantraniliprole applied for control of lepidopterans in Brazilian cotton fields range from approximately 33 mg a.i./L when targeting cotton leafworm, *Alabama argillacea* (Hübner), to 200 mg a.i./L for tobacco budworm, *Chloridea* (= *Heliothis*) *virescens* (Fabr.) (Lepidoptera: Noctuidae), assuming an application volume of 150 L of water per hectare (AGROFIT 2016). The former species is considered the most important defoliator of cotton in Brazil (Silva *et al.* 2011, Ramalho *et al.* 2014), whereas the latter is a polyphagous species attacking various crops in addition to damaging the leaves and bolls of cotton (Bestete *et al.* 2016).

In order to successfully integrate biological and chemical control of arthropod pests, assessment of pesticide non-target effects on natural enemy species is necessary (Stern *et al.* 1959, Theiling & Croft 1988). Many ecotoxicological assessments of diamides have focused on the baseline susceptibility of pest species (Jiang *et al.* 2012, Silva *et al.* 2012, Campos *et al.* 2015), whereas their impacts on beneficial arthropods have been assessed in comparison to those of older chemistries applied at labelled rates (Mills *et al.* 2015, Shearer *et al.* 2016). However, the highest FRs recommended against lepidopteran pests in Brazilian cotton fields greatly exceed the estimated lethal concentrations (LCs) required to control other cotton pests (Caballero *et al.* 2013,

Bird 2015), and are substantially higher than most dosages that have been tested on natural enemies (Castro *et al.* 2013, Mills *et al.* 2015). We hypothesized that 1) chlorantraniliprole is less toxic to *H. convergens* than it is to either *A. argillacea* or *C. virescens* and, 2) that label rates of chlorantraniliprole in cotton are high enough to be harmful to *H. convergens*. To address these issues, we conducted bioassays in which the toxicity of chlorantraniliprole to adult *H. convergens* was assessed in three potential routes of exposure, and others in which larvae of *A. argillacea* and *C. virescens* were exposed to residues of chlorantraniliprole through treated cotton leaves. In addition, we evaluated the residual activity of chlorantraniliprole to both *H. convergens* and *C. virescens* at different times following its application to cotton plants under greenhouse conditions.

### **Material and Methods**

**Insects.** A colony of *H. convergens* was established from ca. 50 adult beetles collected in organically grown kale fields located in Chã Grande, PE (08° 15' 14.4'' S and 35° 30' 0.3'' W). Larvae and pupae of the lepidopteran species *A. argillacea* were collected from cotton fields located in Chapadão do Sul, MS (52° 37' 22" W and 18° 47' 39" S), and pupae of *C. virescens* were ordered from a commercial insectary (Bug Agentes de Controle Biológico, Piracicaba, SP). All these insect populations were inferred to have a low chance of any previous exposure to chlorantraniliprole. Colonies of these insects were all reared separately in a climate-controlled room set to  $25 \pm 2$  °C, 60–70% RH, and a 13:11 (L:D) photoperiod, at the Laboratory of Biological Control of the 'Universidade Federal Rural de Pernambuco (UFRPE)', Recife, Pernambuco State, Brazil. All experiments were carried out in a climate-controlled growth chamber set to these same physical conditions.

***Hippodamia convergens.*** Mated females of *H. convergens* were held in transparent 1-L plastic containers (five per container) covered with screened lids for ventilation and filled with crumpled

paper towel for harborage and to serve as an oviposition substrate. Frozen eggs of the Mediterranean flour moth, *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) (ordered from Bug Agentes Biológicos, Piracicaba, SP, Brazil), were provided *ad libitum* as food, along with a 10% honey solution on a cotton wick, both refreshed every 48 h. Live prey in the form of either cotton aphids, *Aphis gossypii* Glover (Hemiptera: Aphididae), or psyllids, *Isogonoceraia* sp. (Hemiptera: Psyllidae) was provided to stimulate oviposition when a new beetle generation was needed. Eggs were harvested by transferring adult insects to fresh containers; a small amount of *A. kuehniella* eggs was added to containers of eggs as they neared eclosion. Three days after eclosion, larvae were transferred to 80 mL plastic containers (three per container) and fed frozen moth eggs *ad libitum* and refreshed every 48 h until pupation. Adults were used in bioassays when they were 10–13 days old.

***Alabama argillacea* and *Chloridea virescens*.** Plant food for larvae of both lepidopteran species was grown in a greenhouse. Cotton plants (var. BRS Rubi) were cultivated in 5-L pots filled with a mixture of clay soil, sand and humus (2:1:1), plus 10 g of fertilizer (N:P:K = 4:14:8). Adult moths of *A. argillacea* and *C. virescens* were kept in containers (ca. 25 per container) made with sections of PVC pipe (21 cm height x 14 cm diam) lined with a dot matrix paper (MaxPrint®, Tietê, SP, Brazil) that served as a substrate for oviposition. These containers were placed on a plastic plate (16 cm diam) lined with paper towel and were covered with an organdy mesh secured by a rubber band. Moths' hydration was guaranteed by providing a wet cotton wick over the organdy mesh, and both adult species fed a 10% honey solution *ad libitum*, refreshed every two days. At this time, adult insects were transferred to clean PVC containers and the papers bearing eggs were placed in transparent 1-L plastic containers. Young cotton leaves were provided as food to newly hatched larvae. Four days after eclosion, larvae of *A. argillacea* were transferred to 1-L plastic containers (ca. 50 per container) where they were fed young leaves of cotton until

pupation. Because larvae of *C. virescens* are cannibalistic, they were singly reared in 80 mL plastic containers and fed young cotton leaves, buds, and soft bolls until pupation. Early instars of both species were provided food *ad libitum* that was refreshed every two days, whereas food for older larvae was refreshed daily. All experiments were performed using 7 d-old larvae (late second to early third instar).

**Insecticide Rates.** We tested a range of concentrations of chlorantraniliprole (Premio<sup>®</sup>, 200 g a.i./L, Du Pont Brazil S.A., Barueri, SP, Brazil) to establish LCs approaching 0 and 100% for both adult lady beetles and lepidopteran larvae. Desired concentrations were prepared using distilled water containing 0.05% of the surfactant Will Fix<sup>®</sup> (Charmon Destyl Chemical Industry Ltda, Campinas, SP, Brazil), which alone served as the control treatment.

**Concentration-mortality Bioassay of *Hippodamia convergens*.** Given that arthropod predators may be exposed to insecticides in the field by topical contact with spray droplets, dried residues on plant surfaces, and/or ingestion of contaminated prey, we subjected adults of *H. convergens* to a range of chlorantraniliprole concentrations via topical application, freshly dried residues, and ingestion of treated moth eggs. For each route of exposure, 280 adult lady beetles of both sexes were divided equally among eight treatments: seven concentrations of chlorantraniliprole and a control group. Each treatment replication ( $n = 7$ ) comprised five adult beetles in a glass Petri dish (1.5 cm ht x 10 cm diam). Each Petri dish received a volume of 2 mL of control or insecticide solution applied with a Potter precision spray tower (Burkard Scientific, Uxbridge, UK) adjusted to 5 psi. Topical exposure was accomplished by spraying solutions directly on the adult beetles, residue exposure by placing beetles in treated glass Petri dishes (after air-drying), and ingestion by spraying moth eggs and then providing them as food. To ensure consumption of contaminated moth eggs, the beetles in this assay were starved for 24 h prior to testing.

In topical assays, concentrations of chlorantraniliprole varied from 250 to 2,700 mg a.i./L and Petri dishes were covered with a plastic screen (ca. 2 mm diam mesh) glued to a plastic frame (14 cm diam) to contain the lady beetles during the exposure period. After air-drying for one hour, beetles were transferred to clean Petri dishes. By comparison, concentrations ranged from 68 to 1,340 mg a.i./L in the dried residue assay, in which lids and bottoms of the Petri dishes were both sprayed with solution and left to air-dry for one hour before introduction of the beetles. After 24 h in both assays, frozen moth eggs were provided *ad libitum* for two days in a small plastic cup lid (ca. 3 cm diam) to prevent any contamination of the food by chlorantraniliprole residues on the surfaces of treated dishes.

Preliminary tests revealed that ca. 100 mg of frozen moth eggs was sufficient to feed five starved adults of *H. convergens* for 24 h. For the ingestion assay, contaminated moth eggs were prepared in the spray tower by applying solutions ranging from 37.5 to 1,200 mg a.i./L to approximately 250 mg of frozen moth eggs uniformly dispersed on the bottom of a glass Petri dish (10 cm diam). After drying for two hours at room temperature, the treated moth eggs were carefully dislodged with a soft hair brush and ca. 125 mg samples were weighed into small plastic cup lids (as previously described). Each replicate of the experiment received a single sample of the appropriate treatment in a clean glass Petri dish. After 24 h, survivors were transferred to fresh dishes and provisioned with untreated moth eggs *ad libitum* for another two days.

Lady beetles were tallied as dead if they failed to right themselves within five minutes after being placed upside down on their elytra. Lethal-concentration values were calculated from cumulative mortality data three days after exposure.

**Concentration-mortality Bioassay of Lepidopteran Larvae.** Chlorantraniliprole acts against chewing arthropod species via both ingestion and contact (Lahm *et al.* 2009). We cut a series of leaf discs (8.0 cm diam) from young cotton leaves, and rinsed them in a 1% hypochlorite solution



followed by tap water. After evaporating the excess of water, leaf discs were dipped for 10 sec into control or chlorantraniliprole solutions and left to air-dry on paper towel for one hour, whereupon they were transferred to glass Petri dishes lined with lightly moistened filter paper. For *A. argillacea*, each treatment (n = 8, including controls) consisted of nine replications (5 larvae per replication). Seven chlorantraniliprole concentrations were tested, ranging from 0.0002 to 0.2 mg a.i./L. In contrast, concentrations tested against *C. virescens* ranged from 0.014 to 10 mg a.i./L and each treatment had five replications composed of three to five larvae. Larvae of both species were held with their respective leaf discs for three days and then tallied as dead if they did not move a distance equal to their body length when prodded with a soft brush. Lethal concentrations were then calculated from the cumulative mortality data.

**Residual Toxicity of Chlorantraniliprole to *Chloridea virescens* and *Hippodamia convergens*.**

Chlorantraniliprole is reported to range from persistent to very persistent based on its degradation time (DT<sub>50</sub>) in aerobic soil under field and laboratory conditions (204 and 597 d, respectively), but its foliar persistence has not been reported (Pesticide Properties Database 2016), especially in regard to non-target species. We conducted an experiment to assess its residual toxicity to both *C. virescens* and *H. convergens* on leaves of cotton plants at different intervals after application in a greenhouse. We removed any sprouts that emerged after applications, and avoided watering the canopy of the plants to prevent washing the product off the leaves.

Preliminary work indicated that the FR of chlorantraniliprole against *C. virescens* killed all treated caterpillars, which prompted investigation of both its residual toxicity, and the relative effectiveness of the estimated LC<sub>90</sub>. Using a hand sprayer (Leve Ultrajet® Guarany, Itu, SP, Brazil), a chlorantraniliprole solution of 18 mg a.i./L (the upper limit of the LC<sub>90</sub> estimated for *C. virescens*, Table 1) was applied to cotton plants until runoff, while control plants received the surfactant solution alone. Solutions were sprayed on different dates so that residues of age 0 (ca. 2

h), 1, 2, 4, 8, and 16 days were obtained for testing. Leaves were harvested and a series of leaf discs (8 cm diam) were cut from each treatment and transferred to glass Petri dishes lined with lightly moistened filter paper. Each dish (replication) received five larvae. Depending upon the availability of larvae, treatments were established with either four (control and 0, 1 and 2 day intervals) or five replications (4, 8 and 16 day intervals), respectively. Larvae were held with their leaf discs for three days, whereupon mortality was assessed as previously described.

To assess the residual toxicity of chlorantraniliprole at FR to adults of *H. convergens*, we used similar procedures as those for *C. virescens* exposure. Treatments consisted of plants sprayed with only surfactant solution (controls), a chlorantraniliprole solution of 18 mg a.i./L (the LC<sub>90</sub> for *C. virescens*), and another solution of 200 mg a.i./L (the recommended FR against *C. virescens*). Cotton leaves were harvested at different ages post-treatment, specifically 0 (2 h), 1, 2, 4, 8, and 16 days, and all procedures followed as previously described, but using five replications with five to seven adult beetles in each. Insects were fed *ad libitum* on uncontaminated frozen moth eggs provided over the leaf discs, and numbers of survivors were recorded on the third day of exposure.

**Statistical Analysis.** Lethal-concentrations of chlorantraniliprole and their 95% confidence intervals (CIs) were estimated for all three species by Probit analysis (Finney 1971) using the Proc Probit (SAS Institute 2001). Tolerance ratios (TR<sub>50s</sub>) were calculated as proposed by Preetha *et al.* (2010) by dividing the lowest LC<sub>50</sub> of *H. convergens* by those calculated for *A. argillacea* and *C. virescens*, with values > 1 defined as selective. Unlike control treatments in the lethal-concentration tests where mortality was less than five percent, data from residual toxicity bioassays with *C. virescens* and *H. convergens* after 0, 4 and 8 days required correction for control mortality (Abbott 1925). Mortality data for *C. virescens* were analyzed by chi-square goodness of fit test (Proc Freq, SAS Institute 2001) at  $P < 0.05$ , with 90% as the expected

mortality (given exposure to dried residues of chlorantraniliprole at LC<sub>90</sub>). The percent mortality of *C. virescens* larvae was checked for normality and equality of variance using the Kolmogorov-Smirnov and Levene tests, respectively (Proc Univariate and Proc ANOVA, SAS institute 2001) before analysis by one-way ANOVA. Similarly, ANOVA assumptions were checked for *H. convergens* survival data which passed the tests and was subjected to two-way ANOVA to investigate the interaction between concentrations (18 mg a.i./L = upper limit of the calculated LC<sub>90</sub>, and 200 mg a.i./L = FR) and the period after application (0, 1, 2, 4, 8 and 16 days).

## Results

**Concentration-mortality Bioassay of *Hippodamia convergens*.** Chlorantraniliprole-induced mortality data for *H. convergens* fit the Probit model ( $P > 0.05$ ) for all three routes of exposure. The lowest LC values were obtained with dried residues on inert surfaces, and the highest values with topical application, with ingestion of contaminated moth eggs yielding intermediate values (Table 1). However, CI values obtained from dried residues overlapped those from ingestion of contaminated food, suggesting that these routes of exposure may result in comparable levels of toxicity to *H. convergens*, an inference supported by convergence of the lethal-concentration lines (Fig. 1). Based on the LC<sub>50</sub> for adults of *H. convergens* exposed to dried residues (most toxic route), the TR<sub>50</sub> value indicated that chlorantraniliprole is approximately 556-fold more toxic to *C. virescens* larvae when fed similarly treated cotton leaves (Fig. 2). The tolerance ratio was far greater when the LC<sub>50</sub> for *A. argillacea* was used, ca. 30,600-fold.

**Concentration-mortality Bioassay of Lepidopteran Larvae.** Mortality data of the lepidopteran species exposed to chlorantraniliprole-treated leaves also fit the Probit model ( $P > 0.05$ ). The estimated LC<sub>50</sub> for *A. argillacea* was 55-fold lower than that calculated for *C. virescens* (Table 1), a difference in susceptibility also reflected in the lethal-concentration lines (Fig. 2). The currently

recommended FRs against *A. argillacea* and *C. virescens* in Brazil are almost 175- and 11-fold greater than the upper limits of their LC<sub>90</sub>s, respectively, estimated in these experiments.

### **Residual Toxicity of Chlorantraniliprole to *Chloridea virescens* and *Hippodamia convergens*.**

Residues of chlorantraniliprole applied at the upper limit of the LC<sub>90</sub> estimated for *C. virescens* caused larval mortality of this species above 90% as expected ( $\chi^2 = 0.06$ ,  $df = 5$ ,  $P = 1.0$ ), regardless of whether the insecticide solution was applied 2 h or 16 days prior to exposure. Average mortality did not differ significantly among periods after application ( $F_{5, 21} = 0.62$ ,  $P = 0.69$ ), and ranged from  $91.6 \pm 5.16\%$  to 100%.

Survival of *H. convergens* was not affected by the period since insecticide application ( $F_{5, 58} = 1.31$ ,  $P = 0.28$ ), nor did the time since treatment interact with chlorantraniliprole concentration ( $F_{5, 58} = 1.21$ ,  $P = 0.32$ ). However, beetles exposed to residues at the upper limit of the LC<sub>90</sub> calculated for *C. virescens* survived significantly better than did those exposed to the FR ( $91.7 \pm 2.19\%$  vs  $67.9 \pm 6.04\%$ ;  $F_{1, 58} = 14.21$ ,  $P < 0.001$ ).

## **Discussion**

This is perhaps the first effort to estimate lethal concentrations of chlorantraniliprole for a key natural enemy in different routes of exposure while simultaneously evaluating its relative toxicity to target species. Regardless of the exposure route, lethal concentrations of chlorantraniliprole that killed ca. 50% of *H. convergens* adults were substantially higher than those required to cause similar mortality of *A. argillacea* and *C. virescens* larvae when applied to cotton leaf discs. These results would permit classification of this material as potentially harmless to adult *H. convergens*. By comparison, the LC<sub>50</sub> for the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) is ca. 7.0 mg a.i./L for adults, also obtained by the leaf dipping method (Jiang *et al.* 2012). Notably, this value is higher than the largest values

obtained for *Plutella xylostella* L. (Lepidoptera: Plutellidae) (Silva *et al.* 2012) and *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Campos *et al.* 2015, Silva *et al.* 2016), which may reflect a higher toxicity of chlorantraniliprole to lepidopteran compared to coleopteran species.

Previous work has found chlorantraniliprole to be compatible with the predatory bugs *Podisus nigrispinus* (Dallas) and *Supputius cincticeps* (Stal) (Hemiptera: Pentatomidae). Mortality was  $\leq 10\%$  even at concentrations 10-fold the FR, whereas the  $LC_{50}$  for the target pest, *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), was approximately 23- and 33-fold lower than those obtained with methamidophos and deltamethrin, respectively (Castro *et al.* 2013). Given that lepidopteran species are primary targets of chlorantraniliprole, and appear significantly more susceptible (lower  $LC_{50}$ s) than their coleopteran and hemipteran predators, it could be argued that this insecticide has a degree of physiological selectivity.

One might speculate that the lower toxicity of chlorantraniliprole to the lady beetle compared to the lepidopterans results from differences in the structure and function of their RyRs, but this cannot be confirmed without data on the  $Ca^{2+}$  response kinetics of these species. Our knowledge about insect RyR has grown substantially since it was first characterized in *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) (Takeshima *et al.* 1994), and differences in amino acid sequences among and within insect groups have been recognized. For example, amino acid homology between the RyR domains of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) and three other lepidopteran species was  $\geq 93\%$ , whereas homology with *D. melanogaster* ranged from 70 to 88% (Wang *et al.* 2013). Similarly, a phylogenetic analysis of RyR from *L. decemlineata* resulted in coleopterans, lepidopterans, dipterans, hemipterans and hymenopterans all clustering in different clades (Wan *et al.* 2014), indicating that proteins encoded by the RyR gene vary among insect orders. The membrane-spanning domain of the RyR in *P. xylostella* was suggested as a probe binding site for chlorantraniliprole and its derivatives (Lou *et al.* 2014),

whereas studies using specific radioligands demonstrated that anthranilic and phthalic diamides probably share a common binding site on the RyR of *C. virescens* (Qi & Casida 2013, Qi *et al.* 2014). Based on these findings, we suspect that the observed differences between *H. convergens* and the lepidopteran species probably reflect different amino acid sequences in the RyRs of these species that affect the physical configuration of their binding sites, thus resulting in differential susceptibility to diamide insecticides.

The susceptibility of *H. convergens* to chlorantraniliprole varied significantly with route of exposure. Apart from the small plastic lid holding moth eggs, insects in the residue assays had no clean surfaces on which to rest and exhibited similar survival to those fed contaminated food. Adults groom frequently and may have ingested insecticide residues in the course of this behavior. In contrast, beetles receiving topical applications received only a single, fine spray of insecticide droplets on their dorsal surface, which dried in a few minutes, a factor that likely enabled their survival of higher concentrations.

The current FR of chlorantraniliprole against *A. argillacea* in Brazil appears compatible with *H. convergens*, but that recommended against *C. virescens* exceeds both the residual and oral LC<sub>50</sub>s, indicating a risk to this key predator. The FRs against *A. argillacea* and *C. virescens* in Brazilian cotton (AGROFIT 2016) are 380- and 37-fold higher than their respective LC<sub>90</sub>s as estimated by the leaf dipping method. These discrepancies between FRs and the LCs for the two pest species were not unexpected. For another example, the FR against *T. absoluta*, an important pest of tomatoes in South America that is now invasive in Europe, vary from 3 to 40 mg a.i./L, but the highest LC<sub>99</sub> measured in eight different populations was only 0.84 mg a.i./L (Campos *et al.* 2015). Similarly, the recommended rates of chlorantraniliprole against *H. armigera* in Australian cotton fields are 315 and 525 mg a.i./L (APVMA 2008), whereas pooled data from 21 field populations of the pest converge on an LC<sub>99.9</sub> of 0.45 mg a.i./L (Bird 2015). Collectively, these

studies and our data illustrate the high toxicity of chlorantraniliprole to lepidopteran species, leading us to conclude that current FRs in Brazilian cotton should provide adequate control of *A. argillacea* and *C. virescens*.

We found that chlorantraniliprole sprayed on cotton leaves retained residual toxicity for 16 days in a greenhouse, as confirmed by mortality of over 90% of *C. virescens* larvae exposed to the estimated LC<sub>90</sub> rate, regardless the period since application. This result was expected since foliar applications under field conditions have provided excellent control of *Cydia pomonella* L. (Lepidoptera: Tortricidae) and *L. decemlineata* after 14 and 22 days, respectively (Bassi *et al.* 2009), whereas soybean seeds treated with diamides reduced survivorship of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) for up to 51 days after planting (Thrash *et al.* 2013). According to Lahm *et al.* (2009), the movement of diamides into leaf tissues may prevent or delay their degradation due to rain events or photo-oxidation, thus preserving good residual activity. This is consistent with the results of Roubos *et al.* (2014), who found that chlorantraniliprole applied on glass Petri dishes and aged for up to 14 days under greenhouse conditions exhibited low toxicity to *H. convergens*, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) and *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae), whereas pyrethroid and organophosphate materials remained harmful over all application periods. In the present study, *H. convergens* adults exposed to chlorantraniliprole leaf residues at the LC<sub>90</sub> of *C. virescens* averaged 24% better survival than those contacting residues at the FR, indicating that excessive FRs may compromise the desired compatibility of chlorantraniliprole with this lady beetle.

In conclusion, our results demonstrated that *H. convergens* is less susceptible to chlorantraniliprole than either *A. argillacea* or *C. virescens*, the primary targets of diamides in cotton. However, the rates of chlorantraniliprole labelled for both lepidopteran species are several times higher than their LC<sub>90</sub>s, and that recommended against *C. virescens* may be detrimental to

*H. convergens* when beetles contact residues or ingest contaminated food. In addition to its low impact on *H. convergens* at carefully selected concentrations, chlorantraniliprole showed good residual activity against *C. virescens* for up to 16 days under greenhouse conditions, indicating that this insecticide may be simultaneously effective against target pests and compatible with beneficial species, a desirable feature for integrated pest management in cotton.

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Table 1. Mean ( $\pm$  SE) relative susceptibility of *Hippodamia convergens* adults to chlorantraniliprole in three routes of exposure, and 7-day old larvae of *Alabama argillacea* and *Chloridea virescens* exposed to treated cotton leaf discs. CI<sub>95</sub> = 95% confidence interval. The Chi-squared test ( $\chi^2$ ) was used to test goodness-of-fit to the Probit model.

Species (exposure route)	N	df	Slope $\pm$ SE	LC <sub>50</sub> <sup>a</sup> (95% CI)	LC <sub>90</sub> <sup>a</sup> (95% CI)	$\chi^2$ , P-value
<i>H. convergens</i> (topical)	245	5	3.34 $\pm$ 0.36	706.2 (608.6 – 808.6)	1,710 (1,430 – 2,188)	2.96 <sup>0.706</sup>
<i>H. convergens</i> (ingestion)	245	5	1.82 $\pm$ 0.20	198.7 (154.6 – 251.0)	1,010 (721.4 – 1,629)	7.54 <sup>0.183</sup>
<i>H. convergens</i> (dried residues)	245	5	1.72 $\pm$ 0.23	153.0 (108.8 – 197.9)	852.1 (613.3 – 1,409)	3.15 <sup>0.677</sup>
<i>A. argillacea</i> (leaf disc)	315	5	1.03 $\pm$ 0.10	0.005 (0.003 – 0.007)	0.087 (0.049 – 0.189)	7.32 <sup>0.198</sup>
<i>C. virescens</i> (leaf disc)	161	5	0.99 $\pm$ 0.14	0.275 (0.158 – 0.470)	5.452 (2.521 – 18.650)	0.98 <sup>0.975</sup>

<sup>a</sup> Estimated concentration of chlorantraniliprole (mg a.i./L) required to kill 50% or 90% of treated insects.

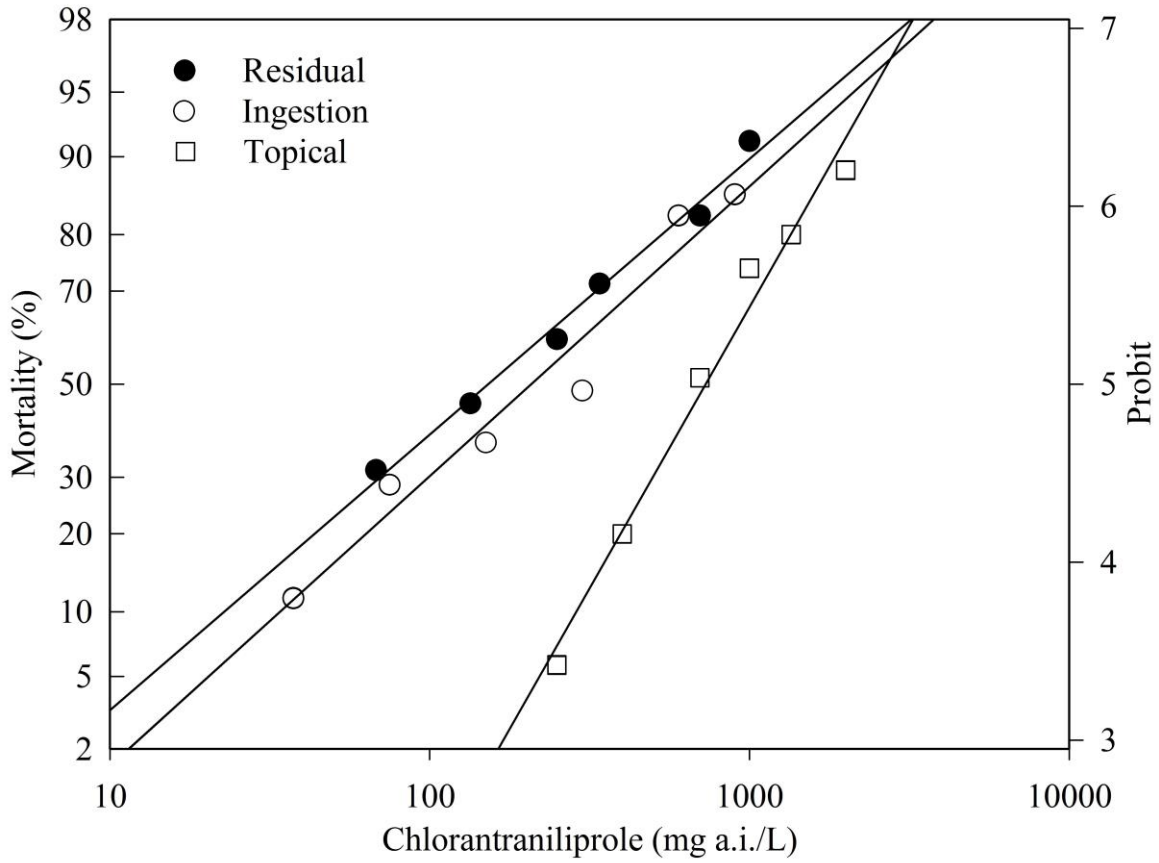


Figure 1. Lethal-concentration lines for a population of *Hippodamia convergens* exposed to a range of chlorantraniliprole concentrations (expressed on a log scale) via dried residues on inert surfaces, ingestion of contaminated moth eggs, or topical application.

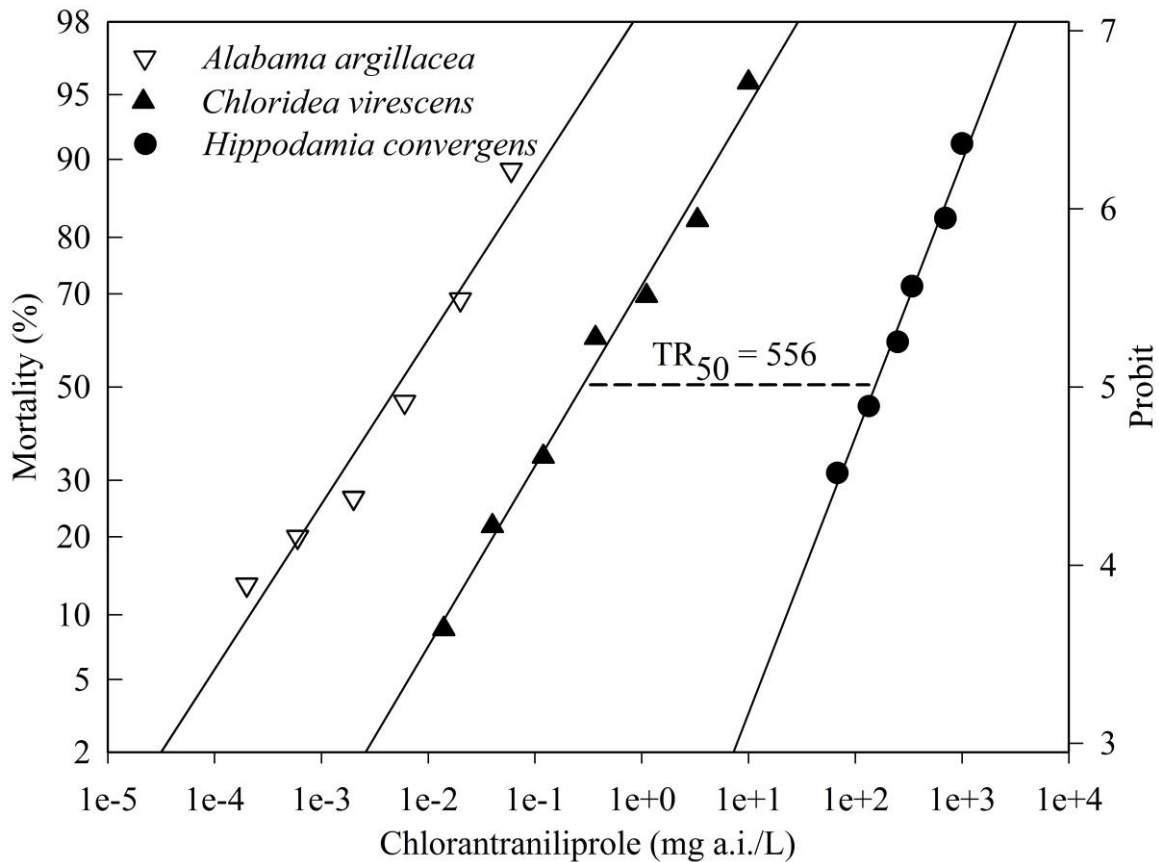


Figure 2. Lethal-concentration lines for *Alabama argillacea* and *Chloridea virescens* exposed to a range of chlorantraniliprole concentrations (expressed on a log scale) via the leaf dipping method. The lethal-concentration line for *Hippodamia convergens* exposed to dried residues on inert surfaces (its most toxic form of exposure) is included to depict the lower susceptibility of this species compared to the lepidopterans. The tolerance ratio ( $TR_{50}$ ) was calculated by dividing the *H. convergens*  $LC_{50}$  by that estimated for *C. virescens*, the least susceptible herbivore.

## CHAPTER 3

### DUAL RESISTANCE TO LAMBDA-CYHALOTHRIN AND DICROTOPHOS IN *Hippodamia convergens* (Guérin-Méneville) (COLEOPTERA: COCCINELLIDAE)<sup>1</sup>

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<sup>1</sup>Barbosa, P.R.R., J.P. Michaud, A.R.S. Rodrigues and J.B. Torres. 2016. Dual resistance to lambda-cyhalothrin and dicrotophos in *Hippodamia convergens* (Coleoptera: Coccinellidae). *Chemosphere* 159: 1-9.



ABSTRACT – Insecticide resistance is usually associated with pests, but may also evolve in natural enemies. In this study, adult beetles of three distinct North American populations of *Hippodamia convergens* (Guérin-Méneville), and the progeny of reciprocal crosses between the resistant and most susceptible population, were treated topically with varying concentrations of lambda-cyhalothrin and dicotophos. In addition, the LD<sub>50</sub> of both insecticides were applied in combination to resistant individuals. The developmental and reproductive performance of each population was assessed in the absence of insecticide exposure to compare baseline fitness. California and Kansas populations were susceptible to both materials, whereas Georgia (GA) beetles exhibited a resistance ratio (RR<sub>50</sub>) of 158 to lambda-cyhalothrin and 530 to dicotophos. Inheritance of lambda-cyhalothrin resistance was linked to female, whereas inheritance of dicotophos resistance was autosomal. Mortality of resistant beetles treated with a mixture of LD<sub>50</sub>s of both materials was twice that of those treated with lambda-cyhalothrin alone, but not significantly different from those receiving dicotophos alone. Life history parameters were largely similar among populations, except that Georgia beetles had higher egg fertility relative to susceptible populations. We conclude that the high levels of resistance to lambda-cyhalothrin and dicotophos in Georgia beetles reflect heavy loads of these insecticides in local environments, most likely the large acreage under intensive cotton cultivation.

KEY WORDS: Convergent lady beetle, mode of inheritance, local adaptation, organophosphates, pyrethroids

## RESISTÊNCIA À LAMBDA-CIALOTRINA E DICROTOFÓS EM *Hippodamia convergens*

(Guérin-Méneville) (COLEOPTERA: COCCINELLIDAE)

RESUMO – Embora também ocorra em inimigos naturais, a resistência a inseticidas é mais frequentemente documentada a espécies praga. No presente estudo, adultos de três populações norte americanas de *Hippodamia convergens* (Guérin-Méneville), bem como as progênies de cruzamentos recíprocos entre a população mais resistente e a mais suscetível, foram tratadas topicamente com várias concentrações de lambda-cialotrina e dicrotofós. Também aplicamos uma mistura das  $DL_{50}$  de cada inseticida em indivíduos da população resistente e acompanhamos o desenvolvimento e performance reprodutiva das três populações na ausência de exposição a inseticidas para averiguar possível custo adaptativo. As populações da Califórnia e do Kansas foram suscetíveis a ambos os inseticidas, enquanto as joaninhas provenientes da Geórgia (GA) apresentaram razão de resistência ( $RR_{50}$ ) de 158 e 530 vezes à lambda-cialotrina e dicrotofós, respectivamente. A herança da resistência à lambda-cialotrina foi ligada a fêmea, enquanto a resistência ao dicrotofós revelou-se autossomal. A mortalidade de joaninhas resistentes tratadas com a mistura das  $DL_{50}$ s de ambos os produtos revelou-se duas vezes maior que a observada no controle apenas com lambda-cialotrina, mas foi semelhante àquelas tratadas apenas com dicrotofós. Não houve grandes diferenças em relação aos parâmetros biológicos avaliados, exceção à viabilidade de ovos que foi maior na população resistente comparada às suscetíveis. Concluimos que os altos níveis de resistência à lambda-cialotrina e dicrotofós na população de *H. convergens* da Geórgia reflete a massiva utilização desses inseticidas nessa região, muito provavelmente nas grandes áreas sob cultivo intensivo de algodão.

PALAVRAS CHAVE: Coccinelídeos predadores, herdabilidade, adaptação local, organofosforados, piretorides

## Introduction

There exists a need to address the impact of pesticides, not only on target pests, but also on non-target arthropods and natural enemies, i.e., parasitoids and predators (Croft & Brown 1975, Croft 1990, Cloyd & Dickinson 2006, Biondi *et al.* 2012). The agronomic value of many insecticides has been diminished due to resistance evolution in numerous pest species (Elzen *et al.* 1992, Jiang *et al.* 2011, Whalon *et al.* 2015). However, it has also become evident that certain natural enemy populations, given repeated exposure to pesticides, can evolve resistance to them in much the same manner as the pests themselves (Croft & Morse 1979, Pree *et al.* 1989, Pathan *et al.* 2008, Rodrigues *et al.* 2013a). The evolution of pesticide resistance can be influenced by many intrinsic factors, such as genetic makeup of the species, metabolic physiology, and behavior patterns, in addition to extrinsic 'operational factors' that relate to pesticide properties, their application frequency and coverage (WHO 1957, Georghiou & Taylor 1977, Forgash 1984, Rosenheim & Tabashnik 1990).

Herbivores are products of an evolutionary 'arms race' with plants; the latter have responded to herbivory by evolving a diversity of physical and chemical defenses, while the former have evolved a variety of detoxifying enzymes and other counter-adaptations to overcome them (Levin 1976, Bowers 1992). By contrast, arthropod predators have faced lower selective pressures to tolerate or detoxify harmful substances in their food supply. The evolution of resistance by members of the third trophic level is further challenged by the fact that any survivors of pesticide applications typically face a scarcity of prey, which may drive them to migration or local extinction (Croft & Brown 1975). Furthermore, the rotation of insecticides with differing modes of action, an approach conventionally recommended for delaying the evolution of resistance in pest populations, may also serve to weaken directional selection for pesticide resistance in natural enemy populations (Georghiou 1972, Pree *et al.* 1989). Collectively, these factors may explain the

low numbers of resistant natural enemies (~ 50) included in an ever-growing database of arthropod species resistant to one or more pesticides (~ 600, Whalon *et al.* 2015).

It is therefore of interest why certain groups of predators appear less susceptible to pesticides than most parasitoids (Theiling & Croft 1988, Tillman & Mulrooney 2000, Williams *et al.* 2003), their prey (Croft & Brown 1975, Gesraha 2007), or even key pest species (Spíndola *et al.* 2013). For example, species of predatory Coccinellidae appear reasonably tolerant to, or compatible with, a variety of pesticides when these are used responsibly in integrated pest management programs (Michaud 2012). There are also notable examples of resistance, such as populations of *Coleomegilla maculata* (DeGeer) that exhibit resistance to both DDT and organophosphates (Atallah & Nettles 1966, Head *et al.* 1977). Similarly, pyrethroid resistance has been reported in populations of *Stethorus gilvifrons* Mulsant (Kumral *et al.* 2011), *Eriopis connexa* (Germar) and *Hippodamia convergens* (Guérin-Méneville) (Rodrigues *et al.* 2013a, 2013b).

*Hippodamia convergens* is a cosmopolitan species that exploits a wide range of aphid prey on graminaceous and herbaceous plants. It is widely recognized as an important biological control agent of aphids in many agroecosystems including various cereal crops (Rice & Wilde 1988, Michaud & Qureshi 2006), cotton (Whitcomb & Bell 1964) and cruciferous vegetables (Rodrigues *et al.* 2013b). Pesticide use regimes vary considerably among these crops, partly as a function of their relative value. For example, low value cereal crops such as wheat and sorghum may be grown for many crop cycles on the High Plains of the USA without receiving a single insecticide application, not because pests are completely absent, but because background biological control is generally sufficient and economic thresholds for treatment are high. On the other hand, the broad diversity of agricultural crops grown in the central valleys of California renders it difficult to infer insecticide exposure history for those *H. convergens* populations that

are routinely harvested in large numbers from their overwintering aggregations in the Sierra Nevada Mountains, and subsequently sold across the rest of North America. Although these beetles migrate from summer feeding sites to overwintering sites within California, the Rocky Mountains effectively isolate these populations from gene flow from central and eastern North America, even though their own genetics are being regularly introduced to disparate geographic locations (Obrycki *et al.* 2001), along with the parasitoids and diseases that infect them (Bjornson 2008). In contrast, cotton and other crops (tobacco, soybean, etc) in southeastern USA have historically received frequent insecticide applications. For instance, cotton fields have experienced average reduction in insecticide applications from 14.4 to about three following the successful boll weevil eradication program and the widespread adoption of *Bt*-cotton (Haney *et al.* 2009, Roberts 2015). It follows that populations of *H. convergens* inhabiting these different cropping systems have radically different histories of pesticide exposure, both quantitatively and qualitatively.

Despite the efficacy of plant-incorporated *Bt* traits against many lepidopteran species, *Bt*-crops are not immune to damage by other herbivores and provide no protection against sucking pests (Torres *et al.* 2009). For example, various stink bug species have emerged as important pests of cotton, soybean and other crops (Pannizi 2015) and their control often requires applications of broad-spectrum insecticides, or combinations thereof (Greene *et al.* 2001, Snodgrass *et al.* 2005, Roberts 2015, Roberts & Toews 2015). Despite the emergence of pyrethroid and OP resistance in many arthropod species (Plapp *et al.* 1990, Jiang *et al.* 2011, Karatolos *et al.* 2012, Bass *et al.* 2014, Whalon *et al.* 2015), these compounds retain efficacy against many pests and can be preferred because of their low cost.

Using collections from cotton fields in Mississippi, Tillman & Mulrooney (2000) reported 77-95.0% survival of *H. convergens* when adults were exposed to the recommended field rate of

lambda-cyhalothrin in residual and topical bioassays. Likewise, Torres & Ruberson (2005a, 2005b) observed good survival of *H. convergens* in Georgia cotton fields following lambda-cyhalothrin applications and high levels of resistance were confirmed in subsequent laboratory assays (Ruberson *et al.* 2007, Rodrigues *et al.* 2013a). In addition, Georgia *H. convergens* survived high rates of dicotophos (ARSR, personal communication). This and other organophosphate materials have a history of use in cotton going back to the 1950s, when boll weevil resistance to DDT forced a shift to materials with other modes of action (Anonymous 1958).

The present study was conducted to compare levels of pyrethroid and OP resistance among three disparate *H. convergens* populations with presumably different histories of insecticide exposure: a population from central Kansas assumed to have a history of minimal exposure, a commercially-sourced population from central California with unknown exposure history, and a population from a cotton-growing region in Georgia assumed to have a history of long-term exposure to both pyrethroids and OPs. We hypothesized that heavy insecticide exposure would be a regionally-specific selective force driving the evolution of resistance in local populations of *H. convergens*. We also performed reciprocal crosses between two of these populations to assess their genetic compatibility, and the mode of resistance inheritance. Insecticide formulations combining both pyrethroids and OPs have been used to manage resistance in pest populations (Martin *et al.* 2003, Ahmad *et al.* 2009). Thus, we also evaluated the susceptibility of resistant Georgia beetles to a mixture of lambda-cyhalothrin and dicotophos, both at their LD<sub>50</sub>. Finally, we compared the life histories of the three populations under standardized laboratory conditions, in the absence of insecticide exposure, in order to observe any baseline differences that might reflect physiological costs of insecticide resistance.

## Material and Methods

**Insects.** Three populations of *H. convergens* were established in the laboratory at the Agricultural Research Center - Hays in April and May of 2015. The first population (hereafter 'KS'), was established from adult beetles collected from wheat fields in Hays, Kansas, USA (38° 51' 31.14" N, 99° 20' 10.86" W). The second one (hereafter 'CA'), was established from adults purchased from Arbico Organics® (Oro Valley, AZ), that collected them from overwintering aggregations in the foothills of Sierra Nevada mountains, California, USA. The third (hereafter 'GA'), was established from adult beetles collected in crimson clover in Tifton County, Georgia, USA (31° 30' 38.99" N, 83° 32' 44.92" W). Prior to breeding or use in experiments, beetles were maintained in reproductive diapause by holding them in 1-L glass mason jars covered with organdy mesh, where they were provisioned with frozen eggs of *Ephestia kuehniella* (Zeller) with water provided on a cotton wick. The jars were held in growth chambers set to  $19 \pm 1$  °C, 50-60% RH, and a 16:8 L:D daylength. During all experiments, beetles were held in a chamber set to  $23 \pm 1$  °C, 50-60% RH, and a 16:8 L:D daylength.

A colony of sugarcane aphids, *Melanaphis sacchari* (Gehntner) (Hemiptera: Aphididae), was initiated from material collected in Matagorda County, TX, and maintained on sorghum seedlings grown in metal trays in a greenhouse. The rationale for use of this aphid prey was two-fold. First, we knew from previous work that this aphid was an adequate and complete diet for KS *H. convergens*, supporting both development and reproduction. Second, the aphid constituted an equally novel prey for all three populations, as *M. sacchari* was not yet present in any of the collection areas at the time. Thus, it was considered a 'neutral' diet, as opposed to an aphid, which might be usual prey for one population, but not the others. Aphids were reared in a growth

chamber set to  $23 \pm 1$  °C under continuous light. Twice weekly, new trays of sorghum were introduced into the chamber and infested clippings placed on the plants.

Adult beetles of the KS and GA populations were in reproductive diapause at the time of collection, and were each transferred directly to a 1-L glass Mason jar, about 150 beetles in each, filled with shredded wax paper for harborage, and covered with organdy mesh. Water was provided on a cotton wick within each jar and the beetles were fed ca. 100 mg of frozen eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) (Beneficial Insectary, Oak Run, CA), twice weekly, supplemented with about 50 mg of palm pollen. Under such crowded conditions with limited food, *H. convergens* can be held in reproductive diapause for several months (Michaud & Qureshi 2006). Adults of the CA population were overwintered beetles of limited reproductive potential and lifespan, so they were observed for a period of five days to isolate any individuals parasitized, whereupon a series of ca. 30 healthy females were isolated in plastic Petri dishes (5.5 cm diameter) and provisioned with *M. sacchari* 'ad libitum' on the excised leaves of their host plants to induce oviposition.

Whenever insects were required for experiments, a series of twenty females was removed from a jar and induced to oviposit as described above. Eggs were collected daily by transferring the female to a new dish. Hatching larvae were reared in the same Petri dishes, five per dish, and provisioned daily with frozen eggs of *E. kuehniella* with water provided on a small cube of sponge. Adult individuals from the first (F1) or second (F2) laboratory generation were used in all experiments, except for the bioassay of simultaneous exposure to both insecticides, which utilized adults from the fourth (F4) generation.

**Insecticides.** The insecticides selected for testing represented two of the most widely used insecticide groups in agriculture, pyrethroids [ $\lambda$ -cyhalothrin, 22.8% a.i., Syngenta Crop Protection, Greensboro, NC (Warrior II®)], and organophosphates [dicotophos, 83.8% a.i.,



AMVAC Chemical Corporation, Los Angeles, CA (Bidrin 8<sup>®</sup>)]. All insecticide doses were prepared using a solution of distilled water with 0.05% of the surfactant Activator 90<sup>®</sup> (Loveland Products, Greeley, CO).

**Dose-mortality Response.** Dosing of adult beetles was accomplished by using a Hamilton<sup>®</sup> syringe (25  $\mu$ L volume), to apply a 0.5  $\mu$ L droplet of the appropriate solution to the ventral surface of the abdomen part of the insects. An initial screening test was conducted in which both insecticides were tested against each population. Materials were diluted according to the highest recommended field rate for cotton in Georgia (lambda-cyhalothrin: ca. 0.46 g a.i./L, dicrotophos: 5.13 g a.i./L), assuming application in 100 L of water per hectare (Roberts & Toews 2015).

Following the initial screening test, we conducted bioassays with several concentrations of both insecticides in order to define the range of doses which resulted in mortality approaching 0% and 100% in each population. From those bioassay results, a set of 6-7 concentrations of lambda-cyhalothrin and dicrotophos was selected to estimate the dose-mortality response curves, and the lethal doses (LDs) for each insecticide. The lambda-cyhalothrin doses varied from 0.0003 to 0.07  $\mu$ g a.i./insect for the CA population, from 0.005 to 0.18  $\mu$ g a.i./insect for the KS population, and from 0.63 to 4.79  $\mu$ g a.i./insect for the GA population. In comparison, dicrotophos concentrations ranged from 0.0105 to 7.69  $\mu$ g a.i./insect for CA, from 0.065 to 15.37  $\mu$ g a.i./insect for KS, and from 38.44 to 166.56  $\mu$ g a.i./insect for GA. A 0.05% aqueous solution of the surfactant was used as a control. At least 25 adult beetles (7-10 d old) were treated with each concentration.

Following treatment, beetles were placed into plastic Petri dishes (as above, 5 per dish), provisioned with a 10% honey solution soaked in a cube of cotton wick (1 cm diam per 1 cm high), and transferred to a growth chamber under the same physical conditions previously described. The mortality was assessed 24 h later each insecticide application, when the beetles

were tallied as dead if they were unable to right themselves and walk within ca. five minutes after being placed upside down on their elytra.

**Inheritance of Resistance.** An experiment was conducted to verify whether the resistance to lambda-cyhalothrin and dicotophos observed in the GA population was autosomal or sex-linked, and also to determine the degree of dominance of the resistance trait. Since dose-mortality results indicated that the CA population was the most susceptible and the GA population the most resistant, reciprocal crosses were made between F1 virgin females (n = 20) and males (n = 20) to yield SR progeny (CA♀ x GA♂) and RS progeny (GA♀ x CA♂). All couples were fed 'ad libitum' *M. sacchari*, and their eggs collected for ca. 15 days accomplishing 400 neonate larvae from each cross (SR and RS). Larvae were reared (5 per dish) until they emerged as adults and, following application of insecticides, mortality was evaluated in the same manner as the previous experiment. New dose-mortality curves and new LDs were determined for each progeny type depending on the insecticide applied. Based on preliminary tests, six concentrations of each insecticide were used, with a 0.05% aqueous solution of surfactant as the control treatment. The dosages of lambda-cyhalothrin ranged from 0.0025 to 0.25 µg a.i./insect for SR progeny, and from 0.045 to 2.0 µg a.i./insect for RS. Concentrations of dicotophos ranging from 0.21 and 150 µg a.i./insect were used for both progeny types. A minimum of 20 beetles were treated per concentration, per cross.

**Simultaneous Exposure of the Resistant Population to Both Insecticides.** We selected a concentration of each insecticide that corresponded to the LD<sub>50</sub> for the GA population, 1.03 µg a.i./insect for lambda-cyhalothrin and 108.35 µg a.i./insect for dicotophos. The materials were topically applied on adult beetles (7-10 d old), as described above, and comprising three insecticide treatments: each insecticide alone or in combination, plus one control treatment. The

insecticides were mixed using a ratio ca. 1:105 of lambda-cyhalothrin to dicotophos. At the time of the trial, the GA population of *H. convergens* was in its fourth generation in the laboratory, with each generation exposed to the pre-determined LD<sub>50</sub> concentration of each insecticide to maintain selective pressure on the laboratory generations. Fifty-two adult beetles (n = 10 replicates of 5-6 beetles per replicate) were treated with each insecticide solution, while 42 beetles (n = 10 replicates of 4-5 beetles per replicate) were treated with a 0.05% aqueous solution of the surfactant as controls. Application and evaluation procedures followed the same methodology as in the previous trials.

**Comparative Life-Histories of Susceptible and Resistant Populations.** An additional experiment was performed, under the same physical conditions as previous experiments, to compare the immature development and adult reproductive performance of susceptible and resistant *H. convergens* populations without insecticide exposure. Couples (n = 20 per treatment) were established from the first laboratory generation in plastic Petri dishes (9 cm diam) after beetles (< 24 h old) were weighed individually on an analytical balance (Mettler Toledo, AG285). Couples were held together for the duration of the experiment and fed *M. sacchari* 'ad libitum'. Petri dishes were inspected twice daily for egg clusters, which were harvested by transferring the couple to a fresh Petri dish. The preoviposition period was determined as the number of days from adult emergence until first oviposition, and fecundity and egg viability were tallied for the next 30 days. The incubation period was estimated as the average number of days from oviposition to eclosion for the first 10 egg clusters laid by each female.

Eighty larvae from each population were reared out to determine developmental times, the fresh mass of male and female adults, and the sex ratio. Four neonate larvae (< 24 h old) from the second egg cluster of each female in all treatments were isolated in plastic Petri dishes (5.5 cm diam) and provisioned with frozen *E. kuehniella* eggs and a wet sponge cube, both refreshed

every two days. Larval developmental time was tallied as the period from eclosion to pupation, and pupation time as the period from pupal formation until adult emergence. Residual moth eggs were removed from each Petri dish at pupation to prevent any consumption by emerging adults. Adults were separated by gender and weighed within 24 h of emergence. Immature survival was tallied as the percentage of neonate larvae that successfully emerged as adults, and sex ratio as percentage female.

**Statistical Analysis.** Data from dose-mortality experiments were subjected to Probit analysis (Finney 1971) at  $P > 0.05$  and a model fit using the software package POLO-Plus 2.0 (LeOra Software 2005). The population yielding the lowest LDs was considered susceptible for purposes of comparisons with others and with F1 progeny from reciprocal crosses. Resistance ratios (RRs) and their 95% confidence intervals (CIs) were calculated as proposed by Robertson & Preisler (1992), and were considered statistically significant when the CI did not include the value 1.0 (Robertson *et al.* 2007). Data from SR and RS progeny were subjected to parallelism and equality tests at  $P > 0.05$ , and their estimated LDs were used to verify sex-linkage or autosomal inheritance of resistance (Robertson *et al.* 2007). When the CI of the RR between heterozygotes included the value 1.0, resistance was inferred to be autosomal, otherwise it was inferred to be sex-linked. Degrees of dominance (DD) were determined according to Stone (1968) and standard errors calculated using the procedure of Lehmann (1966) and compared by superposition (Preisler *et al.* 1990).

Mortality data from the simultaneous exposure experiment were analyzed by Chi-square, Goodness of fit test (PROC FREQ, SAS Institute 2001) with 50% mortality as the expected value (given application of LD<sub>50</sub>s). These data, and those from life history comparisons, were checked for normality and homoscedasticity of variance using the Kolmogorov-Smirnov and Levene tests, respectively (PROC UNIVARIATE, SAS Institute 2001), prior to analysis by one-way ANOVA.

Only egg viability and immature survival data required  $\log(x + 1)$  transformation to fit the assumptions of ANOVA; untransformed means are presented in tables. Means were separated by the Bonferroni test when more than three treatments were compared, and sex ratio was analyzed by Chi-square test.

## Results

**Dose-mortality Responses.** Mortality in all control groups was below five percent, ruling out any need to correct for natural mortality (Abbott 1925). The responses of all populations and crosses to both insecticides fit to the Probit model ( $P > 0.05$ ). Lethal doses (LD<sub>50</sub>s and LD<sub>90</sub>s) were all highest for the GA population, intermediate for the KS population, and lowest for the CA population (Tables 1 and 2). Thus, the CA population was considered susceptible for purposes of comparison and for reciprocal crosses with the GA population. All RRs were statistically significant, except for the RR<sub>90</sub> based on the LD<sub>90</sub> of the KS population treated with lambda-cyhalothrin (Table 1). The slopes of the dose-mortality curves of F1 reciprocal crosses treated with lambda-cyhalothrin were not significantly different ( $\chi^2 = 1.33$ ,  $df = 1$ ,  $P > 0.05$ ), although the intercepts were not equal ( $\chi^2 = 15.58$ ,  $df = 2$ ,  $P < 0.05$ ; Fig. 1), suggesting that levels of resistance differed quantitatively, but not qualitatively. The slopes of the dose-mortality curves for SR and RS progeny treated with dicotophos were parallel ( $\chi^2 = 0.85$ ,  $df = 1$ ,  $P > 0.05$ ) and the intercepts equal ( $\chi^2 = 1.87$ ,  $df = 2$ ,  $P > 0.05$ ; Fig. 2), so these data were pooled and new LDs were estimated (Table 2).

**Inheritance of Resistance.** LD values for lambda-cyhalothrin indicated recessive inheritance with sex-linkage in females (RR<sub>50</sub> = 3.20, CI = 1.67 – 6.14 and RR<sub>90</sub> = 5.06, CI = 2.00 – 12.75, respectively; Table 1). In contrast, resistance to dicotophos exhibited autosomal dominant

inheritance ( $RR_{50} = 1.28$ ,  $CI = 0.82 - 1.99$  and  $RR_{90} = 0.84$ ,  $CI = 0.32 - 2.22$ , respectively; Table 2).

**Simultaneous Exposure of the Resistant Population to Both Insecticides.** Mortality of resistant beetles was as expected, regardless of whether lambda-cyhalothrin or dicotophos were applied alone at their  $LD_{50}$ , or in combination ( $\chi^2 = 3.07$ ,  $df = 2$ ,  $P = 0.22$ ). Each insecticide alone produced similar mortality in resistant beetles, but simultaneous exposure to both significantly increased mortality compared to lambda-cyhalothrin alone ( $F_{2,27} = 3.51$ ,  $P = 0.04$ , Fig. 3).

**Comparative Life Histories of Susceptible and Resistant Populations.** Both male and female GA beetles had lower fresh mass than their counterparts from the KS and CA populations, but all populations had similar pre-oviposition periods and 30-day fecundity, although CA females oviposited on more days than did KS females, with GA females intermediate (Table 3). The eggs of GA females had higher viability than those of the KS population, with CA intermediate. Although eggs of CA females hatched faster than those of the other two populations, their larvae took longer to complete development. Pupation time was shortest in the KS population, but there was no difference in immature survival among populations. Neither sex ratio nor the fresh mass of progeny varied among populations, with the exception of males of the GA population that were significantly lighter than others.

## Discussion

Our results demonstrated multiple resistance to lambda-cyhalothrin and dicotophos in *H. convergens* collected from a cotton-growing area in Georgia, USA, a trait not shared by either of the two other populations studied. Other collections of *H. convergens* from Georgia have demonstrated resistance to pyrethroids (Ruberson *et al.* 2007, Rodrigues *et al.* 2013a), as have populations of *E. connexa* from cotton fields and vegetable gardens in Brazil (Rodrigues *et al.*

2013b). Similarly, multiple resistance to pyrethroids and OPs was detected in a population of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) collected from cotton fields in Pakistan (Pathan *et al.* 2008), albeit at lower levels, and populations of *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae) from cruciferous fields in China (Tang *et al.* 2015). Collectively, these results suggest that dependency on these insecticides can drive the evolution of resistance in natural enemy populations in diverse agroecosystems around the world.

Although the present study did not address the mechanism(s) of resistance in GA beetles, it seems reasonable to speculate on the involvement of both metabolic detoxification and target site insensitivity. Pyrethroids act on voltage-sensitive sodium channels (Soderlund and Bloomquist 1989, Vais *et al.* 2001), and mutations in that target site are known to confer knockdown resistance (Kdr type). By contrast, OP's cause irreversible inhibition of the enzyme acetylcholinesterase (AChE) (Fukuto 1990), which is also the target of carbamates. Thus, alterations of AChE that confer resistance to OP's may also confer resistance to carbamates (Gunning *et al.* 1998, Zhang *et al.* 2013). Both pyrethroids and OP's contain ester linkages which are susceptible to attack and inactivation by enzymes such as esterases and cytochrome P450 monooxygenases (David *et al.* 2013, Yu 2015). For example, resistance to OP's and carbamates in *Myzus persicae* Sulzer (Hemiptera: Aphididae), and to a lesser extent pyrethroids, is associated with an overproduction of carboxylesterases that sequester and metabolize these molecules before they reach the insect nervous system (Bass *et al.* 2014).

Without data from backcrosses or dose-mortality response from each gender tested separately, the inference of sex-linked inheritance of pyrethroid resistance in the present study is only tentative. Although the RRs between RS and SR progenies indicated this inheritance as X-linked, their degrees of dominance were not conclusive, with the former revealed as partially dominant and the latter as partially recessive. However, using lambda-cyhalothrin, Rodrigues *et*

*al.* (2013a) characterized pyrethroid resistance in a different Georgia population of *H. convergens* as X-linked, and recessive. They also suggested that heterozygous female ( $X^R X^S$ ) in the resistant population would produce F<sub>1</sub> RS progeny with resistant male ( $X^R y$ ), and susceptible female ( $X^R X^S$ ), whereas the F<sub>1</sub> SR progeny would yield both male ( $X^S y$ ) and female ( $X^R X^S$ ) susceptible. It was not our purpose to investigate the complete inheritance of resistance to pyrethroids in *H. convergens*, firstly because it was already done (Rodrigues *et al.* 2013a); and because sex distinction of *H. convergens* newly emerged is a time-consuming process that would make this experiment unpractical. Since the reciprocal crosses in the present study were performed using F<sub>1</sub> individuals without previous selection to reduce any heterogeneity within the populations of *H. convergens*, we believe that sex ratios in the bioassays with SR and RS progenies may have affected estimation of the degrees of dominance of resistance to pyrethroids.

Besides characterizing the inheritance of pyrethroid resistance in a population of *H. convergens*, Rodrigues *et al.* (2013a) studied its response to a mixture of lambda-cyhalothrin and the synergist piperonyl butoxide (PBO). Due to incomplete suppression of resistance by PBO, they suggested that this adaptive trait would be a possible combination of target site insensitivity and detoxification. Their population was collected in April, 2011 approximately 140 kilometers distant from our GA collection, but both studies used commercially distributed *H. convergens* from California as susceptible controls and obtained similar resistance ratios to lambda-cyhalothrin. The consistency of these findings is indicative of generally sustained selective pressure by lambda-cyhalothrin in Georgia agroecosystems and suggests that the resistance trait is quite stable under field conditions.

The GA population of *H. convergens* exhibited autosomal inheritance of resistance to dicotophos as a dominant trait. Autosomal inheritance of pesticide resistance with variable degrees of dominance has been reported in field populations of various arthropod species.



Examples include resistance to abamectin in *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) in North Carolina (Argentine & Clark 1990), and *Tetranychus urticae* Koch (Acari: Tetranychidae) in Brazil (Ferreira *et al.* 2015); to permethrin and methomyl in *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in Florida (Yu 1993); and to lambda-cyhalothrin in *E. connexa* in Brazil (Rodrigues *et al.* 2013c). Mode of inheritance influences the evolution of resistance ratios and depends to some degree on sex determination and on the relative dominance of the resistance trait in heterozygous individuals (Daly & Fisk 1998). In the present study, only beetles homozygous for susceptibility to dicotophos would have a susceptible phenotype, which may have contributed to the high resistance ratios observed for this insecticide.

The increased mortality of resistant *H. convergens* when exposed to a mixture of lambda-cyhalothrin and dicotophos in a ratio of ca. 1:105 could reflect either the saturation of enzymes that act to detoxify both materials, and the interference of one molecule with enzymes that act on the other. LD<sub>50</sub>s of lambda-cyhalothrin and dicotophos corresponded to 4.5 and 42.3 times the highest concentrations recommended for application to cotton in Georgia, respectively. Thus, mixtures of these insecticides at recommended field rates would be less harmful to resistant *H. convergens* than was our highly concentrated combination. Although not calculated in the present study, the combination index proposed by Chou & Talalay (1984) is a method often used to reveal interactions between components of a mixture. Based on dose-mortality responses to each product used alone, this method permits determination of whether the lethal dose of the mixture is equal to, lower or higher than the expected indicating additive, synergistic, or antagonistic interactions, respectively. Additive and synergistic interactions among combinations of some pyrethroids and OPs were reported for *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in West Africa (Martin *et al.* 2003), *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in Pakistan (Ahmad *et al.* 2009), and Brazilian populations of *Sitophilus zeamais* Motschulsky (Coleoptera:

Curculionidae) (Corrêa *et al.* 2011). Thus, the interaction between insecticides in the mixture resulted in increased toxicity to lambda-cyhalothrin, probably by means of competitive substrate inhibition, where OPs inhibit oxidases (Kulkarni & Hodgson 1980) and/or esterases (Gunning *et al.* 1999), thereby preventing degradation of pyrethroids.

In proposing the fundamental concept of IPM, Stern *et al.* (1959) envisioned the potential for harmonious integration of pesticide use and biological control. In this vein, some have viewed insecticide resistance in natural enemies as a path towards improved pesticide integration in IPM (Croft & Morse 1979), even advocating selection for increased resistance levels in mass-rearing programs (Pree *et al.* 1989, Rodrigues *et al.* 2013c), and the use of strains resistant to more than one insecticide (Tang *et al.* 2015, Torres *et al.* 2015). However, naturally high levels of allelic variation may render resistance traits unstable over evolutionary time (Robertson *et al.* 1995), and resistant populations can also present altered life-history traits compared to susceptible ones, depending on the trait being measured (Konopka *et al.* 2012, Ferreira *et al.* 2013, Mansoor *et al.* 2013, Abbas *et al.* 2014). Costs associated with resistance generally take the form of changes in physiology and/or life-history traits that reduce the fitness of resistant individuals in the absence of insecticide pressure (Coustau *et al.* 2000). Despite the multiple resistance to lambda-cyhalothrin and dicotophos in *H. convergens* from Georgia, life history traits were quite similar among the three populations. However, because laboratory-rearing conditions approach the optimal and protect insects from virtually all forces of natural selection, a failure to measure life history differences under these conditions does not imply that no costs exist for these resistant beetles in nature. The apparent widespread resistance in GA populations would seem consistent with the apparent low fitness cost of these traits and, if costs are truly low or negligible, resistance may persist in these populations for some time in the absence of insecticidal selection pressure.

Given the resistance profile of the GA population, and its similar, if not higher, baseline fitness compared to susceptible populations, one might argue that this population is an ideal candidate for mass-rearing and release into agroecosystems with high reliance on these materials. However, rear-and-release programs that employ coccinellids in open field settings have a generally poor record of success, and the primary contributions of coccinellids to biological control occur via natural recruitment within 'healthy' agroecosystems (Michaud 2012). Field-evolved insecticide resistance in natural enemies is likely indicative of heavy insecticide burdens in local environments, although in the present study it could also reflect historical exposure patterns, as the lack of a fitness cost would suggest continued insecticide selection pressure might not be necessary to maintain these traits. Information on resistance levels in other predatory species prevalent in GA cotton and other predominant crops would be useful to determine whether modifications to IPM programs are advisable to reduce insecticide dependency.

Our results revealed that levels of insecticide resistance in coccinellid populations vary in accordance with their history of exposure in the field. Georgia *H. convergens* are resistant to both lambda-cyhalothrin and dicrotophos, whereas those from California and Kansas are susceptible to both molecules. Inheritance of lambda-cyhalothrin resistance is recessive and X-linked, whereas inheritance of dicrotophos resistance is autosomal dominant, which reflects independent underlying genetics. Simultaneous exposure to the LD<sub>50</sub>s of both insecticides significantly increases mortality of resistant individuals compared to exposure of lambda-cyhalothrin alone, suggesting either shared involvement of, or interference among, the enzyme systems involved in their detoxification. In the absence of insecticide exposure, Georgia *H. convergens* had higher egg viability compared to the other populations, with no other apparent differences in life history.

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1 Table 1. Mean ( $\pm$  SE) relative susceptibility to lambda-cyhalothrin of three North American populations of *Hippodamia*  
 2 *convergens*, and the F1 progeny of two reciprocal crosses (SR = CA $\text{\textcircled{f}}$  x GA $\text{\textcircled{m}}$  and RS = GA $\text{\textcircled{f}}$  x CA $\text{\textcircled{m}}$ ), evaluated 24 h after topical  
 3 treatment with various dosages. The chi-squared test ( $\chi^2$ ) was used to test goodness-of-fit to the Probit model. CI<sub>95</sub> = 95% confidence  
 4 interval, RR = resistance ratio, DD = resistance degree of dominance.

Population or cross	N	Df	Slope $\pm$ SE	LD <sub>50</sub> (CI <sub>95</sub> ) <sup>a</sup>	RR <sub>50</sub> (CI <sub>95</sub> )	DD <sub>50</sub> $\pm$ SE	LD <sub>90</sub> (CI <sub>95</sub> ) <sup>a</sup>	RR <sub>90</sub> (CI <sub>95</sub> )	DD <sub>90</sub> $\pm$ SE	$\chi^2$
CA	180	4	1.39 $\pm$ 0.17	0.005 (0.005 – 0.01)			0.05 (0.03 – 0.12)			2.40
KS	150	4	1.87 $\pm$ 0.27	0.025 (0.02 – 0.03)	3.67 (2.27 – 5.96)*		0.11 (0.07 – 0.23)	2.14 (0.91 – 5.05)		3.88
GA	180	4	2.91 $\pm$ 0.43	1.03 (0.82 – 1.23)	157.83 (102.75 – 242.46)*		2.84 (2.27 – 4.09)	52.52 (25.10 – 109.87)*		0.60
SR	132	4	1.91 $\pm$ 0.27	0.035 (0.02 – 0.06)	5.46 (3.27 – 9.12)*	-0.33 $\pm$ 0.02	0.17 (0.08 – 0.64)	3.08 (1.25 – 7.58)*	-0.43 $\pm$ 0.02	4.38
RS	120	4	1.48 $\pm$ 0.27	0.11 (0.06 – 0.17)	17.47 (8.91 – 34.23)*	0.13 $\pm$ 0.02	0.84 (0.48 – 2.29)	15.60 (5.79 – 41.98)*	0.39 $\pm$ 0.02	1.07

5 <sup>a</sup>Estimated dose of lambda-cyhalothrin ( $\mu\text{g a.i./insect}$ ) required to kill 50% or 90% of treated insects.

6 \*RR significant (Robertson *et al.* 2007).

7 Table 2. Mean ( $\pm$  SE) relative susceptibility to dicotophos of three North American populations of *Hippodamia convergens*, and  
8 the F1 progeny of two reciprocal crosses (SR = CA $\text{\textcircled{f}}$  x GA $\text{\textcircled{m}}$  and RS = GA $\text{\textcircled{f}}$  x CA $\text{\textcircled{m}}$ ), evaluated 24 h after topical treatment with a  
9 range of dosages. The chi-squared test ( $\chi^2$ ) was used to test goodness-of-fit to the Probit model. CI<sub>95</sub> = 95% confidence interval, RR =  
10 resistance ratio, DD = resistance degree of dominance.

Population or cross	N	Df	Slope $\pm$ SE	LD <sub>50</sub> (CI <sub>95</sub> ) <sup>a</sup>	RR <sub>50</sub> (CI <sub>95</sub> )	DD <sub>50</sub> $\pm$ SE	LD <sub>90</sub> (CI <sub>95</sub> ) <sup>a</sup>	RR <sub>90</sub> (CI <sub>95</sub> )	DD <sub>90</sub> $\pm$ SE	$\chi^2$
CA	210	5	1.66 $\pm$ 0.24	0.20 (0.12 – 0.30)			1.21 (0.77 – 2.41)			1.21
KS	150	4	0.88 $\pm$ 0.15	0.67 (0.35 – 1.20)	3.26 (1.57 – 6.76)*		19.16 (7.60 – 104.27)	15.67 (4.19 – 58.64)*		1.19
GA	210	4	4.18 $\pm$ 0.91	108.34 (76.68 – 131.53)	530.07 (338.84 – 829.23)*		219.43 (167.54 – 601.39)	181.13 (97.76 – 335.61)*		4.24
SR	132	4	1.61 $\pm$ 0.32	55.40 (23.56 – 107.21)	270.95 (157.12 – 467.25)*	0.79 $\pm$ 0.01	347.90 (154.58 – 9865.52)	286.69 (113.22 – 725.91)*	1.18 $\pm$ 0.01	5.72
RS	122	4	2.08 $\pm$ 0.42	70.70 (51.92 – 94.65)	345.83 (207.92 – 575.24)*	0.86 $\pm$ 0.01	291.71 (185.36 – 757.14)	240.54 (105.47 – 548.55)*	1.11 $\pm$ 0.01	3.49
Pooled <sup>b</sup>	254	4	1.78 $\pm$ 0.25	62.54 (34.01 – 106.45)	304.80 (188.74 – 492.22)*	0.82 $\pm$ 0.01	327.71 (164.28 – 3160.93)	269.04 (129.03 – 560.99)*	1.15 $\pm$ 0.01	8.95

11 <sup>a</sup> Estimated dose of dicotophos ( $\mu\text{g}$  a.i./insect) required to kill 50% or 90% of treated insects.

12 <sup>b</sup> Data pooled for SR and RS progeny, after passing tests for parallelism and equality.

13 \*RR significant (Robertson *et al.* 2007).

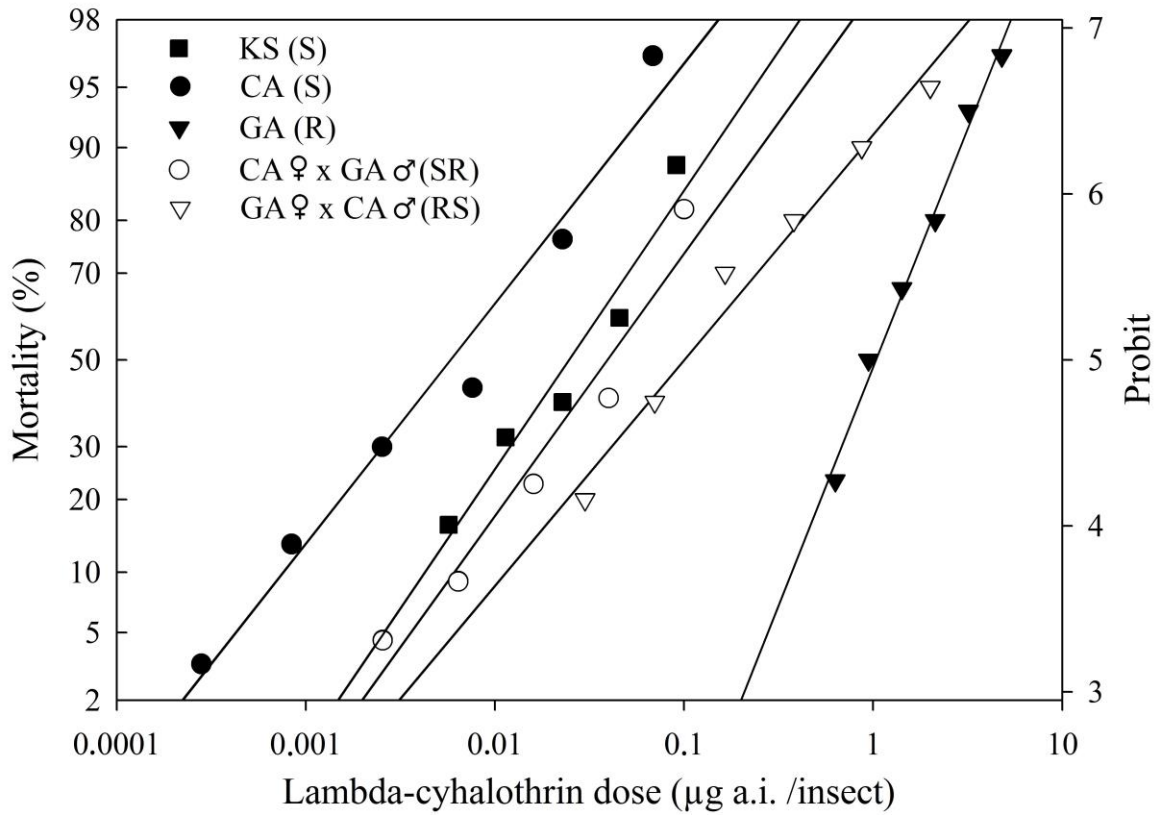
14 Table 3. Mean ( $\pm$  SE) reproductive parameters for three *Hippodamia convergens*  
 15 populations (without insecticide exposure) and developmental parameters of their offspring.

Parameter	Population			<i>F</i> or $\chi^2$	df	<i>P</i>	
	Kansas	California	Georgia				
No. couples established	20	20	20				
Parental fresh mass (mg)	Male	14.9 $\pm$ 0.3a	15.3 $\pm$ 0.4a	13.2 $\pm$ 0.3b	9.88	2,57	< 0.001
	Female	18.6 $\pm$ 0.3a	18.5 $\pm$ 0.4a	17.1 $\pm$ 0.3b	6.32	2,57	0.003
Pre-oviposition period (days)		7.5 $\pm$ 0.4a	8.0 $\pm$ 0.2a	7.4 $\pm$ 0.28a	1.32	2,57	0.274
Fecundity (no. eggs female <sup>-1</sup> ) <sup>a</sup>		405.7 $\pm$ 41.9a	413.8 $\pm$ 22.6a	471.7 $\pm$ 33.4a	1.15	2,57	0.324
No. reproductive days		18.4 $\pm$ 1.4b	23.3 $\pm$ 0.7a	20.1 $\pm$ 1.3ab	4.46	2,57	0.016
Egg viability (% hatching)		58.7 $\pm$ 4.9b	69.0 $\pm$ 3.4ab	77.4 $\pm$ 2.6a	5.38	2,57	0.007
Eclosion time (days)		4.7 $\pm$ 0.1a	4.3 $\pm$ 0.1b	4.6 $\pm$ 0.04a	10.07	2,57	< 0.001
Larval developmental time (days)		13.4 $\pm$ 0.1b	14.1 $\pm$ 0.2a	13.6 $\pm$ 0.1b	6.23	2,215	0.002
Pupation time (days)		6.7 $\pm$ 0.1b	7.1 $\pm$ 0.1a	7.1 $\pm$ 0.05a	25.48	2,215	< 0.001
Immature survival (%)		92.5 $\pm$ 3.0a	85.0 $\pm$ 4.0a	95.0 $\pm$ 2.5a	2.63	2,237	0.074
Sex ratio (percent female)		55.4a	55.9a	55.3a	0.006*	2	0.997
Progeny fresh mass (mg)	Male	15.5 $\pm$ 0.3a	15.6 $\pm$ 0.3a	14.6 $\pm$ 0.3b	3.49	2,94	0.035
	Female	18.9 $\pm$ 0.4a	18.6 $\pm$ 0.4a	18.4 $\pm$ 0.3a	0.54	2,118	0.585

16 <sup>a</sup> Monitored over a period of 30 days, tallied from onset of oviposition of each female.

17 Analysis by one-way ANOVA or Chi-square (\*). Means followed by the same letter were not  
 18 significantly different within rows (Bonferroni,  $\alpha = 0.05$ ).

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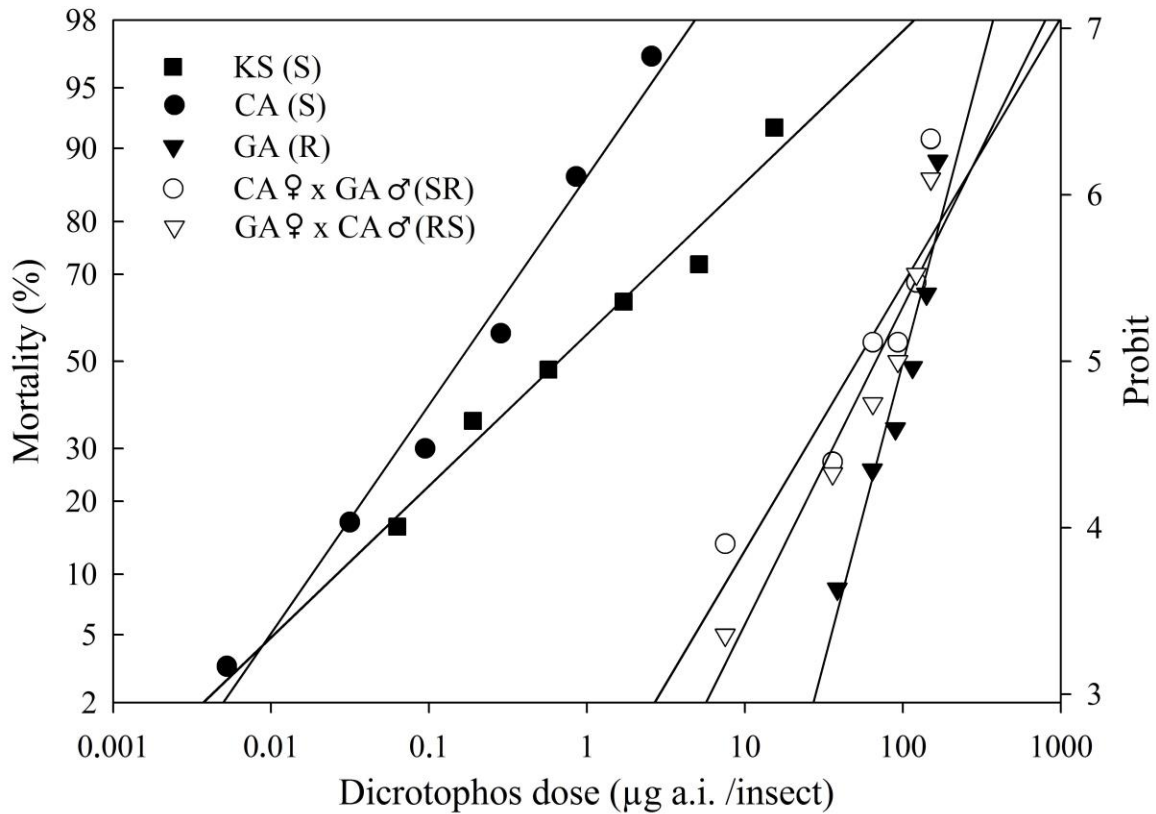
21 Figura 1. Dosage-mortality lines for three *Hippodamia convergens* populations (KS = Kansas, CA

22 = California, GA = Georgia, S = susceptible, R = resistant), and the progeny of reciprocal crosses

23 (SR and RS) between the GA and CA populations, when treated with a range of dosages of

24 lambda-cyhalothrin, expressed on a log scale.

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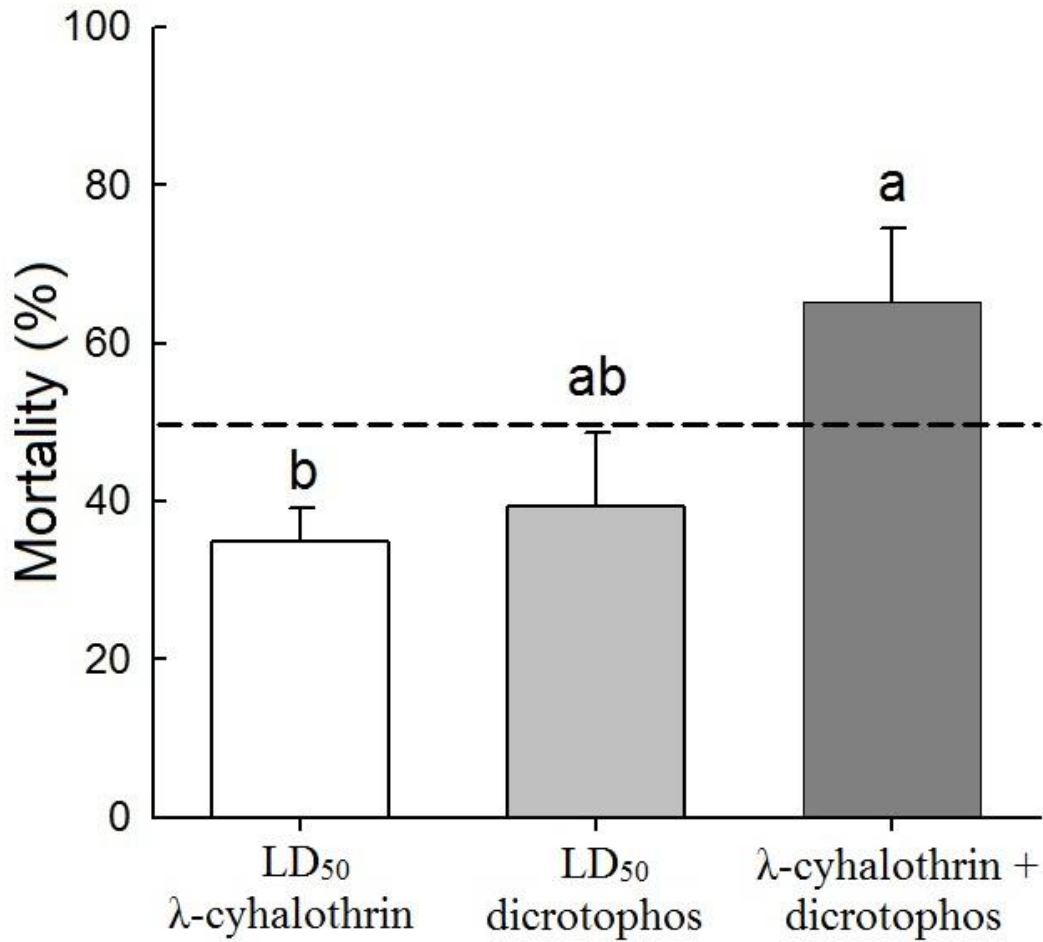
27 Figura 2. Dosage-mortality lines for three *Hippodamia convergens* populations (KS = Kansas, CA

28 = California, GA = Georgia, S = susceptible, R = resistant), and the progeny of reciprocal crosses

29 (SR and RS) between the GA and CA populations, when treated with a range of dosages of

30 dicrotophos, expressed on a log scale.

31



32  
 33 Figure 3. Mortality (+SE) of resistant *Hippodamia convergens* 24 h after exposure to LD<sub>50</sub> of  
 34 lambda-cyhalothrin, dicrotophos, or a combination of both. Columns bearing the same letter were  
 35 not significantly different (Bonferroni,  $\alpha = 0.05$ ). Dashed line indicates expected mortality at LD<sub>50</sub>  
 36 (50%).

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## CHAPTER 4

41

### FINAL CONSIDERATIONS

42

43       Integrated pest control can be achieved by using selective insecticides, i.e., those that act  
44 against the target species without inflicting detrimental effects on beneficials, such as pollinators,  
45 parasitoid and predators. More than a desire, this issue has become a requirement for maintaining  
46 agroecosystem sustainability, not only because farmers are concerned about production costs, but  
47 also because society has demanded healthier and environmentally safer solutions to pest  
48 problems.

49       Despite this study focused only on the lady beetle *H. convergens* and its role as a predator in  
50 cotton, the main results and information obtained can be easily extended to other agroecosystems.  
51 We showed that use of high rates of chlorantraniliprole, an anthranilic diamide selective to  
52 lepidopterans, may reduce its compatibility with *H. convergens* in cotton fields, thus potentially  
53 impacting biological control of aphids by this species. However, this insecticide would be less  
54 toxic to *H. convergens*, and probably to other natural enemies, if applied at concentrations lower  
55 than those recommended against *A. argillacea* and *C. virescens*, the primary target pests.

56       We also showed that resistance to broad spectrum insecticides such as pyrethroids and  
57 organophosphates may evolve in populations of *H. convergens* in the field, depending on  
58 exposure history. Although some might consider pesticide resistance a desirable trait that permits  
59 beneficial species to survive chemical applications, it should not be considered a license to  
60 increase insecticide usage because pest populations will be subjected to similar or even higher  
61 selective pressure than their natural enemies. Furthermore, we provided information that a



62 mixture of pyrethroid and organophosphate is potentially more harmful to *H. convergens* than is  
63 either insecticide alone, again indicating that more selective insecticides, rather than broad  
64 spectrum molecules or their combinations, will be preferred options for controlling pests.