VINICIUS DE ABREU D’ÁVILA

ESTRESSE CAUSADO POR INSETICIDAS EM DUAS ESPÉCIES DE INIMIGOS NATURAIS (*Aphidius colemani* e *Eriopis connexa*) DE PULGÕES

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Doctor Scientiae*.

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APROVADA: 15 de dezembro de 2017.

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“Estudar não é encontrar o mundo que eu concordo, as ideias que apoiam meu universo, autores que confirmam o que eu já sei. Estudar é expandir, entrar em contradição, pensar, buscar os limites de cada pensamento. Estudar não é abrir um espelho para seu rosto ser contemplado no seu esplendor, mas uma janela para sua mente olhar mais longe e além do seu mundo."

(Leandro Karnal)

Aos meus pais Denarte e Silvana;

Pelo amor e paciência,

Dedico

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SUMÁRIO

RESUMO

D’ÁVILA, Vinícius de Abreu, D.Sc., Universidade Federal de Viçosa, dezembro de 2017. **Estresse causado por inseticidas em duas espécies de inimigos naturais (*Aphidius colemani* e *Eriopis connexa*) de pulgões.** Orientador: Raul Narciso Carvalho Guedes. Coorientadores: Gerald Christopher Cutler e Wagner Faria Barbosa.

Diante a necessidade de diminuir os efeitos deletérios do uso contínuo de inseticidas e ao mesmo tempo maximizar a produção agrícola, surge a necessidade de se integrar o controle biológico e o controle químico de maneira eficiente e harmoniosa. Assim, o objetivo desse trabalho foi avaliar o efeito de concentrações subletais de inseticidas em inimigos naturais do pulgão *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) e a possibilidade da utilização dos dois métodos simultaneamente. No primeiro capítulo foi avaliado o efeito da exposição residual de dois inseticidas convencionais, imidacloprid e lambda-cialotrina, e do biopesticida spinosad sobre a longevidade e reprodução do parasitoide *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae). A curva de concentração-mortalidade mostrou que o parasitoide foi 20 vezes mais suscetível ao spinosad que ao imidacloprid e ao lambda-cialotrina, com concentrações menores que a recomendada a campo reduzindo mais da metade a longevidade do parasitoide. Por outro lado, embora imidacloprid e lambda-cialotrina tenham comprometido a taxa de parasitismo do *A. colemani*, o lambda-cialotrina não alterou o número de vespas produzidas. No segundo e terceiro capítulo duas linhagens do predador *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae), uma resistente e outra suscetível a piretroides, foram expostas a concentrações subletais de lambda-cialotrina sendo avaliado o seu efeito na longevidade, reprodução, mobilidade e forrageamento desses predadores. Bioensaios de sobrevivência com lambda-cialotrina permitiram a estimativa dos tempos de exposição subletais na maior concentração recomendada a campo tanto para resistentes, expostas por 48 horas, quanto para suscetíveis, expostas por 45 minutos. Em relação aos aspectos reprodutivos, sem a exposição prévia ao lambda-cialotrina os machos de ambas linhagens mostraram mais rapidez para a monta precisando de menos tentativas para isto. A exposição ao lambda-cialotrina prolongou o tempo de cópula e diminuiu o tempo de tremulação do corpo da fêmea no início da cópula. As fêmeas suscetíveis também apresentavam um tempo maior na cópula quando comparadas as resistentes resultando em uma maior fertilidade e um maior pico de produção de progênie mantendo a produção por muito mais tempo. A exposição ao lambda-cialotrina reduz o pico de produção da linhagem resistente e o período para linhagem suscetível. Em relação a mobilidade, quando confinadas em áreas parcialmente tratadas com lambda-cialotrina a linhagem suscetível apresentou uma redução na velocidade e percorreu uma menor distância, o que pode ser um resultado da desordenação dos movimentos do predador diante a exposição ao inseticida. Não foi detectada repelência, porem 40% dos indivíduos apresentaram irritabilidade. Entretanto, os insetos permaneceram por mais tempo na porção contaminada com inseticida da arena favorecendo sua exposição prolongada. Em relação aos indivíduos expostos previamente ao inseticida, apesar de não apresentarem diferença em relação ao manuseio da presa, a busca de presas por adultos resistentes foi significativamente prolongada como consequência da exposição ao inseticida, esse resultado apresenta uma correlação significativa com o tempo em repouso e a distância percorrida, assim como uma correlação significativa negativa com a velocidade. Assim, a exposição subletal afetam o forrageamento por predadores resistentes ao piretroide exibindo busca de presas prolongadas associadas a uma maior distância caminhada com maior intervalo de repouso e baixa velocidade, reduzindo sua performance predatória.

ABSTRACT

D'ÁVILA, Vinícius de Abreu, D.Sc., Universidade Federal de Viçosa, December, 2017. **Stress caused by insecticides on two species of natural enemies (*Aphidius colemani* and *Eriopis connexa*) of aphids.** Advisor: Raul Narciso Carvalho Guedes. Co-advisors: Gerald Christopher Cutler and Wagner Faria Barbosa.

In view of the need to reduce the deleterious effects of the continuous use of insecticides and at the same time maximize agricultural production, there is a need to integrate biological control and chemical control in an efficient and harmonious way. Thus, the objective of this work was to evaluate the effect of sublethal concentrations of insecticides on natural enemies of the *Myzus persicae* (Sulzer, 1776) aphid (Hemiptera: Aphididae) and the possibility of using both methods simultaneously. In the first chapter the effect of the residual exposure of two conventional insecticides, imidacloprid and lambda-cyhalothrin, and the spinosad biopesticide on the longevity and reproduction of the parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae) were evaluated. Based on the concentration-mortality curve, the parasitoid was more than 20 times susceptible to spinosad than to imidacloprid and lambda-cyhalothrin, with concentrations lower than that recommended in the field, reducing the longevity of the parasitoid by more than half. On the other hand, although imidacloprid and lambda-cyhalothrin compromised the parasitism rate of *A. colemani*, lambda-cyhalothrin did not change the number of wasps produced. In the second and third chapters, two strains of the predator *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae), one resistant and another susceptible to pyrethroids, were exposed to sublethal concentrations of lambda-cyhalothrin, being evaluated their effect on longevity, reproduction, mobility and foraging of these predators. Lambda-cyhalothrin survival bioassays allowed the estimation of sublethal exposure times at the highest recommended concentration in the field for both resistant, exposed for 48 hours, and susceptible, exposed for 45 minutes. Regarding the reproductive aspects, without the previous exposure to lambda-cyhalothrin, the males of both lines showed a faster rate of mating, requiring fewer attempts to copulate. Exposure to lambda-cyhalothrin prolonged the copulation time and decreased the flutter time of the female body at the beginning of intercourse. Susceptible females also had a longer copulation time when compared to resistant females, resulting in higher fertility and a higher progeny production peak, maintaining production for much longer. Exposure to lambda-cyhalothrin reduces the production peak and the production period, respectively for the resistant and susceptible strains. Regarding mobility, when confined to areas partially treated with lambda-cyhalothrin, the susceptible line presented a reduction in speed and a shorter distance, which may be a result of the disorganization of the movements of the predator on exposure to the insecticide. No repellency was detected, but 40% of the individuals presented irritability. However, the insects remained longer in the insecticide-contaminated portion of the arena favoring their prolonged exposure. Regarding the individuals previously exposed to the insecticide, although they did not present differences in relation to prey handling, the search for prey by resistant adults was significantly prolonged as a consequence of insecticide exposure; this result shows a significant correlation with the time at rest and the distance traveled, as well as a significant negative correlation with velocity. Thus, sublethal exposure affect foraging by predators resistant to pyrethroid exhibiting a search for prolonged prey associated with a longer walking distance with a longer resting time and slower speed, reducing predatory performance.

INTRODUÇÃO GERAL

Um dos problemas trazidos com a globalização do último século, especialmente devido a viagens internacionais e comercio, foi a crescente introdução local de espécies exóticas. Por sua vez, grande parte desses invasores se tornam pragas agrícolas por encontrarem boas condições de sobrevivência (Liebhold et al. 1995). A abundante disponibilidade de alimentos em monoculturas e a inexistência de fatores que limitavam seu crescimento populacional, como a ausência de inimigos naturais nos locais de invasão contribuem significativamente para o estabelecimento e propagação dessas espécies exóticas, com destaque especial aos insetos, causando danos econômicos aos sistemas agrícolas (Corn et al. 2002, Walther et al. 2009). Dessa maneira, a partir da década de 40, com o intuito de aumentar a produção agrícola e minimizar os prejuízos causados pelas pragas foi disseminado o uso de inseticidas orgânicos sintéticos para o controle de pragas (Carson 1962, Van Den Bosch 1978).

Como nem tudo são flores, o uso crescente, constante e/ou indiscriminado de inseticidas acarretou diversos problemas para o ambiente e os sistemas de produção agrícola, como surgimento de populações resistentes a inseticidas (Che et al. 2013, Koo et al. 2014, Charaabi et al. 2016, Guedes et al. 2017), efeitos nocivos a organismos não alvos (Zhou et al. 2014, Dorneles et al. 2017, Regan et al. 2017) e surto populacional de pragas (Dutcher 2007, Szczepaniec et al. 2011, Cordeiro et al. 2013, Guedes et al. 2016, 2017). Essas consequências acarretam novos prejuízos ambientais e econômicos assim como aceleram a perda do inseticida com a resistência.

Foi no contexto acima que, a partir da década de 70, introduziram-se os fundamentos do Manejo Integrado de Pragas (MIP) com intuito de reduzir os efeitos nocivos de inseticidas no ambiente, além de minimizar os danos econômicos causados pelos ataques das pragas. Além da utilização do controle químico de maneira consciente e estratégica, outras medidas alternativas de controle de pragas passaram a ser preconizadas de maneira planejada e harmoniosa nas tomadas de decisão, como por exemplo o controle biológico (Flint e Van Den Bosch 1981, Kogan 1998).

Muito antes do surgimento do MIP, contudo, já havia registro de sucesso do controle biológico clássico. Este se refere à importação de inimigo natural geralmente da região de origem da espécie exótica, sendo posteriormente liberado em quantidades inoculativas para se estabelecer em um novo ambiente. O primeiro registro foi em 1888 com a espécie de joaninha *Rodolia cardinalis* (Mulsant) (Coccinellidae: Coleoptera) para controle da cochonilha *Icerya purchasi* (Maskell) (Hemiptera: Monophlebidae) em pomares da Califórnia nos Estados Unidos (Van Den Bosch et al. 1982, Caltagirone e Doutt 1989). Por outro lado, foi após a introdução do MIP que o controle biológico passou a ser visto como uma possibilidade real para o controle de pragas, principalmente em cultivos em ambiente protegido, como casas-de-vegetação.

Ainda que o controle biológico seja uma alternativa com aplicabilidade crescente, ele não é necessariamente excludente ao controle químico e nem livre de problemas ambientais (Howarth 1991). Quando combinados de maneira correta, um pode aumentar a eficiência do outro (Stern et al. 1959). Entretanto, para que isso ocorra, é necessário um profundo conhecimento tanto do inseticida quanto do inimigo natural, assim como suas interações. Isso porque essas relações podem determinar toda logística de manejo, incluindo a época de aplicação do inseticida e liberação dos inimigos naturais. Por exemplo, Tremblay et al. (2008) constaram que ao usar sabão inseticida e o parasitoide *Aphidius colemani* (Hymenoptera: Braconida) para o controle de pulgão de maneira simultânea, é necessário que a liberação das vespas seja antecipada em um dia em relação a aplicação do inseticida, caso contrário há prejuízo ao desempenho do parasitoide.

Além do controle biológico clássico e do controle biológico aumentativo, onde inimigos naturais são liberados periodicamente, uma proposta mais recente é a de manipulação do ambiente para preservação do inimigo natural em campo, o chamado controle biológico conservativo (Tscharntke et al. 2007, Aguiar-Menezes et al. 2008, Straub et al. 2008, Begg et al. 2017). Essa preservação pode ser conseguida pela oferta de alimentos alternativos, como a presença de recursos florais servindo de alimento alternativo nos períodos de baixa populacional da presa preferencial (Lands et al. 2000, Venzon et al. 2006, Charles e Paine 2016, Davila et al. 2016), e/ou a utilização de inseticidas seletivos em favor dos inimigos naturais (Croft 1990).

De maneira geral, a seletividade pode ser resultado de diferenças fisiológicas e morfológicas entre as espécies, desde diferenças no sítio alvo do inseticida a outras que promovam uma menor taxa de penetração ou favoreça a desintoxicação, sequestro e excreção do inseticida. Somado a isso diferenças comportamentais podem levar a redução do período de exposição ao inseticida levando à seletividade ao produto. Por fim, endossimbiontes presentes no interior dos insetos podem desempenhar papeis relevantes e determinantes que favoreçam a seletividade de seu hospedeiro a estes compostos (Tremblay et al. 2008, Cordeiro et al. 2009, Guedes et al. 2016).

Dessa maneira, no caso de controle biológico somado ao controle químico, a principal busca é por inseticidas que numa mesma concentração são tóxicos para as pragas e não para os inimigos naturais (Ruberson et al. 1998). A maximização do manejo pode também ser beneficiada além da seletividade, com a busca por populações de inimigos naturais resistentes a inseticidas (Rodrigues et al. 2013ab, Barbosa et al. 2016). Dentro desse cenário de busca por populações resistentes, por vezes a CL_{50} ou a DL_{50} é usada como único parâmetro para determinar a toxicidade ou a seletividade de um inseticida em relação a pragas e inimigos naturais, ignorando as consequências dos efeitos subletais às populações.

A importância de efeitos subletais é ilustrado pelo fato de que algumas espécies que apresentam alta taxa de mortalidade podem se recuperar rapidamente se mostrarem gerações curtas e reprodução acelerada (Banks et al. 2011). Por outro lado, espécies que apresentam baixa mortalidade na mesma concentração podem sofrer prejuízos na sua capacidade reprodutiva devido a efeitos subletais na espécie levando a população gradativamente a extinção local (Desneux et al. 2007, Banks et al. 2011). Além disso, devido a degradação do inseticida no ambiente, os inimigos naturais, que não são os alvos da aplicação destes, são normalmente expostos a concentrações menores do que requeridas para a mortalidade deles, tornando-se importante a avaliação do efeito ou resposta a estas concentrações e mesmo concentrações abaixo das recomendadas ou aplicadas (Eijaza et al. 2015, Guedes et al. 2016, 2017). Outro equívoco frequente quando se fala de seletividade de inseticida é associar a origem do composto à sua toxicidade. Compostos orgânicos, como por exemplo óleos essenciais, podem apresentar toxicidade igual ou superior a de compostos sintéticos (Castilhos et al. 2017).

Dois comportamentos importantes a serem avaliados após exposição de inimigos naturais a concentrações subletais inseticidas são os comportamentos de forrageamento e reprodutivo. Afinal, a alteração destes comportamentos pode prejudicar a sobrevivência e persistência dos agentes de controle biológico a campo, assim como prejudicar a sua finalidade de aplicação: o controle populacional de pragas. Por exemplo, a espécie de joaninha predadora *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) tem seu crescimento populacional reduzido pelo prejuízo na fertilidade das fêmeas expostas a concentrações subletais de spinosad e imidacloprid (Galvan et al. 2005).

Outro fenômeno causado pelo uso de inseticidas em concentrações subletais e que vem ganhando bastante destaque é conhecido como hormese. Este fenômeno se refere ao fato de que um determinado composto químico tóxico ao indivíduo quando em altas doses, ser benéfico a ele quando em baixas doses distorcendo a curva de dose-resposta tornando-a bifásica (Kendig et al. 2010, Cutler 2013, Guedes e Cutler 2014, Cutler e Guedes 2017). Um exemplo é o trabalho de Mallqui et al. (2014), onde se verificou que fêmeas do caruncho-pequeno-do-feijão, *Zabrotes subfasciatus* Bohemann (Coleoptera, Bruchidae), aumentavam sua fecundidade diária compensando a redução da longevidade causada pela exposição azadiractina, alterando assim o balanço fisiológico do indivíduo, favorecendo um processo (i.e., reprodução) em detrimento de outro (i.e., longevidade).

Entre as várias pragas agrícolas, os afídeos (ou pulgões) merecem destaque. Mais de 100 espécies de pulgões são consideradas pragas em diversas culturas em todo o mundo (Vam Emden e Harrington 2007). Além dos danos que causam ao sugar a seiva, estes insetos são vetores frequentes de doenças de plantas, particularmente de viroses vegetais. São espécies praga de controle difícil devido a suas estratégias de reprodução (partenogênese), alta taxa de crescimento além de fácil dispersão, podendo migrar longas distâncias através dos ventos (Vam Emden and Harrington 2007).

Um inimigo natural bastante citado na literatura como alternativa no controle de pulgões é o parasitoide *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae). Vàsquez et al. (2006) apontaram que esta espécie de parasitoide foi tão eficaz quanto o inseticida imidacloprid para controle de pulgões da espécie *Aphis gossypii* (Hemiptera: Aphididae) em casas de vegetação, apesar de apresentar um custo mais elevado. No intuito de reduzir os custos e ao mesmo tempo retardar a seleção de populações resistentes ao inseticida, muitos pesquisadores vem avaliando o efeito inseticida nesse parasitoide a fim de encontrar produtos seletivos que possam ser usados conjuntamente a esta espécie parasitoide em programas de manejo de pulgões (Bostanian and Akalach 2004, Bostanian et al. 2005, Stara et al. 2010).

Outro inimigo natural de pulgões em evidencia no momento é a joaninha predadora *Eriopis connexa* (Germar, 1824) (Coleoptera: Coccinelidae). Alguns autores vêm pesquisando a eficiência predatória de população resistente a piretroides no intuito de conciliar os dois métodos de controle. O inseticida piretroide objetiva o controle de lagartas e o predador objetiva o controle de pulgões, desta maneira busca-se evitar surtos populacionais de afídeos após aplicação de inseticida e eliminação de competidores pelo alimento (Rodrigues 2012). Apesar de elucidada a origem da resistência, assim como avaliação dos custos adaptativos associados a ela, são necessários estudos que avaliem o efeito da exposição do predador a concentrações subletais desse produto e como isso altera o seu comportamento reprodutivo e de predação, além de seus efeitos temporais e espaciais na comunidade devido essas alterações (Spindola et al. 2013, Rodrigues et al. 2013ab, 2014, Torres et al. 2015, Lira et al. 2016, Santos et al. 2016, Guedes et al. 2017).

Frente ao contexto descrito acima, o objetivo desse trabalho foi avaliar a longevidade, reprodução e forrageamento de duas espécies de inimigos naturais de pulgões, o parasitoide *A. colemani* e duas populações do predador *E. connexa*, frequentemente sujeitos a exposição subletal de inseticidas. Este esforço busca contribuir com a viabilização de uso simultâneo de ambos métodos de controle integrando em programas de manejo integrado de pragas.

REFERÊNCIAS

AGUIAR-MENEZES, E. L.; LIXA, A. T.; RESENDE, A. L. S. 2008. Joaninhas predadoras, as aliadas do produtor no combate às pragas. **A Lavoura**, Rio de Janeiro. 111: 38-41.

BANKS, J. E.; STARK, J. D.; VARGAS, R. I.; ACKLEH, A. S. 2011. Parasitoids and ecological risk assessment: Can toxicity data developed for one species be used to protect entire guild? **Biological Control**. 59: 336-339.

BARBOSA, P. R. R.; MICHAUD, J. P.; RODRIGUES, A. R. S.; TORRES, J. B. 2016. Dual resistance to lambda-cyalothrin and dicrotophos in *Hippodamia convergens* (Coleoptera: Coccinellidae). **Chemosphere**. 159: 1-9.

BEGG, G. S.; COOK, S. M.; DYE, R.; FERRANTE, M.; FRANCK, P.; LAVIGNE, C.; LÖVEI, G. L.; MANSION-VAQUIE, A.; PELL, J. K.; PETIT, S.; QUESADA, N.; RICCI, B.; WRATTEN, S. D.; BIRCH, A. N. E. 2017. A functional overview of conservation biological control. **Crop Protection**. 97: 145-158.

BOSTANIAN, N.; AKALACH, M. 2004. The contact toxicity of indoxacarb and fiver other insecticides to *Orius insidiosus* (Hemiptera: Anthocorida) and *Aphidius Colemani* (Hymenoptera: Braconidae), beneficials used in the greenhouse industry. **Pest Management Science**. 60: 1231-1236.

BOSTANIAN, N. J.; AKALACH, M.; CHIASSON, H. 2005. Effects of a *Chenopodium*-based botanical insecticide/acaricide on *Orius insidiosus* (Hemiptera: Anthocoridae) and *Aphidius colemani* (Hymenoptera: Braconidae). **Pest Management Science.** 61: 979-984.

CALTAGIRONE, L. E.; DOUTT, R. L. 1989. The history of the vedalia beetle importation to California and its impact on the developmento of biological control. **Annual Review of Enetomology**, Palo Alto. 34: 1-16.

CARSON, R. 1962. Silent Spring. **Boston: Houghton Mifflin Co**. 368p.

CASTILHOS, R. V., GRÜTZMACHER, A. D., & COATS, J. R. 2017. Acute Toxicity and Sublethal Effects of Terpenoids and Essential Oils on the Predator *Chrysoperla externa* (Neuroptera: Chrysopidae). **Neotropical Entomology**. 1-7.

CHARAABI, K.; BOUKHRIS-BOUHACHEM, S.; MAKNI, M.; FENTON, B.; DENHOLM, I. 2016. Genetic variation in target-site resistance to pyrethroids and pirimicarb in Tunisian populations of the peach potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). **Pest management science**. 72: 2313-2320.

CHARKES, J. J.; PAINE, T. D. 2016. Fitness Effects of Food Resources on the Polyphagous Aphid Parasitoid, *Aphidius colemani* Viereck (Hymenoptera: Braconidae: Aphidiinae). **PloS one**. 11: e0147551.

CHE, W.; SHI, T.; WU, Y.; YANG, Y. 2013. Insecticide resistance status of field populations of *Spodoptera exigua* (Lepidoptera: Noctuidae) from China. **Journal of economic entomology**. 106: 1855-1862.

CORDEIRO, E. M. G.; DE MOURA, I. L. T., FADINI, M. A. M.; GUEDES, R. N. C. (2013). Beyond selectivity: Are behavioral avoidance and hormesis likely causes of pyrethroid-induced outbreaks of the southern red mite *Oligonychus ilicis*?. **Chemosphere**. 93: 1111-1116.

CORDEIRO, E. M. G.; CORRÊA, A. S.; GUEDES, R. N. C. 2014. Insecticide-mediated shift in ecological dominance between two competing species of grain beetles. **PloS one.** 9: e100990.

CORN, M. L.; BUCK, E. H.; RAWSON, J. M.; SEGARRA, A.; FISCHER, E. A. 2002. Invasive non-native species: Background and issues for Congress. RL30123, **Congressional Research Service**. 99p.

CROFT, B. A. 1990. Arthropod biological control agents and pesticides. **John Wiley & Sons**, New York, NY.

D'ÁVILA, V. A.; AGUIAR-MENEZES, E. L.; GONÇALVES-ESTEVES, V.; MENDONÇA, C. B. F.; PEREIRA, R. N.; SANTOS, T. M. 2016. Morphological characterization of pollens from three Apiaceae species and their ingestion by twelve-spotted lady beetle (Coleoptera: Coccinellidae). **Brazilian Journal of Biology**. 76: 796-803.

DESNEUX, N.; DECOURTYE, A.; DELPUECH, J. M. 2007. The sublethal effects of pesticides on beneficial arthropods. **Annual Review of Entomology**. 58: 81-106.

DORNELES, A. L.; DE SOUZA ROSA, A.; BLOCHTEIN, B. 2017. Toxicity of organophosphorus pesticides to the stingless bees *Scaptotrigona bipunctata* and *Tetragonisca fiebrigi*. **Apidologie**. 48: 612-620.

DUTCHER, J. D. 2007. A review of resurgence and replacement causing pest outbreaks in IPM. In General concepts in integrated pest and disease management. **Springer Netherlands**. pp. 27-43.

EIJAZA, S.; KHAN, M. F.; MAHMOOD, K.; ANWAR, M.; ALAMGIR, A.; KHATRI, I. 2015. Studies on degradation and efficacy of synthetic pesticides on okra crop. **Academic Journal of Entomology**. 8:12-18.

FLINT, M. L.; VAN DEN BOSCH, R. 1981. Introduction to integrated pest management. **New York: Plenum Press**. 240 p.

GALVAN, T. L.; KOCH, R. L.; HUTCHISON, W. D. 2005. Effects of spinosad and indoxacarb on survival, development, and reproduction of the multicolored Asian lady beetle (Coleoptera: Coccinellidae). **Biological Control**. 34: 108-114.

GUEDES, R. N. C.; SMAGGHE, G.; STARK, J. D.; DESNEUX, N. 2016. Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. **Annual review of entomology**. 61: 43-62.

GUEDES, R. N. C.; WALSE, S. S.; THRONE, J. E. 2017. Sublethal exposure, insecticide resistance, and community stress. **Current Opinion in Insect Science**. 21: 47-53.

HOWARTH, F. G. 1991. Environmental impacts of classical biological control. **Annual review of entomology**. 36: 485-509.

KENDIG, E. L.; LE, H. H.; BLECHER, S. M. 2010. Defining hormesis: evaluation of a complex concentration response phenomenon. **International Journal of Toxicology**. 29: 235-246.

KOGAN, M. 1998. Integrated pest management: historical perspectives and contemporary developments. **Annual review of entomology**, 43: 243-270.

KOO, H. N.; AN, J. J.; PARK, S. E.; KIM, J. I.; KIM, G. H. 2014. Regional susceptibilities to 12 insecticides of melon and cotton aphid, *Aphis gossypii* (Hemiptera: Aphididae) and a point mutation associated with imidacloprid resistance. **Crop Protection**. 55: 91-97.

LANDIS, D. A.; WRATTEN, S. D.; GURR, G. M. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. **Annual review of entomology**. 45: 175-201.

LIEBHOLD, A. M.; MACDONALD, W. L.; BERGDAHL, D.; MASTRO, V. C. 1995. Invasion by exotic forest pests: a threat to forest ecosystems. **Forest Science**. 41.

LIRA, R.; RODRIGUES, A. R. S.; TORRES, J. B. 2016. Fitness Advantage in Heterozygous Ladybird Beetle *Eriopis connexa* (Germar) Resistant to Lambda-Cyhalothrin. **Neotropical entomology**. 45: 573-579.

MALLQUI, K. S.; VIEIRA, J. L.; GUEDES, R. N. C.; GONTIJO, L. M. 2014. Azadirachtin-Induced Hormesis Mediating Shift in Fecundity-Longevity Trade-Off in the Mexican Bean Weevil (Chrysomelidae: Bruchinae). **Journal of Economic Entomology**. 107: 860-866.

REGAN, K.; ORDOSCH, D.; GLOVER, K. D.; TILMON, K. J.; SZCZEPANIEC, A. 2017. Effects of a pyrethroid and two neonicotinoid insecticides on population dynamics of key pests of soybean and abundance of their natural enemies. **Crop Protection**. 98: 24-32.

RODRIGUES, A. R. D. S. 2012. Caracterização da resistência de joaninhas predadoras ao lambda-cialotrina. **Tese de Doutorado**, Universidade Federal Rural de Pernambuco, Recife, PE, Brasil.

RODRIGUES, A. R. S.; SPINDOLA, A. F.; TORRES, J. B.; SIQUEIRA, H. A. A.; COLARES, F. 2013a. Response of different populations of seven lady beetle species to lambda-cyhalothrin with record of resistance. **Ecotoxicology and Environmental Safety**. 96: 53-60.

RODRIGUES, A. R. S.; TORRES, J. B.; SIQUEIRA, H. A. A.; LACERDA, D. P. A. 2013b. Inheritance of lambda-cyhalothrin resistance in the predator lady beetle *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae). **Biological Control** (Print). 64: 217-224.

RODRIGUES, A. R. S.; SIQUEIRA, H. A. A.; TORRES, J. B. 2014. Enzymes Mediating Resistance to Lambda-Cyhalothrin in *Eriopis connexa* (Coleoptera: Coccinellidae). **Pesticide Biochemistry and Physiology**. 110: 36-43.

RUBERSON, J. R.; NEMOTO, H.; HIROSE, Y. 1998. Pesticides and conservation of natural enemies in pest management. **Conservation biological control**. pp. 207-220.

SANTOS, D. S.; RODRIGUES, A. R. S.; TORRES, J. B.; LIRA, R. 2016. Performance of *Eriopis connexa* (Coleoptera: Coccinellidae) resistant to lambda-cyhalothrin after extended recovery from knockdown. **Neotropical entomology**. 45: 718-724.

SPÍNDOLA, A. F., SILVA-TORRES, C. S. A., RODRIGUES, A. R. S.; TORRES, J. B. 2013. Survival and behavioural responses of the predatory ladybird beetle, *Eriopis connexa* populations susceptible and resistant to a pyrethroid insecticide. **Bulletin of entomological research**. 103: 485-494.

STERN, V. M. R. F.; SMITH, R.; VAN DEN BOSCH, R.; HAGEN, K. 1959. The integration of chemical and biological control of the spotted alfalfa aphid: the integrated control concept. **California Agriculture**. 29: 81-101.

STRAUB, C. S.; FINKE, D. L.; SNYDER, W. E. 2008. Are the conservation of natural enemy biodiversity and biological control compatible goals?. **Biological control**. 45: 225-237.

SZCZEPANIEC, A.; CREARY, S. F.; LASKOWSKI, K. L.; NYROP, J. P.; RAUPP, M. J. 2011. Neonicotinoid insecticide Imidacloprid causes outbreaks of spider mites on Elm Trees in urban landscapes. **PLoS ONE**. 6: e20018.

TSCHARNTKE, T.; BOMMARCO, R.; CLOUGH, Y.; CRIST, T. O.; KLEIJN, D.; RAND, T. A.; TYLIANAKIS, J. M.; VAN NOUHUYS, S.; VIDAL, S. 2007. Conservation biological control and enemy diversity on a landscape scale. **Biological control**. 43: 294-309.

TREMBLAY, E.; BÉLANGER, A.; BROSSEAU, M.; BOIVIN, G. 2008. Toxicity and sublethal effects of an insecticidal soap on *Aphidius colemani* (Hymenoptera: Braconidae). **Pest Management Science.** 64: 249-254.

TORRES, J B.; RODRIGUES, A. R. S.; BARROS, E. M.; SANTOS, D. S. 2015. Lambda-Cyalothrin Resistance in the Lady Beetle *Eriopis conexa* (Coleoptera: Coccinellidae) Confers Tolerance to Other Pyrethroids. **Journal of Economic Entomology**. 108: 60-68.

VAM EMDEN, H. F.; HARRINGTON, R. 2007. Aphids as crops pests. **CABI**, Cambridge, MA.

VAN DEN BOSCH, R. 1978. The pesticide conspiracy. **New York: Doubleday & Company**. 226p.

VAN DEN BOSCH, R.; MESSENGER, P. S.; GUTIERREZ, A. P. 1982. The history and development of biological control. In: VAN DEN BOSCH, R.; MESSENGER, P. S.; GUTIERREZ, A. P. (eds.). An introduction to biological control. **New York: Plenum Press**. pp. 21-36.

VÁSQUEZ, G. M.; ORR, D. B.; BAKER, J. R. 2006. Efficacy Assessment of *Aphidius colemani* (Hymenoptera: Braconidae) for Suppression of *Aphis gossypii* (Homoptera: Aphididae) in Greenhouse-Grown Chrysanthemum. **Journal of Economic Entomology**. 99: 1104-1111.

VENZON, M.; ROSADO, M. C.; EUZÉBIO, D. E.; SOUZA, B.; SCHOEREDER, J. H. 2006. Suitability of leguminous cover crop pollens as food source for the green lacewing *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae). **Neotropical Entomology**. 35: 371-376.

WALTHER, G. R.; ROQUES, A.; HULME, P. E.; SYKES, M. T.; PYSYŠEK, P.; KÜHN, I; ZOBEL, M.; BACHER, S.; BOTTA-DUKÁT, Z.; BUGMANN, H.; CZÚCZ, B.; DAUBER, J.; HICKLER, T.; JAROSÍK, V.; KENIS, M.; KLOTZ, S.; MINCHIN, D.; MOORA, M.; NENTWIG, W.; OTT, J.; PANOV, V. E.; REINEKING, B.; ROBINET, C.; SEMENCHENKO, V.; SOLARZ, W.; THUILLER, W.; VILÀ, M.; VOHLAND, K.; SETTELE, J. 2009. Alien species in a warmer world: risks and opportunities. **Trends in ecology & evolution.** 24: 686-693.

ZHOU, K.; HUANG, J.; DENG, X.; VAN DER WERF, W.; ZHANG, W.; LU, Y.; WU, K.; WU, F. 2014. Effects of land use and insecticides on natural enemies of aphids in cotton: First evidence from smallholder agriculture in the North China Plain. **Agriculture, ecosystems & environment**, 183: 176-184.

ARTIGO 1

Effects of Spinosad, Imidacloprid, and Lambda-cyhalothrin on Survival, Parasitism, and Reproduction of the Aphid Parasitoid *Aphidius colemani*

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Abstract

Insecticides can affect biological control by parasitoids. Here, we examined the lethal and sublethal effects of two conventional insecticides, imidacloprid and lambda-cyhalothrin, and a reduced-risk bioinsecticide, spinosad, on the aphid parasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae). Concentration-mortality curves generated from insecticide residue bioassays found that wasps were nearly 20-fold more susceptible to spinosad than imidacloprid and lambda-cyhalothrin. Imidacloprid and lambda-cyhalothrin compromised adult parasitoid longevity, but not as dramatically as spinosad: concentrations >200 ng spinosad/cm^2 reduced wasp longevity by half. Imidacloprid and lambda-cyhalothrin also compromised aphid parasitism by wasps. Although increasing imidacloprid concentrations led to increased host viability and reduced progeny production, lambda-cyhalothrin did not affect viability of parasitized hosts or parasitoid progeny production in a dose-dependent manner. Our results demonstrate that reduced risk bioinsecticide products like spinosad can be more toxic to biological control agents than certain conventional insecticides.

Keywords: Biological control, reduced-risk insecticide, sublethal effects, spinosyn.

Introduction

The green peach aphid, *Myzus persicae* (Sulzer) (Aphididae) attacks plant hosts belonging to over 40 different families (Tingey and Andaloro 1983, Blackman and Eastop 2000). In addition to direct damage *M. persicae* causes to host plants by sap-feeding, this aphid is an important vector of plant diseases, including more than 100 plant viruses (Tingey and Andaloro 1983). *Myzus persicae* is also difficult to control due to its varied reproductive strategies, rapid growth rate, and ease of dispersion over long distances by wind (Blackman and Eastop 2000, Van Emden and Harrington 2007).

The aphid parasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae) is distributed throughout many parts of North America, Europe, Asia, Africa, and Oceania (Stary 1975) and is commercially produced as biocontrol agent (Sampaio et al. 2001, Van Driesche et al. 2008, Khatri et al. 2016, 2017). This parasitoid has become an important biological control agent, particularly for the greenhouse industry, due to its high mobility and fecundity, and short generation time (Van Schelt 1994). The efficacy of *A. colemani* for aphid management in greenhouses can be equivalent to the use of chemical control, though at a higher cost (Vásquez et al. 2006).

The principal control method for green peach aphid is application of insecticides (Umina et al. 2014), but over-reliance on chemical control is problematic. This is so because *M. persicae* has developed resistance to many insecticides with different modes of action (Devonshire and Moores 1982, Field et al. 1988, Little et al. 2017). In addition, insecticide use frequently has unintended deleterious effects on nontarget organisms, such as valued parasitoids, predators, and other natural enemies of agricultural pests (Desneux et al. 2007, Biondi et al. 2012). Insecticides targeting primary pests may also inadvertently cause outbreaks of nontargeted pest populations due to their differential susceptibility, level of exposure, or a range of indirect effects (Hardin et al. 1995, Szczepaniec et al. 2011, Cordeiro et al. 2013, Guedes et al. 2016). The integration of chemical control and biological control can reduce some of these problems, by, for example, reducing costs and undesirable effects (Stern et al. 1959, Roubos et al. 2014, Dara 2017).

In the search for new active ingredients to combat insect pests, so called biopesticides — pesticidal compounds of natural origin — have gained attention. This is in large part because of their alleged increased safety toward nontarget organisms, humans, and the environment relative to conventional insecticides that are based on active ingredients synthesized in a laboratory (Chandler et al. 2011, Guedes et al. 2016, EPA 2017). However, this generalization may not hold true in many cases, given that the origin of an insecticide does not determine its toxicity, spectrum of activity, or safety (Guedes et al. 2016).

In the current study, we assessed the effects of two conventional insecticides —imidacloprid and lambda-cyhalothrin — and a biopesticide, spinosad, on the survival, parasitism, and reproduction of *A. colemani*. These insecticides are used against many aphid pests, including *M. persicae*, in a variety of greenhouse and field scenarios (Vásquez et al. 2006). Certain formulations of spinosad can also be used in organic production, which is more restrictive in its insecticide options for arthropod pest control. We predicted the conventional insecticides imidacloprid and lambda-cyhalothrin would have strong effects on *A. colemani*, although their impacts may vary with the parasitoid developmental stage (Tremblay et al. 2008). Spinosad is considered a reduced-risk (bio)insecticide, and we therefore predicted it would have milder effects on *A. colemani* relative to imidacloprid and lambda-cyhalothrin, although there is evidence that spinosad can adversely affect nontargeted arthropods (Biondi et al. 2012, Barbosa et al. 2015, Tomé et al. 2015). Although we expected variable deleterious effects on *A. colemani* to occur, we were cognizant that insecticide induced hormesis — a response where very low doses of insecticide can stimulate biological processes in insects (Cutler 2013, Guedes and Cutler 2014, Cutler and Guedes 2017) — might also be observed.

Material and Methods

Colonies of Insects

Myzus persicae nymphs and adults used in this study were from a colony maintained on cabbage (*Brassica oleracea* var. capitata L.) at the Dalhousie University Faculty of Agriculture. The *M. persicae* colony was maintained and bioassays were performed under controlled environmental conditions of 25 ±

2°C, 25 ± 5% R.H., and a 16:8 (L:D) photoperiod. Cabbage plants were grown in 7.5 cm diameter pots filled with Pro-Mix (Halifax Seed, Halifax, NS, Canada) potting soil in a greenhouse. Aphids on cabbage plants (approximately 3 wk old) were contained within polyethylene plastic cages (92 × 48 × 48 cm) with mesh for ventilation (about 20 plants per cage) to prevent contamination by other species. Aphid-infested cabbage plants were replaced weekly with fresh, uninfested plants.

A. colemani was originally purchased from Koppert Canada (Scarborough, Ontario, Canada). For rearing of parasitoids, aphid infested plants were placed inside another cage containing adult parasitoids. After 3–4 d, when the most *M. persicae* on the host plant were mummified, the plants were removed from the cage and parasitized aphids were transferred to 1 liter plastic jars covered with organza tissue to allow air circulation and prevent insect escape. Emerging adult parasitoids (<24 h old) were collected using a small vacuum cleaner (EL-BT13BK model - ECOLA©), and were used in bioassays at the same day. Some mummified aphids were kept in the plants for *A. colemani* colony maintenance.

Insecticides

Three formulated insecticides used to manage green peach aphid were used: imidacloprid (240 g a.i./L; Admire 240 SC; Bayer Crop Science Canada, AB, Canada), lambda-cyhalothrin (120 g a.i./L; Matador 120 EC; Syngenta Crop Protection Canada, Guelph, ON, Canada), and spinosad (240 g a.i./L; Entrust 240 SC; Dow AgroSciences, Calgary, AL, Canada).

Concentration-mortality Bioassays

Concentration-mortality bioassays were carried out to assess the toxicity of each insecticide to *A. colemani*. A variation of a standard method of residual exposure commonly used to test toxicity of pesticides to parasitoids was used (Hassan et al. 1985). Two milliliters of each insecticide in water (without surfactant) was applied to acrylic Petri dishes (9 cm diameter; 2 cm high) using a Potter spray tower (Burkard Scientific, Uxbridge, UK), applied at a pressure of 78 kPa. The spray covered a circular area of 176.71 cm^2 (base and lid), and dishes were left to dry on the bench top for two hours after treatment. Each Petri dish had four opposing holes (1 cm diameter). Plastic petri dishes were used to allow holes to be made in its sides. Two of the holes were covered with organza tissue for ventilation and the other two were used to provision the parasitoids with sugar water solution (10%) via soaked cotton wicks (Bostanian et al. 2005, Charles and Paine 2016). Five adult parasitoid wasps (<24 h old) were released into each Petri dish using the small vacuum cleaner and mortality was assessed after 48 h. Insects were considered dead if they did not respond to prodding with a fine hairbrush. At least five different concentrations and a water control were used for each insecticide, at a range of concentrations that caused 0–100% mortality. Each bioassay was replicated seven times (five wasps per petri dish and seven petri dishes per concentration per insecticide).

Time-mortality Bioassays

Time-mortality bioassays were performed with the same concentrations used in the concentration-mortality bioassays, as well as with additional concentrations that extended into the no observable effect concentration (NOEC) range for each compound. Methods used were generally as described above, but insect mortality was measured daily following an initial 24 h exposure to the dried insecticide residue, and transfer of the exposed adult parasitoids to uncontaminated Petri dishes. Seven replicates were used for each concentration and insecticide. The concentrations tested ranged from: 240 - 0.43 ng i.a./cm^2 for spinosad; 960 - 1.88 ng/cm^2 for imidacloprid; and 960 - 3.75 ng i.a./cm^2 for lambda-cyhalothrin. Adult survival was recorded to determine survival curves and estimates of median survival time (LT$_{50}$).

Bioassays of Parasitism and Parasitoid Progeny Production

In cases where the insecticide label rate was less than the estimated median lethal concentration (LC$_{50}$), determined from the concentration-mortality bioassays, insects were subjected to additional bioassays to examine sublethal effects of these compounds on parasitism, host viability, and parasitoid progeny production. This was done by exposing the insects as previously described, and then placing 30 of them of a given treatment in 1 liter plastic containers along with a provision of sugar water (10%) through of a piece of cotton by a hole in the top of the containers. Male and female parasitoids were released together (30 wasps/container) and allowed to mate over 72 h. The insects were removed from the containers and males were distinguished from females by the shape of the abdomen. This was done under a microscope after collecting each parasitoid with a vacuum aspirator and reducing their mobility in a refrigerator (−20°C) for 20 s; the sex ratio was approximately 1:1 (Vargas 2010). Each female was then individually placed in a 500 ml glass jar. Each jar contained: a moist filter paper on the bottom to prevent desiccation; a cotton wick saturated with sugar solution; and a cabbage leaf infested with 35 s or third-instar aphids, with a moistened cotton swab placed around the petiole to provide the leaf with a water source. Each glass jar was covered with organza tissue to allow air circulation and prevent insect escape. Parasitoid females were allowed to parasitize the aphids for 48 h and then removed from jars using a small vacuum cleaner. After 10 d, we recorded in each jar the number of parasitized aphids, the percentage mummified hosts, the number of parasitoid progeny emerged, and the parasitoid sex ratio.

Statistical Analyses

Lethal concentrations of insecticide to parasitoids in concentration-nmortality bioassays were estimated by probit analyses using PROC PROBIT (SAS 9.4; SAS Institute, Cary, NC); these data were corrected for natural mortality using Abbott's Formula (Abbott 1925) prior to analysis. Data from the time-mortality bioassays were subjected to survival analyses using Kaplan–Meier estimators, with a Log Rank test to verify if there were statistical differences among concentrations (PROC LIFETEST; SAS 9.4; SAS Institute). The estimated LT_{50}'s for each concentration of each insecticide were subsequently subjected to regression analyses to allow estimating the rate longevity decreased with concentration. Data on parasitism rates, viability of parasitized hosts, and parasitoid emergence and sex ratio were also subjected to regression analyses using insecticide concentration as the independent variable and the curve-fitting procedure of TableCurve 2D (Systat, San Jose, CA). Model selection was performed based on parsimony, high F-values, and steep increases in R^2 with model complexity, always testing the models from the simplest to most complex.

Results

Concentration-mortality Bioassays

Probit analyses on the concentration-mortality data produced low X^2 and high P-values (≥0.05) for all insecticides, indicating a good fit of the data to the probit model used. Based on LC_{50} values, wasps were approximately 19- and 37-fold more susceptible to spinosad than imidacloprid or lambda-cyhalothrin, respectively (Fig. 1). Slopes of the concentration-mortality curves were relatively steep for all insecticides, but somewhat lower for imidacloprid, suggesting a more heterogeneous response of *A. colemani* adults to that insecticide (Fig. 1).

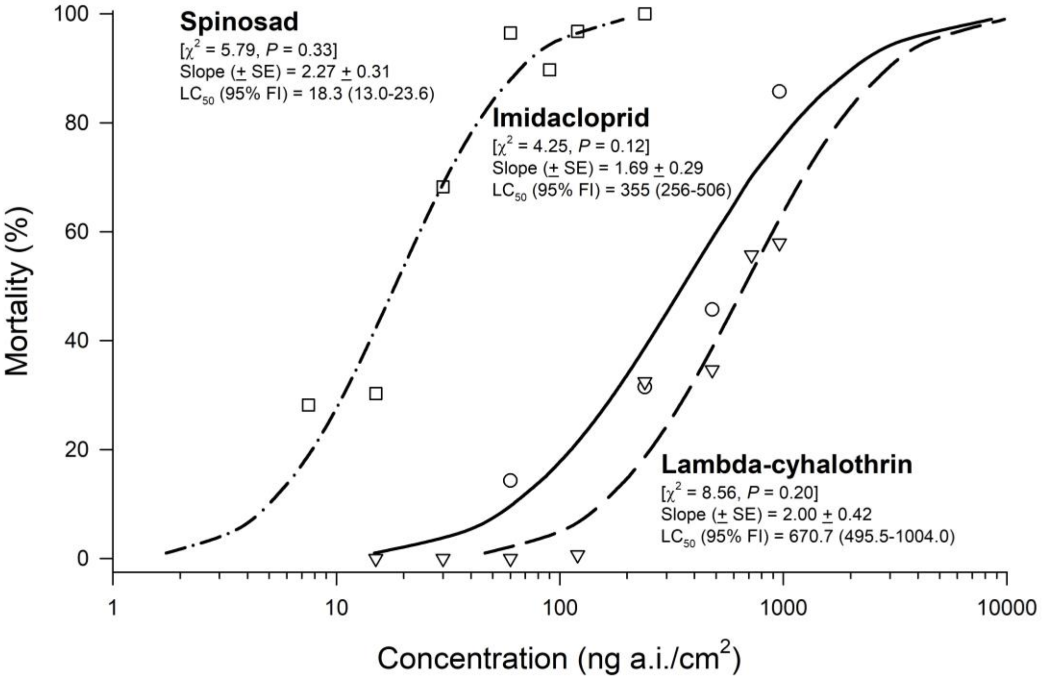

Fig. 1. Concentration-mortality curves showing adult *Aphidius colemani* susceptibility to dry residues (24 h exposure) of imidacloprid (circles), spinosad (squares), and lambda-cyhalothrin (triangles). Each data point on the figure is the mean of seven replicates.

Time-mortality Bioassays

The log-rank test found that adult parasitoid longevity varied significantly with concentration for each of the three insecticides tested (imidacloprid: χ^2 = 122.7, df = 6, $P < 0.001$; lambda-cyhalothrin: χ^2 = 81.1, df = 8, $P < 0.001$; spinosad: χ^2 = 174.0, df = 7, $P < 0.001$). Regression analyses for LT_{50} estimates of each insecticide showed that exposure to spinosad concentrations of 100–250 ng a.i./cm^2 led to rapid mortality of adults, whereas only slight deleterious effects on longevity were found with exposure to imidacloprid and lambda-cyhalothrin at this range of concentrations (Fig. 2). The LT_{50} dose–response curves for imidacloprid and lambda-cyhalothrin were similar, with a rather gradual decrease in adult longevity along concentrations ranging 200–1,000 ng a.i./cm^2, whereas the LT_{50} dose–response curve with spinosad was very steep along concentrations ranging from 0.42 to 7.50 ng a.i./cm^2 (Fig. 2).

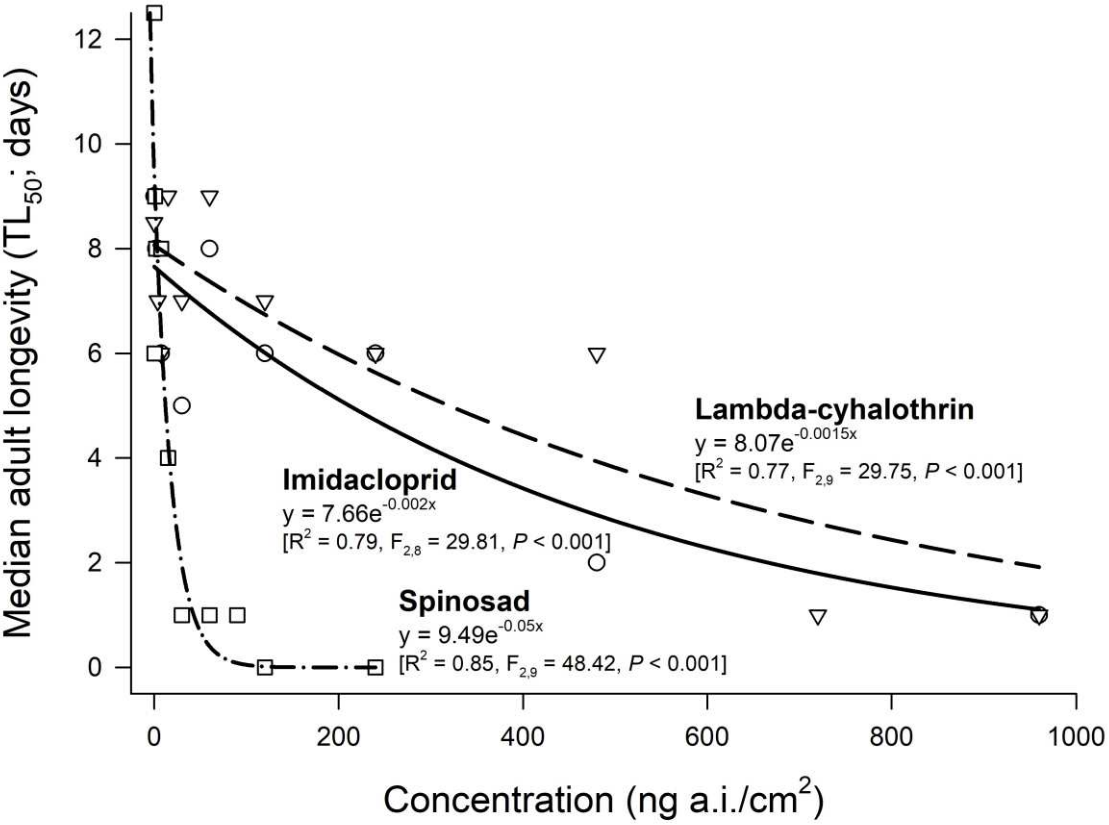

Fig. 2. Median adult *Aphidius colemani* longevity (LT_{50}) following exposure to dry insecticide residues, estimated by using Kaplan–Meier estimators.

Parasitism and Parasitoid Progeny Production

Spinosad was very potent to parasitoid adults, with toxicity >20-fold that of imidacloprid or lambda-cyhalothrin, and an estimated LC_{50} (18.26 ng a.i./cm^2) lower than the label rate (364 ml/ha, or 480 ng a.i./cm^2). Therefore, we did not subject parasitoids to experiments with spinosad assessing effects on parasitism and progeny production.

In contrast, label rates of imidacloprid (200 ml/ha, or 480 ng a.i/cm^2) and lambda-cyhalothrin (233 ml/ha, or 279.6 ng a.i./cm^2) were close or lower than their respective LC_{50} values of 355.15 and 670.70 ng a.i./cm^2, respectively. Both imidacloprid and lambdacyhalothrin impaired parasitism in a dose-dependent manner (Fig. 3). However, the effect was stronger for imidacloprid, with host parasitism dropping more than twofold at concentrations of 2 ng imidacloprid/cm^2 or greater (Fig. 3). Consequently, aphids had increased viability when exposed to parasitoids that were treated with sublethal concentrations of imidacloprid, whereas no such changes in host viability were observed with exposure to our test concentrations of lambda-cyhalothrin (Fig. 4). Our lambda-cyhalothrin sublethal treatments also did not affect parasitoid progeny production, unlike imidacloprid, which greatly impaired this endpoint (Fig. 5). The sex ratio (female/total) of the emerging progeny was not affected by either insecticide (overall mean 0.60 ± 0.03; imidacloprid: $F_{1,37} = 0.006$, $P = 0.94$; lambda-cyhalothrin: $F_{1,38} = 0.49$, $P = 0.49$).

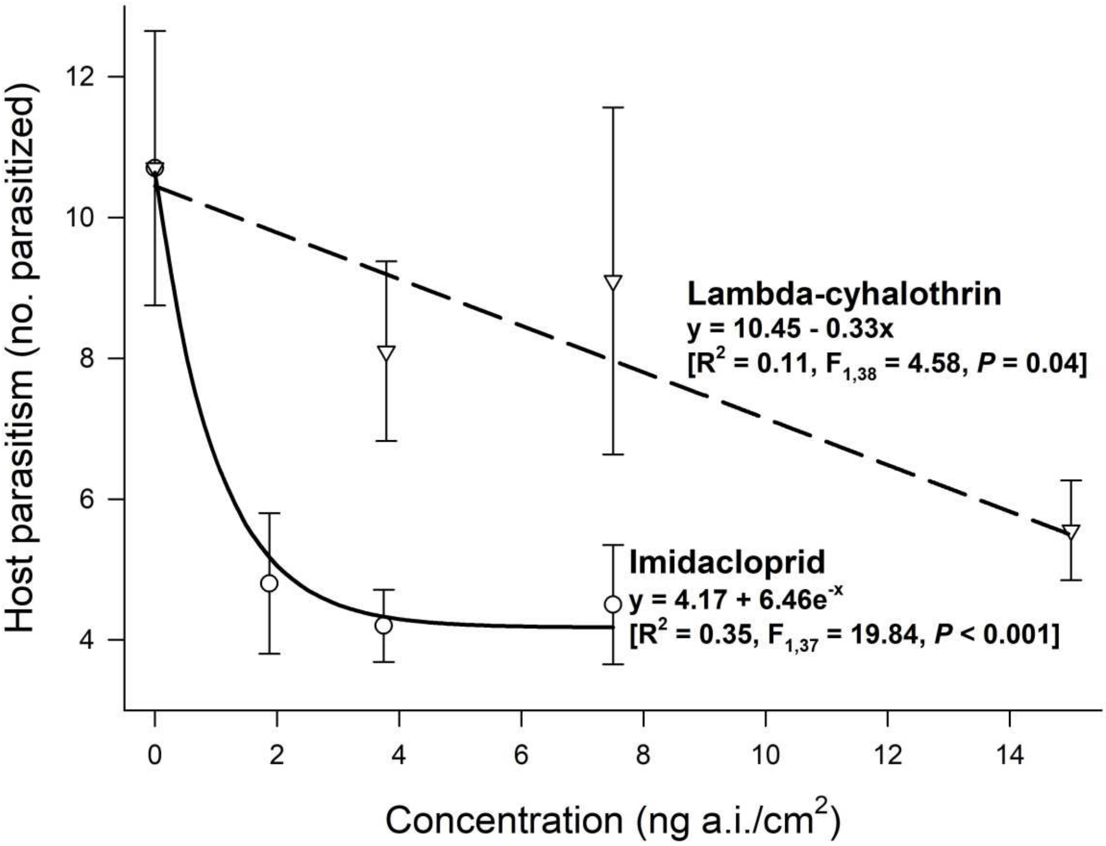

Fig. 3. Host parasitism (± SE) by adults of the parasitioid wasp *Aphidius colemani* surface exposed to dry insecticide residues.

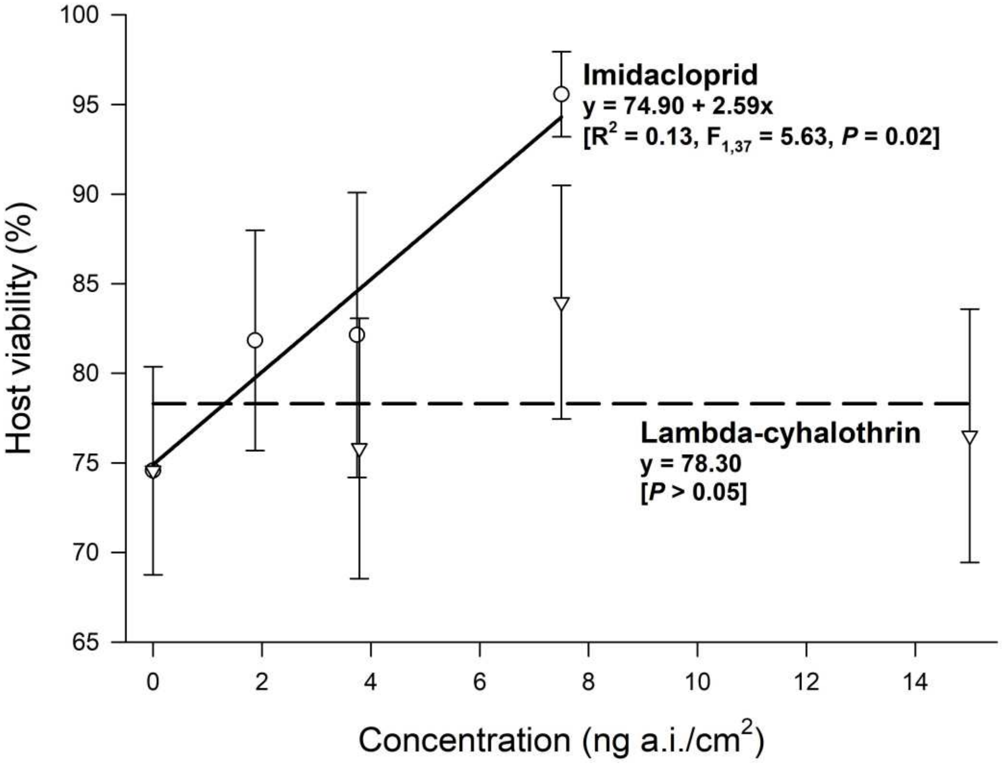

Fig. 4. Viability of aphids (± SE) parasitized by adults of the parasitioid wasp *Aphidius colemani* surface exposed to dry insecticide residues.

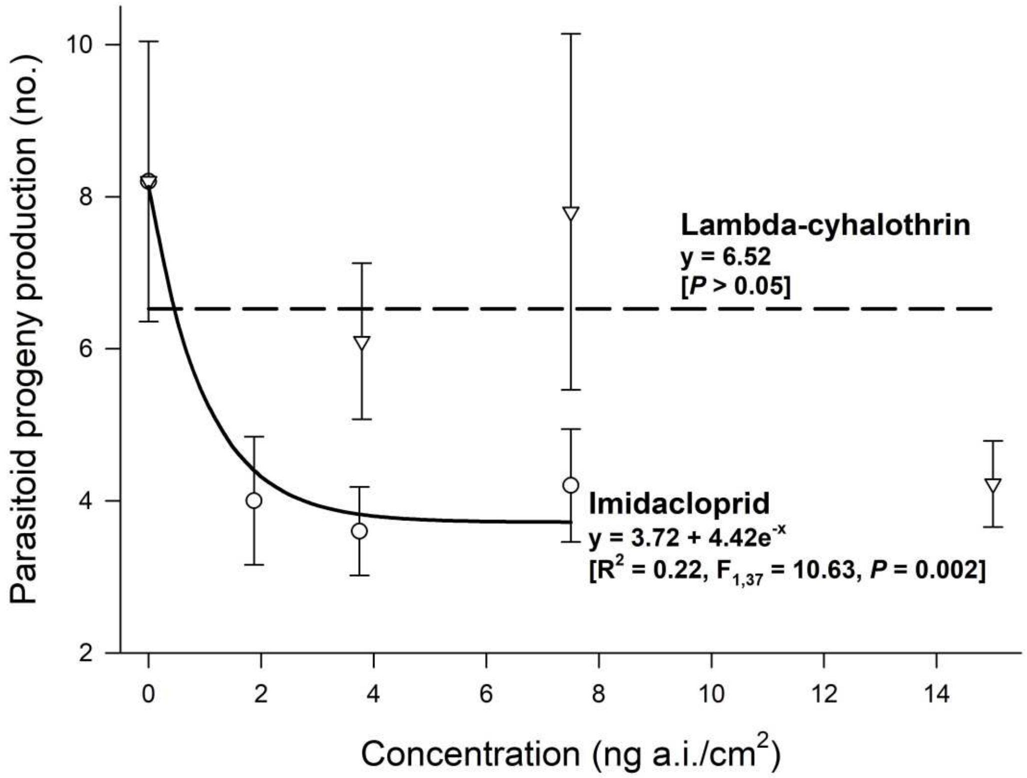

Fig. 5. Progeny production (± SE) by adults of the parasitioid wasp *Aphidius colemani* surface exposed to dry insecticide residues.

Discussion

In this study, we wanted to assess the lethal and sublethal effects of two conventional insecticides and a biopesticide to the aphid parasitoid *A. colemani*. Biopesticides are generally thought of as being reduced-risk and safer for nontarget organisms (e.g., Villaverde et al. 2014, EPA 2017), and we therefore predicted that imidacloprid and lambda-cyhalothrin would have stronger effects on the parasitoid than spinosad, a biopesticide. However, in our experiments, the residual contact lethal toxicity of spinosad was approximately 20-fold greater than that of both conventional insecticides. The effects of spinosad on adult *A. colemani* longevity were also more severe than the effects of imidacloprid and lambda-cyhalothrin on adult longevity. Our results show that the perceived higher safety of bioinsecticides to nontarget species may not always be true and that the source of an insecticidal molecule is not a determinant of its toxicity or safety (Barbosa et al. 2011).

The recognition of the natural origin of an insecticide is primarily associated with its aimed use in organic agriculture systems rather than to its safety to nontargeted species. Our initial expectation of an improved safety profile of spinosad toward *A. colemani* was not due to its natural origin as a fermentation product from the actinomycete *Saccharopolyspora spinosa* (Mertz & Yao), but its recognition as a reduced-risk insecticide by some regulatory agencies, including the EPA (EPA 2017). Similar to our results, others have shown that spinosad can elicit a range of lethal and sublethal effects on beneficial arthropods (Sparks et al. 2001, Miles 2006, Biondi et al. 2012, Barbosa et al. 2015, Tomé et al. 2015). Takahashi et al. (2005) found that applications of spinosad recommended in strawberry cultivation left 0.194 $\mu g/cm^2$ of active ingredient on the leaves up to 5 d after application, and 0.046 $\mu g/cm^2$ after 40 d, with mortality of *A. colemani* exposed to strawberry leaves reaching 95.1%. These concentrations are higher than our estimated LC_{50} (18.3 ng a.i./cm^2) for *A. colemani*. Miles (2006) also reported that spinosad was toxic to *A. colemani* when exposed shortly after application, but that toxicity is reduced 1 wk after spraying.

We found that imidacloprid and lambda cyhalothrin were not as acutely lethal to *A. colemani* as spinosad, and did not impair adult longevity as greatly as spinosad. The imidacloprid LC_{50} (355.2 ng a.i./cm^2) was close to that expected from a recommended maximum foliar application of this insecticide in several crops (480 ng a.i./cm^2), but concentrations four times lower than the LC$_{50}$ caused a significant reduction in *A. colemani* longevity. It was previously shown that spinosad is also more toxic to *Aphidius ervi* (Haliday) than imidacloprid (Araya et al. 2010). Although the rate of spinosad penetration into the insect body is lower than when injected, the same is true imidacloprid, which also exhibits higher oral rather than contact exposure, while pyrethroid contact penetration is superior to that of spinosad (Sparks et al. 2001). Thus, the prevailing route of (contact) exposure does not explain the higher acute toxicity of spinosad compared with imidacloprid and lambda-cyhalothrin.

Both spinosad and imidacloprid target nicotinic acetylcholine receptors (nAChR), but spinosad is an allosteric neuromodulator whose binding site differs from that of imidacloprid, although this has not yet been characterized (Salgado 1997, Crouse et al. 2001, Lester et al. 2004). *Aphidius* spp. may have more or greater sensitivity of nAChR subunits targeted by spinosad than those targeted by imidacloprid. Spinosad also has secondary effects as an agonist of the neurotransmitter gamma-amino-butyric acid (GABA) (Watson 2001), which in *Aphidius* spp. might also be particularly sensitive to chemical agonists. In addition, distinct detoxification of both insecticides by the parasitoid may also contribute for the reported differences in susceptibility.

Lower target site sensitivity and enhanced detoxification, but not reduced penetration, are potential causes of lower toxicity of lambdacyhalothrin to *A. colemani*, compared to spinosad. Lambda cyhalothrin was the least toxic insecticide tested in our study, and more than twice its label rate did not significantly alter longevity when compared to the control treatment. Although lambda-cyhalothrin reduced host parasitism at concentrations lower than label rate, in contrast to imidacloprid the viability of parasitized hosts and progeny production was unaffected. This finding counters the usual notion that pyrethroids are very toxic to natural enemies (Hull et al. 1985, Clarke et al. 1992), and indicates that effects of pyrethroids vary considerably depending on the active ingredient and the arthropod species.

We included treatments around the NOEC, as determined in our concentration-response mortality bioassays, because natural enemies are frequently exposed to low concentrations of insecticide following its degradation in the field (Eijaza et al. 2015). As expected, some deleterious sublethal effects were observed at these concentrations for certain insecticides. However, we also used NOEC concentrations in order to determine if such exposure would induce hormesis in *A. colemani*. In some cases, insecticide exposure at low or sublethal concentrations does not cause deleterious effects, but may stimulate, include in beneficial insects, the reproduction, longevity, or stimulated growth (Cutler 2013, Cutler and Guedes 2017).

In commercial products tests, the ratio between the label rate and the LC_{50} gives an indication of the product risk, i.e., Hazard Quotient. The higher the hazard quotient indicates the greater the risk of the product, on the other hand, if the Hazard Quotient is lower than one, the adverse effects are no expected (Eppo 1999, Desneux et al. 2004). While spinosad presented a ratio of 26.29, imidacloprid and lambda-cyhalothrin presented ratios close to or less than one, in this case, 1.35 and 0.42, respectively. This suggests that while exposure to residues of imidacloprid and lambda cyhalothrin would present no or low hazard *A. colemani*, exposure to residues or spinosad would pose a hazard to the parasitoid, supporting the findings of our experiments.

Insecticides and biological control are often thought of as incompatible, but is not always the case. It has long been appreciated that the correct integration of natural enemy and insecticide can lead to integrated management success (Stern et al. 1959, Hoyt 1969). Many new insecticides, in particular, demonstrate good selectivity in favor of natural enemies (Gentz et al. 2010) and there are certainly reports of certain insecticides having low toxicity to parasitoids, including *A. colemani* (Kim et al. 2006, Stara et al. 2011, Bengochea et al. 2012). We suggest that due to the relatively low toxicity of lambda cyhalothrin to *A. colemani* in our laboratory experiments, it may be possible to simultaneously use lambda-cyhalothrin and the parasitoid A. colemani in integrated pest management (IPM) programs against aphids. If both are to control the same pest, lambda cyhalothrin could be applied only when the damage is imminent, since the parasitoid would keep aphid populations below the economic threshold for most of the time. Chemical control provides adequate control, but does not necessarily eradicate prey, allowing parasitoids to remain effective at low densities. It is also possible that lambda-cyhalothrin and *A. colemani* could be used in the same system for different target pests.

Additional work is needed to confirm this hypothesis given that other factors in the field may alter the interaction of the natural enemy with the insecticide (Banks and Stark 2011).

In summary, the reduced-risk bioinseciticde spinosad exhibited higher acute lethal toxicity to the parasitoid *A. colemani* than the conventional insecticides imidacloprid and lambda-cyhalothrin. The predicted residual exposure with the spinosad label rate was nearly 50-fold higher than the spinosad LC_{50} we recorded to *A. colemani*, emphasizing its potential risk to this biocontrol agent. In contrast, predicted residual exposures from label-rate applications of imidacloprid and lambda-cyhalothrin were close or below the LC_{50} values estimated from our bioassays, and calculated HQ values were below or near 1. Both conventional insecticides compromised aphid longevity and parasitism, but lambda-cyhalothrin did not affect the viability of parasitized hosts or parasitoid progeny production, unlike imidacloprid. Thus, the pyrethroid lambda-cyhalothrin exhibited the most favorable safety profile to *A. colemani*. Our results suggest that there may be opportunities to use this insecticide in conjunction with *A. colemani* in IPM programs against aphid pests.

Acknowledgments

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References

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 3: 302–303.

Araya, J. E., M. Araya, and M. A. Guerrero. 2010. Effects of some insecticides applied in sublethal concentrations on the survival and longevity of *Aphidius ervi* (Haliday) (Hymenoptera: Aphidiinae) adults. Chil. J. Agric. Res. 70: 221–227.

Banks, J. E. and, J. D. Stark. **2011**. Effects of a nicotinic insecticide, imidacloprid, and vegetation diversity on movement of a common predator, *Coccinella septempunctata*. Biopestic Int, 7: 113–122.

Barbosa, F. S., G. L. D. Leite, S. M. Alves, A. F. Nascimento, V. de A. D'Ávila and, C. A. D. Costa. 2011. Insecticide effects of *Ruta graveolens*, *Copaifera langsdorffii* and *Chenopodium ambrosioides* against pests and natural enemies in commercial tomato plantation. Acta Sci. Agron. 33: 37–43.

Barbosa, W. F., H. V. Tomé, R. C. Bernardes, M. A. Siqueira, G. Smagghe, and R. N. Guedes. 2015. Biopesticide-induced behavioral and morphological alterations in the stingless bee *Melipona quadrifasciata*. Environ. Toxicol. Chem. 34: 2149–2158.

Bengochea, P., P. Medina, F. Amor, M. Cánovas, P. Vega, R. Correia, F. García, M. Gómez, F. Budia, E. Viñuela, et al. 2012. The effect of emamectin benzoate on two parasitoids, *Aphidius colemani* Viereck (Hymenoptera: Braconidae) and *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae), used in pepper greenhouses. Span. J. Agric. Res. 10: 806–814.

Biondi, A., V. Mommaerts, G. Smagghe, E. Viñuela, L. Zappalà, and N. Desneux. 2012. The non-target impact of spinosyns on beneficial arthropods. Pest Manag. Sci. 68: 1523–1536.

Blackman, R. L., and V. F. Eastop. 2000. Aphids on the World's crops: an identification and information guide. 2nd ed. John Wiley & Sons Ltd., Chichester, UK.

Bostanian, N. J., M. Akalach, and H. Chiasson. **2005**. Effects of a *Chenopodium*-based botanical insecticide/acaricide on *orius insidiosus* (Hemiptera: Anthocoridae) and *aphidius colemani* (Hymenoptera: Braconidae). Pest Manag. Sci. 61: 979–984.

Chandler, D., S. B. Alastair, G. M. Tatchell, G. Davidson, J. Greaves, and W. P. Grant. 2011. The development, regulation and use of biopesticides for integrated pest management. Phil. Trans. R. Soc. B. 366: 1987–1998.

Charles, J. J., and T. D. Paine. 2016. Fitness effects of food resources on the polyphagous aphid parasitoid, *Aphidius colemani* viereck (Hymenoptera: Braconidae: Aphidiinae). Plos One. 11: e0147551.

Clarke, S. R., J. F. Negron, and G. L. Debarr. 1992. Effects of four pyrethroids on scale insect (Homoptera) populations and their natural enemies in loblolly and shortleaf pine seed orchards. J. Econ. Entomol. 85: 1246–1252.

Cordeiro, E. M. G., I. L. T. Moura, M. A. M. Fadini, and R. N. C. Guedes. 2013. Beyond selectivity: are behavioral avoidance and hormesis likely causes of pyrethroid-induced outbreaks of the southern red mite *Oligonychus ilicis*? Chemosphere. 93: 1111–1116.

Crouse, G. D., T. C. Sparks, J. Schoonover, J. Gifford, J. Dripps, T. Bruce, L. L. Larson, J. Garlich, C. Hatton, R. L. Hill, et al. 2001. Recent advances in the chemistry of spinosyns. Pest Manag. Sci. 57: 177–185.

Cutler, G. C. 2013. Insects, insecticides and hormesis: evidence and considerations for study. Dose. Response. 11: 154–177.

Cutler, G. C., and R. N. C. Guedes. 2017. Occurrence and significance of insecticide-induced hormesis in insects. in pesticide dose: effects on the environment and Target and non-target organisms. ACS Symp. Ser. 1249: 101–119.

Dara, S. K. 2017. Managing western flower thrips (Thysanoptera: Thripidae) on lettuce and green peach aphid and cabbage aphid (Hemiptera: Aphididae) on broccoli with chemical insecticides and the entomopathogenic fungus *Beauveria bassiana* (Hypocreales: Clavicipitaceae). Open Plant. Sci. J. 10: 21–28.

Desneux, N., H. Rafalimanana, and L. Kaiser. 2004. Dose-response relationship in lethal and behavioural effects of different insecticides on the parasitic wasp *Aphidius ervi*. Chemosphere. 54: 619–627.

Desneux, N., A. Decourtye, and J. M. Delpuech. 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52: 81–106.

Devonshire, A. L., and G. D. Moores. 1982. A carboxylesterase with broad substrate specificity causes organophosphorus, carbamate and pyrethroid resistance in peach-potato aphids (*Myzus persicae*). Pestic. Biochem. Physiol. 18: 235–246.

Eijaza, S., M. F. Khan, K. Mahmood, M. Anwar, A. Alamgir, and I. Khatri. 2015. Studies on degradation and efficacy of synthetic pesticides on okra crop. Acad. J. Entomol. 8: 12–18.

Environmental Protection Agency (EPA). 2017. Reduced Risk and Organophosphate Alternative Decisions for Conventional Pesticides [accessed July 21, 2017] https://www.epa.gov/pesticide-registration/reduced-risk-and-organophosphate-alternative-decisions-conventional

EPPO. 1999. Decision-making scheme for the environmental risk assessment of plant protection products. Bull. OEPP/EPPO. 23: 59–65.

Field, L. M., A. L. Devonshire, and B. G. Forde. 1988. Molecular evidence that insecticide resistance in peach-potato aphids (*Myzus persicae* Sulz.) results from amplification of an esterase gene. Biochem. J. 251: 309–312.

Gentz, M. C., G. Murdoch, and G. F. King. 2010. Tandem use of selective insecticides and natural enemies for effective, reduced-risk pest management. Biol. Control. 52: 208–215.

Guedes, R. N. C., and G. C. Cutler. 2014. Insecticide-induced hormesis and arthropod pest management. Pest Manag. Sci. 70:690–697.

Guedes, R. N., G. Smagghe, J. D. Stark, and N. Desneux. 2016. Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. Annu. Rev. Entomol. 61: 43–62.

Hardin, M. R., B. Benrey, M. Coll, W. O. Lamp, G. K. Roderick, and P. Barbosa. 1995. Arthropod pest resurgence: an overview of potential mechanisms. Crop Prot. 14:3–18.

Hassan, S. A., F. Bigler, P. Blaisinger, H. Bogenschütz, J. Brun, P. Chiverton, E. Dickler, M. A. Easterbrook, P. J. Edwards, W. D. Englert, et al. 1985. Standard methods to test the side-effects of pesticides on natural enemies of insects and mites developed by the IOBC/WPRS working group ‘pesticides and beneficial organisms’. EPPO Bull. 15: 214–255.

Hoyt, S. C. 1969. Integrated chemical control of insects and biological control of mites on apple in Washington. J. Econ. Entomol. 62: 74–86.

Hull, L. A., E. H. Beers, and R. L. Meagher Jr. **1985**. Impact of selective use of the synthetic pyrethroid fenvalerate on apple pests and natural enemies in large-orchard trials. J. Econ. Entomol. 78: 163–168.

Khatri, D., X. Z. He, and Q. Wang. 2016. Trade-off between fitness gain and cost determines profitability of a peach aphid parasitoid. J. Econ. Entomol. 109: 1539–1544.

Khatri, D., X. Z. He, and Q. Wang. 2017. Effective biological control depends on life history strategies of both parasitoid and its host: evidence from *Aphidius colemani-myzus persicae* System. J. Econ. Entomol. 110: 400–406.

Kim, J. J., D. K. Seo, and G. H. Kim. 2006. Evaluation of toxicity of 83 pesticides against aphid parasitoid, *Aphidius colemani* (Hymenoptera: Braconidae), and control effects of the green peach aphid, *Myzus persicae* with a combination of aphid parasitoid and pesticides. Korean J. Appl. Entomol. 45: 217–226.

Lester, H. A., M. I. Dibas, D. S. Dahan, J. F. Leite, and D. A. Dougherty. 2004. Cys-loop receptors: new twists and turns. Trends Neurosci. 27: 329–336.

Little, S. C., O. Edwards, A. R. Van Rooyen, A. Weeks, and P. A. Umina. 2017. Discovery of metabolic resistance to neonicotinoids in green peach aphids (*Myzus persicae*) in Australia. Pest Manag. Sci. doi:10.1002/ps.4495

Longley, M., and J. D. Stark. 1996. Analytical techniques for quantifying direct, residual, and oral exposure of an insect parasitoid to an organophosphate insecticide. Bull. Environ. Contam. Toxicol. 57: 683–690.

Miles, M. 2006. The effects of spinosad on beneficial insects and mites used in integrated pest management systems in greenhouses. IOBC/wprs Bulletin. 29: 53–59.

Roubos, C. R., C. Rodriguez-Saona, R. Holdcraft, K. S. Mason, and R. Isaacs. **2014**. Relative toxicity and residual activity of insecticides used in blueberry pest management: mortality of natural enemies. J. Econ. Entomol. 107: 277–285.

Salgado, V. L. 1997. The modes of action of spinosad and other insect control products. Down to Earth [Dow AgroSciences]. 52: 35–43.

Sampaio, M. V., V. H. P. Bueno, and J. C. Van Lenteren. 2001. Preferência de *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae) por *Myzus persicae* (Sulzer) e *Aphis gossypii* Glover (Hemiptera: Aphididae). Neotrop. Entomol. 30: 655–660.

Sparks, T. C., G. D. Crouse, and G. Durst. 2001. Natural products as insecticides: the biology, biochemistry and quantitative structure-activity relationships of spinosyns and spinosoids. Pest Manag. Sci. 57: 896–905.

Stara, J., J. Ourednickova, and F. Kocourek. 2011. Laboratory evaluation of the side effects of insecticides on *Aphidius colemani* (Hymenoptera: Aphidiidae), *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae), and *Neoseiulus cucumeris* (Acari: Phytoseidae). J. Pest. Sci. 84: 25–31.

Stary, P. 1975. *Aphidius colemani* Viereck: its taxonomy, distribution and host range (Hymenoptera: Aphidiidae). Acta Entomol. Bohemoslov. 72: 156–163.

Stern, V. M. R. F., R. Smith, R. Van den Bosch, and K. Hagen. 1959. The integration of chemical and biological control of the spotted alfalfa aphid: the integrated control concept. Calif. Agric. 29: 81–101.

Szczepaniec, A., S. F. Creary, K. L. Laskowski, J. P. Nyrop, and M. J. Raupp. **2011**. Neonicotinoid insecticide imidacloprid causes outbreaks of spider mites on elm trees in urban landscapes. Plos One. 6: e20018.

Takahashi, Y., T. Kojimoto, H. Nagaoka, Y. Takagi, and M. Oikawa. 2005. Tests for evaluating the side effects of chlorothalonil (TPN) and spinosad on the parasitic wasp (*Aphidius colemani*). J. Pest Sci.30: 11–16.

Tingey, W. M., and J. T. Andaloro. 1983. Green peach aphid. New York State IPM Program. 760–761.

Tomé, H. V., W. F. Barbosa, G. F. Martins, and R. N. Guedes. 2015. Spinosad in the native stingless bee *Melipona quadrifasciata*: regrettable non-target toxicity of a bioinsecticide. Chemosphere. 124: 103–109.

Tremblay, E., A. Bélanger, M. Brosseau, and G. Boivin. **2008**. Toxicity and sublethal effects of an insecticidal soap on *Aphidius colemani* (Hymenoptera: Braconidae). Pest Manag. Sci. 64: 249–254.

Umina, P. A., O. Edwards, P. Carson, A. Van Rooyen, and A. Anderson. 2014. High levels of resistance to carbamate and pyrethroid chemicals widespread in Australian *Myzus persicae* (Hemiptera: Aphididae) populations. J. Econ. Entomol. 107: 1626–1638.

Vam Emden, H. F., and R. Harrington. 2007. Aphids as crops pests. CABI, Cambridge, MA.

Van Driesche, R. G., S. Lyon, J. P. Sanderson, K. C. Bennett, E. J. Stanek III, and R. Zhang. 2008. Greenhouse trials of *Aphidius colemani* (Hymenoptera: Braconidae) banker plants for control of aphids (Hemiptera: Aphididae) in greenhouse spring floral crops. Fla. Entomol. 91: 583–591.

Van Schelt, J. 1994. The selection and utilization of parasitoids for aphid control in glasshouses. Proc. Exp. Appl. Entomol. NEV Amsterdam. 5: 151–157.

Vargas, E. V. R. 2010. Ciclo biologico y cria de (*Aphidius colemani*) parasitoide de adultos del Pulgon Harinoso (*Brevicoryne brassicae*), plaga del brocolis. Bachelor's thesis, Escuela Superior Politecnica de Chimborazo. Riobamba, Equador.

Vásquez, G. M., D. B. Orr, and J. R. Baker. 2006. Efficacy assessment of *Aphidius colemani* (Hymenoptera: Braconidae) for suppression of *Aphis gossypii* (Homoptera: Aphididae) in greenhouse-grown chrysanthemum. J. Econ. Entomol. 99: 1104–1111.

Villaverde, J. J., B. Sevilla-Morán, P. Sandín-España, C. López-Goti, and J. L. Alonso-Prados. 2014. Biopesticides in the framework of the European pesticide regulation (EC) No. 1107/2009. Pest Manag. Sci. 70: 2–5.

Watson, G. B. 2001. Actions of insecticidal spinosyns on γ-aminobutyric acid responses from small-diameter cockroach neurons. Pestic. Biochem. Physiol. 71: 20–28.

ARTIGO 2

Lambda-cyhalothrin exposure, mating behavior and reproductive output of pyrethroid-susceptible and resistant lady beetles (*Eriopis connexa*)

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Abstract

Insecticide resistance is a ubiquitous consequence in arthropod pest species subjected to insecticide use in agricultural fields. The widespread use of insecticides also allows selection of insecticide resistance among nontarget arthropod species, such as natural enemies. Nonetheless, the potential consequences of sublethal insecticide exposure in resistant natural enemies are frequently neglected. The detected pyrethroid resistance in the lady beetle *Eriopis connexa* (Germar) in Brazilian agricultural fields afford the opportunity of assessing the consequences of the sublethal exposure of the broadly used pyrethroid insecticide lambda-cyhalothrin in the mating behavior and reproductive output of susceptible and resistant populations of this species. Survival bioassays with lambda-cyhalothrin allowed estimation of sublethal exposure times at the maximum labeled rate to assess the reproductive consequences of such exposure in pyrethroid-susceptible and resistant strains of *E. connexa*. Such sublethal exposures led to significant difficulties in female mounting by male of both populations. Pyrethroid exposure also extended the duration of female body tremulation and of coupling, while latency to mate, tremulation, coupling and female shaking to dislodge the males after coupling differed between strains with interacting effect of insecticide exposure only for the latter behavior. As a consequence mainly of latency to mate, progeny production was significantly smaller among pyrethroid-resistant females where lambda-cyhalothrin exposure exhibited a negligible effect. Thus, population rather than exposure itself prevailed in determining reproductive output of *E. connexa* and pyrethroid resistance incurred in reproductive costs in this species that may counterweight the benefits of its survival.

Keywords: Biocontrol agents, predator, insecticide resistance, insecticide stress, sublethal exposure.

Introduction

Insecticide resistance is a frequent consequence of pest control in crop production systems by means of insecticide use, and overuse (Guedes et al. 2016, 2017a,b). This phenomenon is essentially a genetic change in response to selection by insecticide use among individuals of a species (Sawicki 1987, Whalon et al. 2008, IRAC 2017). The management shortcoming associated with insecticide resistance is that its development can potentially compromise chemical control used against a pest species (Sawicki 1987, Whalon et al. 2008, IRAC 2017, Guedes 2017). Nonetheless, the phenomenon may also take place among species not targeted by the insecticide application, including among them natural enemies of the pest species (Croft and Morse 1979, Hoy 1990, Bielza 2016).

Selection for insecticide resistance is usually associated with the use of lethal insecticide concentrations eliminating susceptible individuals. However, sublethal concentrations are also important in selecting resistant genotypes favoring the survival and reproduction of resistant individuals (Guedes et al. 2017b). Natural enemies are frequently subject to sublethal insecticide concentrations as non-target species. Furthermore, sublethal exposure may also be achieved due to the peculiar behavioral traits of the non-targeted species that may minimize exposure as compared with that of the target pest species (Cordeiro et al. 2010, Lima et al. 2013).

Lady beetles (Coleoptera: Coccinelidae) are common aphid predators in agricultural fields and used as a pest management tactic in aiding the management of several aphid species. An example is the lady beetle species *Eriopis connexa* (Germar), an important aphid predator widely distributed in various crop ecosystems in South America (e.g., maize, sorghum, soybean, wheat) and introduced into North America, which is frequently exposed to pyrethroid insecticides (Rodrigues et al. 2013a, Costa et al. 2017). This scenario has led to a relatively high frequency of insecticide resistance among populations of *E. connexa* in field crops (Costa et al. 2017). As a consequence, pyrethroid resistance was observed in *E. connexa* with an autosomal and semi-dominant pattern of inheritance associated with enhanced activity of detoxification enzymes (Rodrigues et al. 2013a, 2013b, 2014). Life-history and behavioral differences were also reported in pyrethroid resistant *E. connexa* and the resistance was not restricted to a single pyrethroid, but extended to different members of this insecticide group (Torres et al. 2015). However, the effect of sublethal exposure on pyrethroid-resistant *E. connexa* remains unknown, particularly regarding the species reproductive behavior, what we targeted in the present study.

Survival bioassays with the pyrethroid lambda-cyhalothrin were performed in a pyrethroid-susceptible and a resistance population of the lady beetle *E. connexa*. Such bioassays allowed the determination of a suitable sublethal length of exposure to the insecticide label rate to assess the potential behavioral effects of such exposure and its reproductive consequences in both populations. We hypothesized that the pyrethroid was likely to impair mating in both populations compromising their progeny production, particularly in the susceptible population, as the resistant population would be better able to cope with the sublethal stress imposed.

Material and methods

Insects

The two populations (lambda-cyhalothrin resistant and susceptible) of the lady beetle *E. connexa* used in the experiments were obtained from populations maintained at the Entomology Unit of the Federal Rural University of Pernambuco in Recife (State of Pernambuco, Brazil). Both populations were originally field-collected by 2009 and have been maintained in laboratory with periodical introduction of newly-collected field insects and tested for insecticide resistance, as described elsewhere (Rodrigues et al. 2013a, 2014, Torres et al. 2015).

The insecticide-susceptible population was originally collected from cotton fields in Frei Miguelinho county (07°55′90.1″S and 35°51′45.6″W; State of Pernambuco, Brazil). The pyrethroid resistant population was originally collected from cabbage fields subject to intensive pyrethroid use in Viçosa county (20°75′73″S and 42°86′96″W; State of Minas Gerais, Brazil). The former population has always been maintained free from insecticide exposure and its susceptibility status is periodically checked. The pyrethroid resistant population also periodically received field insects, and the resistance status is also periodically checked with eventual selection for resistance to the pyrethroid lambda-cyhalothrin to maintain the original levels of insecticide resistance; the level of pyrethroid resistance has remained around 40-fold compared with the susceptible population (Rodrigues et al. 2014, Spíndola et al. 2013, Torres et al. 2015).

Both lady beetle populations were maintained apart from each other in Viçosa under controlled environmental conditions of 25 ± 1 °C temperature, 70 ± 10% relative humidity, and 12:12 h (L:D) photoperiod. The insects were provided with eggs of the Mediterranean flour moth [*Ephesti*a (= *Anagasta*) *kuehniella* (Zeller) (Lepidoptera: Pyralidae)] ad libitum, and collard green leaves infested with cabbage aphids (*Brevicoryne brassicae* (L.) (Hemipera: Sternorrhyncha: Aphididae)) were provided every other day. In addition, 10% honey solution was also provided during the adult stage of the lady beetles to enhance reproduction.

Survival bioassays

Adult lady beetles (5–7 days-old) were subjected to time- and concentration-mortality bioassays with a commercial formulation of the pyrethroid insecticide lambda-cyhalothrin (Karate® 50 EC; 50 g a.i./L, encapsulate suspension; Syngenta Prot. Cult., São Paulo, SP, Brazil). The aqueous insecticide solution (2 mL) was applied to glass vials (250 mL volume; 178.15 cm^2 of inner surface), which were maintained in a heavy-duty rotator (Roto-Torque model 7637, ColeParmer, Vernon Hills, IL, USA) for rotation until drying to coat the inner walls of each jar with insecticide residue. The upper portion of each glass vial was coated with Teflon PTFE (DuPont, Wilmington, DE, USA) to prevent the insects from escaping. Ten adult insects were placed in each vial at the concentrations of 0, 10, 75, 150, 300 and 600 mL commercial formulation/ha, corresponding to 0, 5.0, 37.5, 75.0, 150.0 and 300.0 ng a.i./cm^2 of lambda-cyhalothrin; the highest concentration corresponds to the maximum label rate for the agriculture field use of this insecticide in Brazil (MAPA, 2017). Mortality was recorded every 15 min for the 1st hour, then at 2, 4, 8, 24 and 48 h. The insects surviving longer than 48 h were transferred to 500 mL plastic containers (used for the regular rearing of the insects) and their mortality was recorded daily until no more live insects remained. The 48 h upper threshold of exposure was used because lambda-cyhalothrin is a fast-acting (pyrethroid) insecticide that requires short exposure for insecticidal activity (Sunderland 2010, Casida and Durkin 2013). Insect mortality was recorded until no more live insects remained and the insects were considered as dead when unable to respond to prodding with a fine hairbrush (Santos et al. 2016). The bioassay was replicated three times for each population (and insecticide concentration), with a vial of 10 insects considered as a replicate.

Reproductive bioassays

Each adult insect was maintained separate from other insects from emergence until the females started exhibiting abdomen enlargement enabling their sex-recognition (7-days after emergence), as no sexual dimorphism is evident in this species. Subsequently, virgin females and males of the lady beetle (7 days-old) were exposed to the maximum label rate of lambda-cyhalothrin (i.e., 30 g a.i./ha corresponding to 300 ng a.i./cm^2) for either 45 min or 48 h depending on the population (susceptible or resistant, respectively), which were the longer lengths of exposure not compromising the survival of each population, as observed in the survival bioassays previously described. The use of different lengths of exposure with the same concentration allowed for a similar level of sublethal effect for both populations. Suitable water-exposed insects were used as controls in each assessment of each exposure time.

The insecticide exposure followed the methods described for the survival bioassays using a single insecticide concentration and exposure periods specific for each population. At least 20 virgin couples were obtained for assessing their mating behavior and the female reproductive output. However, after insecticide exposure the insects were removed and couples were maintained individually in Petri dish arenas (9 cm diameter) with their bottoms covered with filter paper and inner walls coated with Teflon. The mating behavior of each couple was digitally recorded from their initial release in the arena until the eventual separation after copulation using a digital video camcorder (HDRXR520V, Sony, Tokyo, Japan). The behaviors were recorded based on preliminary observations and included: walking (i.e., latency to interact), contact between female and male, mounting of female by male, female body tremulation, copulation, female body shaking with mounted male, and separation of the couple. If the male initially paired with the female did not start interaction within 15 min, it was replaced as were the females that failed to mate with three consecutive males offered.

The males of each coupling pair were discarded after the mating and the females were daily observed until they did not lay eggs for a succession of 10 consecutive days. The eggs laid by each female were removed from the Petri dish and observed for up to 10 days after hatching started in each egg cluster.

Statistical analyses

The adult longevity results obtained with the survival bioassays were subjected to survival analyses using Kaplan-Meier estimators to obtain the median longevity of the insects from both populations subjected to the different observations (PROC LIFETEST; SAS software, SAS Institute, Cary, NC, USA). The estimated median lethal times (LT_{50}) of each combination of insect population and insecticide concentration were subsequently subjected to regression analysis with insecticide concentration as the independent variable (TableCurve 2D, Systat, San Jose, CA, USA).

The behavior sequence and frequency during mating were depicted as simplified ethograms based on 1st order behavioral transitions. The frequency of the behavioral transitions for each population (pyrethroid-susceptible and resistant) and insecticide treatment (treated x nontreated) were tested using $\chi2$ contingency table (4 × 7; $P < .05$; PROC FREQ; SAS). Eventual pointed differences in the proportion of behavioral transitions between treated and untreated couples of each population were compared using $\chi2$-test with Yates' correction for continuity ($P < .05$).

The time budget data of each behavior were checked for the assumptions of homoscedasticity and normality and the latency to interact and duration of mounting were log10-transformed. The data were subsequently subjected to two-way multivariate analysis of variance (2 populations x 2 exposure conditions) followed by univariate analysis of variance for each parameter, when appropriate (PROC GLM with MANOVA statement; SAS). Tukeys's HSD test ($P < .05$) was used to separate means when a fixed effect was significant.

Total progeny production was also checked for the assumptions of analysis of variance and was log10-transformed. Univariate analysis of variance was subsequently carried out (PROC GLM, SAS). The results of daily progeny emergence were subjected to regression analysis with time as independent variable and the descriptive models were tested and selected based on parsimony, F and P-values, and steep increase in R^2 with model complexity using the curve-fitting procedure of the software TableCurve 2D (Systat, San Jose, CA, USA). Correlation analysis between latency for coupling and progeny production was also performed using SAS, as was the correlation between coupling time and progeny production (PROC CORR, SAS).

Results

Adult survival

The adult longevity of susceptible lady beetles varied with pyrethroid exposure ($\chi^2 = 47.53$, df = 5, $P < .001$), unlike pyrethroid resistant lady beetles exposed to increasing concentrations of lambdacyhalothrin ($\chi^2 = 6.75$, df = 5, $P = .24$). The median longevity estimates (LT_{50}) obtained in the survival analyses provided an exponential decrease with lambda-cyhalothrin concentration for the susceptible population, but no significant variation on longevity was observed for the resistant insects regardless of the insecticide concentration, which exhibited a median longevity of 43.67 ± 2.11 days (Fig. 1).

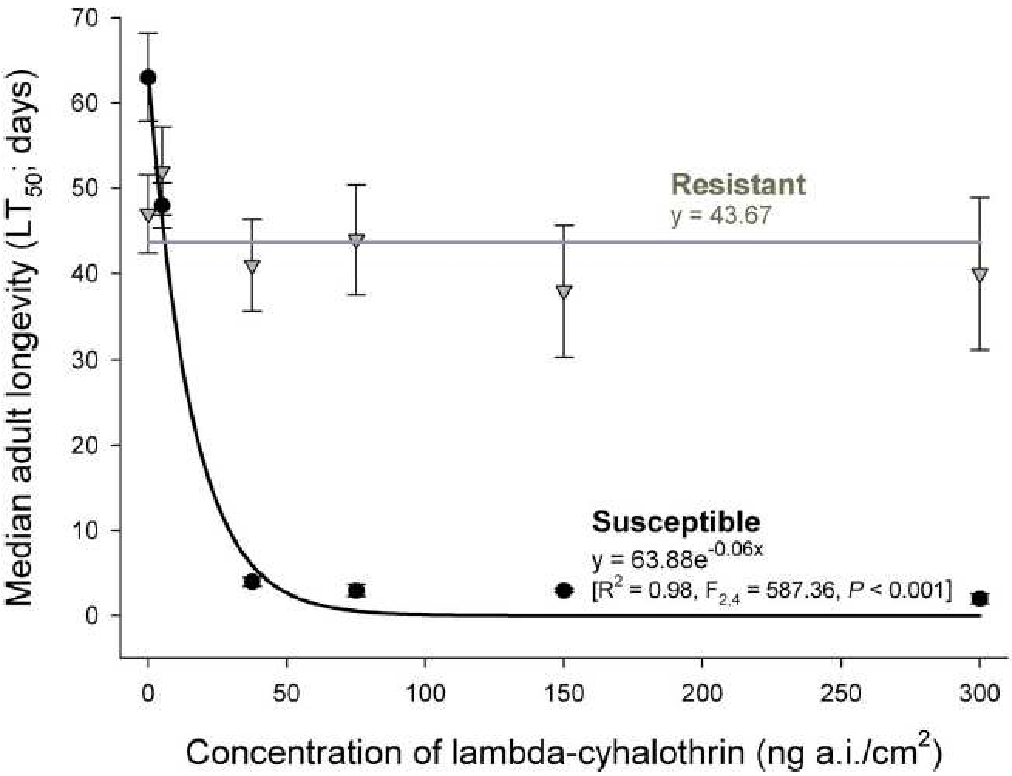

Fig. 1. Median adult longevity (LT_{50}) (± SE) of pyrethroid-susceptible and –resistant populations of the lady beetle *Eriopis connexa* exposed to increasing concentrations of lambda-cyhalothrin.

Mating behavior: sequential analyses

The lady beetle simplified ethograms representing the 1st order behavioral transitions were represented as diagrams of mating for each population and condition of insecticide exposure (Fig. 2). The overall frequency of these 1st order transitions significantly differ among treatments (χ^2 = 237.83, df = 36, P < .001). The major difference observed concerned the female mounting by the male when the insects were exposed to lambda-cyhalothrin, regardless of the population. Exposed lady beetles were more successful in the mounting behavior than unexposed ones where subsequent attempts to mount was required at 2.7- and 6.7-higher frequency for the resistant and susceptible populations respectively (χ^2 ≥ 4.91, df = 1, P < .01) (Fig. 2).

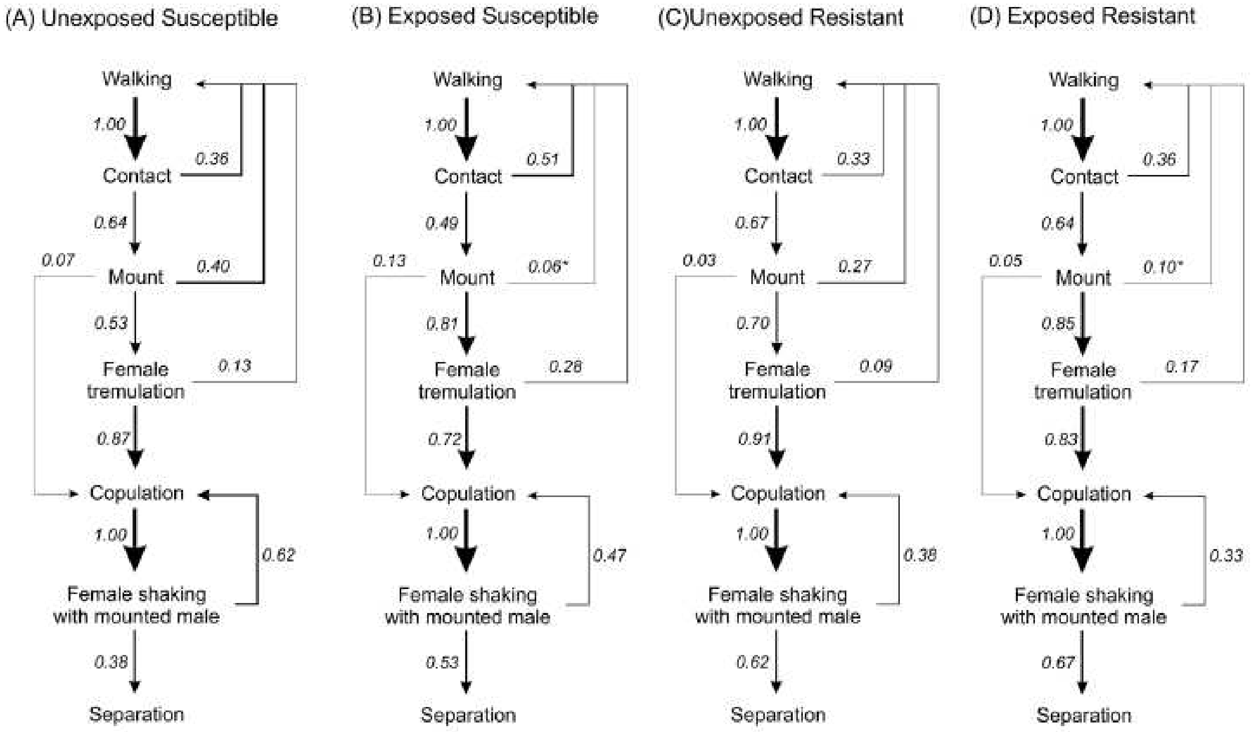

Fig. 2. Ethogram of the mating behavior of the lady beetle *Eriopis connexa* subjected or not to lambda-cyhalothrin exposure represented as first order transition diagrams. The solid arrows indicate each behavioral transition. The relative thickness of each arrow represents the frequency of each behavioral transition, which is indicated in italic. Only the significantly differing transitions between exposed and unexposed insects are indicated by an asterisk (χ^2 test at $P < .05$).

Mating behavior: time budgets

The two-way multivariate analyses of variance indicated significant interaction between population and insecticide exposure for the recorded behaviors (Wilks' lambda = 0.84, $F_{app.} = 2.95$, $df_{num,den} = 5; 80$, $P = .02$). The subsequent (univariate) analyses of variance performed indicated significant differences in all behaviors except mounting time ($F_{3,84} = 0.12$, $P = .94$). Latency to interact varied significantly only between populations ($F_{1,84} = 6.33$, $P = .01$), while the duration of female tremulation and of coupling varied among insecticide treatments ($F_{1,84} \geq 4.54$, $P \leq .04$) and populations ($F_{1,84} \geq 9.95$, $P \leq .002$). The interaction between population and insecticide exposure was significant only for the duration of female shaking ($F_{3,84} = 3.20$, $P = 0.03$). The pyrethroid resistant population exhibited longer latency to interact (Fig. 3A), the females tremulated for longer (Fig. 3B), and coupling was shorter (Fig. 3C) than in the susceptible population. Insecticide exposure decreased the time spent by the females tremulating (Fig. 3B), and extended the coupling time (Fig. 3C). The females shacked their body to dislodge males for longer if they were unexposed resistant insects, and shorter if exposed resistant or unexposed susceptible (Fig. 3D).

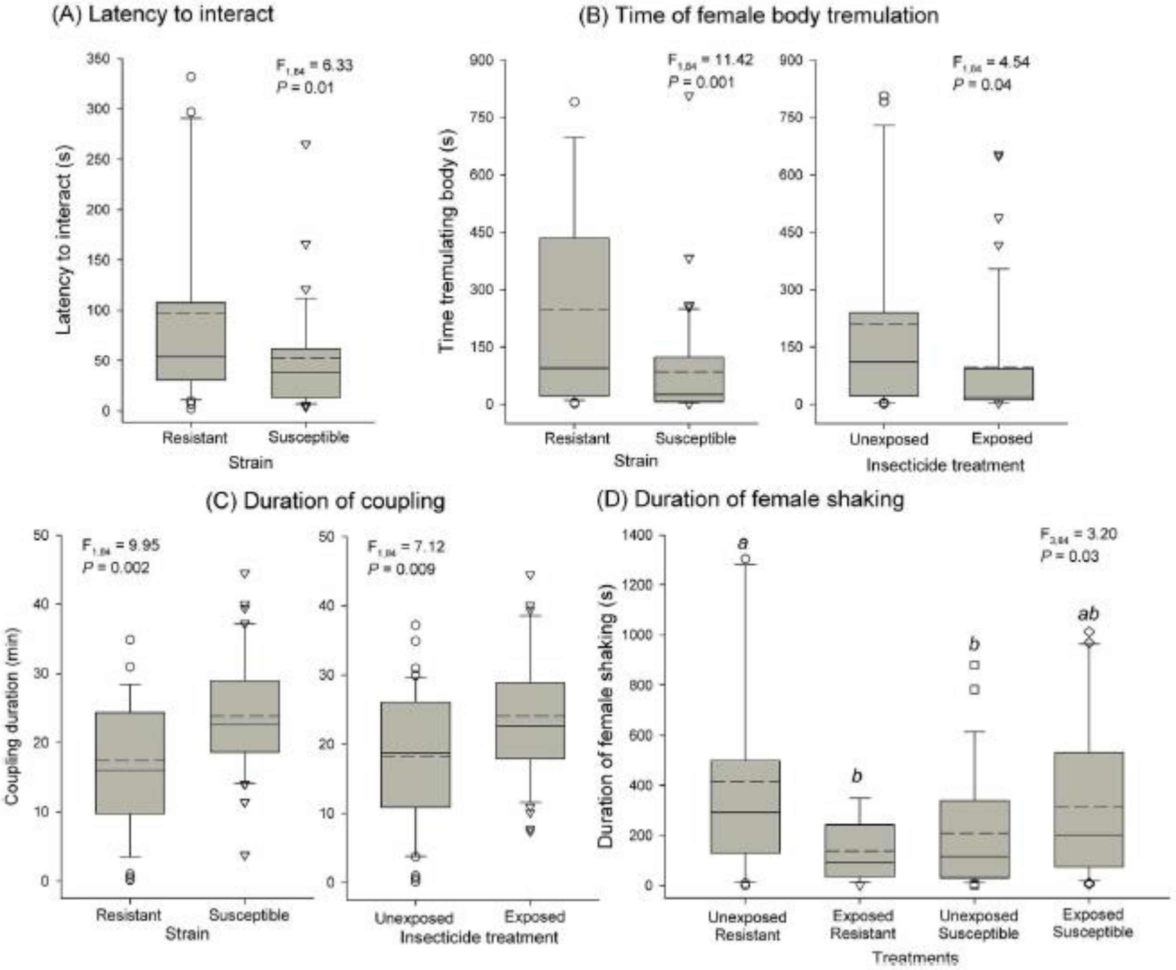

Fig. 3. Duration (±SE) of latency to interact (A), female body tremulation (B), coupling (C), and female shaking to dislodge the male at the end of coupling (D) in couples of pyrethroid-susceptible and -resistant lady beetle *Eriopis connexa* exposed or not to lambda-cyhalothrin. Box plots indicate the median (solid line), mean (dashed line), and dispersal (lower and upper quartiles, and outliers) of the duration values. The box plots with similar letter in (D) are not significantly different by Tukey's HSD test ($P < .05$).

Progeny production

The total larva progeny produced per female lady beetle differed between populations regardless of insecticide exposure, with the pyrethroid susceptible females exhibiting significantly higher overall fertility than the resistant females

(Fig. 4 insert). However, when daily progeny production was considered, the effect of insecticide exposure was also noticeable in addition to the population effect (Table 1; Fig. 4). The susceptible females were again more fertile than the pyrethroid-resistant females exhibiting a higher peak of progeny production and maintaining a higher rate of progeny production for much longer (up to 30 days). Exposure to lambda-cyhalothrin exhibited a milder but significant effect by shortening the period of progeny production for the susceptible population and reducing the peak of production for the resistant population (Fig. 4). The consequence of such profiles is a reduced rate of population growth for the resistant insects with lambda-cyhalothrin exposure also compromising population growth.

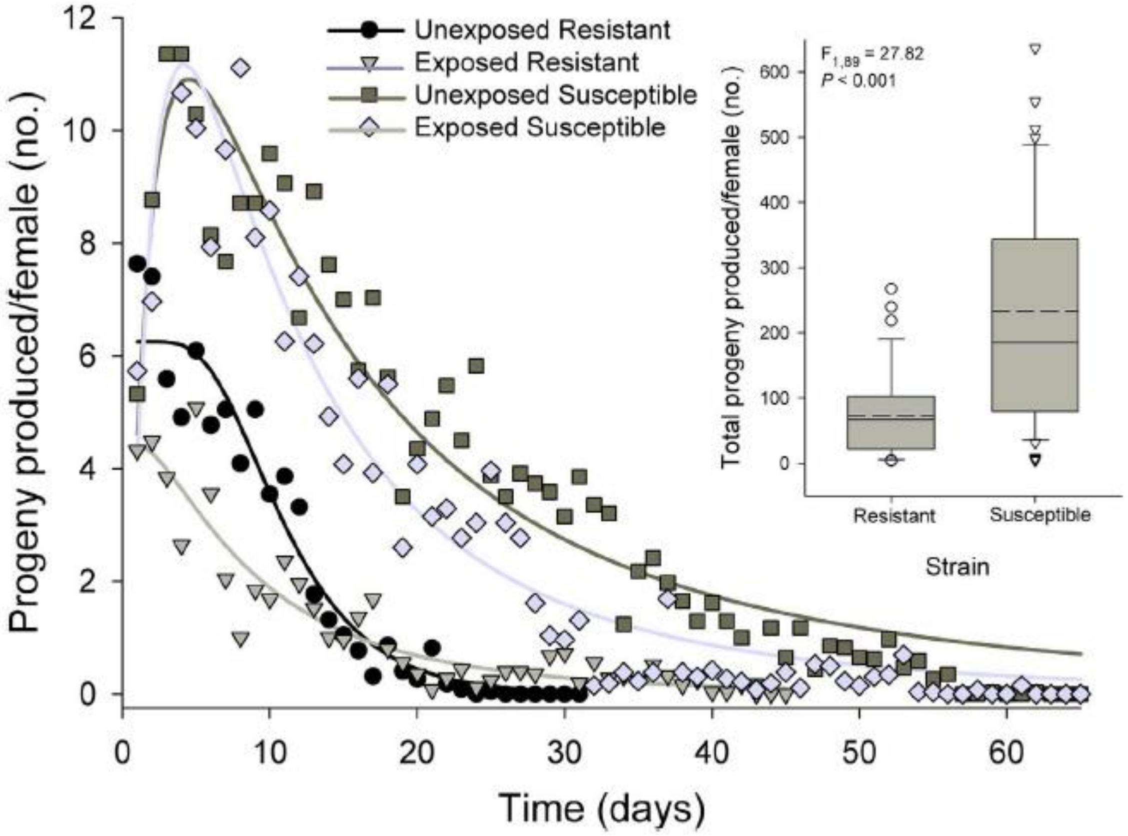

Fig. 4. Daily progeny production per adult couple of pyrethroid-susceptible and –resistant lady beetle *Eriopis connexa* subjected or not to lambda-cyhalothrin exposure. The symbols indicated average determinations of individual pairs in each condition; the equation parameters of each regression curves are presented in Table 1. Insert: Box plot of total progeny produced per couple of *E. connexa*; box plots indicate the median (solid line), mean (dashed line), and dispersal (lower and upper quartiles, and outliers) of the duration values.

Table 1. Summary statistics of non-linear regressions curves of progeny produced per female (Fig. 4). All of the equation parameters are significant at $P < 0.05$ by Student's *t* test.

Population	Insecticide treament	Model	Parameters (± SE)			df_{error}	F	*P*	R^2
			a	*B*	*c*				
Resistant	Unexposed	Log normal cumulative [3 parameters]	6.25 ± 0.28	10.78 ± 0.53	-0.42 ± 0.06	28	247.99	< 0.001	0.94
	Exposed	Log normal cumulative [3 parameters]	4.46 ± 0.38	8.49 ± 1.13	-0.83 ± 0.11	42	145.25	< 0.001	0.86
Susceptible	Unexposed	Log normal [3 parameters]	10.92 ± 0.33	4.49 ± 0.25	1.14 ± 0.04	70	476.27	< 0.001	0.93
	Exposed	Log normal [3 parameters]	11.19 ± 0.34	4.13 ± 0.20	1.00 ± 0.03	68	543.89	< 0.001	0.94

Latency to couple and progeny production

Among the behaviors recorded during mating, only the pooled data of latency to coupling correlated significantly with progeny production (r = −0.20, P = 0.05, n = 88). A longer latency to couple was associated with lower progeny production per female, regardless of the population and insecticide exposure. Coupling duration was not significantly correlated with progeny production when data were pooled (r = 0.08, P = 0.44, n = 88), nor when each strain was considered separately (Susceptible: r = −0.01, P = 0.93, n = 50; resistant: r = −0.26, P = 0.12, n = 38).

Discussion

Sublethal insecticide exposure is a frequent condition faced in crop production fields by natural enemies in general, and biological control agents in particular, as consequence of insecticide use against arthropod pest species. The consequences of such exposure potentially differ between populations of biocontrol agents, particularly if they exhibit distinct susceptibility to insecticides. This knowledge gap was addressed in our study in which we expected that the pyrethroid lambda-cyhalothrin would more likely impair the susceptible population considering a similar sublethal level of exposure. Curiously, insecticide exposure was not as important as the insect population, regardless the said exposure, although sublethal insecticide stress did compromise mating and reproductive output in both susceptible and resistant populations.

Lambda-cyhalothrin exhibited higher toxicity to the susceptible lady beetles, compromising adult longevity with increased insecticide concentrations. This trend was not detected among resistant lady beetles. Longevity of resistant predators remained unaffected by lambda-cyhalothrin exposure even at concentrations as high as the maximum label rate of this insecticide for field use (MAPA 2017). Therefore, lambda-cyhalothrin use for pest control is highly detrimental for susceptible lady beetles at concentrations as low as 6-fold less than the maximum label rate (which did not affect the resistant insects). This scenario favors the use of pyrethroid-resistant *E. connexa* as biocontrol agents in aphid pest management programs (Penman and Chapman 1988, Kidd and Rummel 1997, Deguine et al. 2000). Nonetheless, despite not affecting survival and adult longevity, lambda-cyhalothrin exposure may still compromise predator mating and reproduction, therefore limiting its potential use for biological control when pyrethroids are used. This concern was addressed through mating experiments involving pyrethroid-susceptible and –resistant populations of *E. connexa* subject or not to similar levels of lambda-cyhalothrin sublethal stress.

Lambda-cyhalothrin exposure, but not insect population, significantly affected mounting during mating. This was unexpected as exposure to neurotoxic compounds is more likely to impair insect coordination (Casida and Durkin 2013, Guedes et al. 2016). Nonetheless, such exposure may have arrested female movement while compromising excitatory stimuli with the progression of the neurotoxic effect of pyrethroids in the central nervous system (Sunderland, 2010; Casida and Durkin, 2013). Our finding was consistent with Spíndola et al. (2013) who reported reduced walking activity in lambda-cyhalothrin exposed *E. connexa*. Curiously, the reduced female body tremulation and increased duration of coupling observed with the pyrethroid exposure, which also affected the male dislodging after coupling, did not impact reproductive output of *E. connexa*. The population differences were more revealing.

The pyrethroid-resistant *E. connexa* took longer to interact, the females exhibited longer tremulation activity after mounting, and reduced coupling than the susceptible insects. In truth, the pooling of latency behaviors prior to coupling significantly correlated with fertility where longer latency to coupling resulted in lower reproductive output, regardless of insect population and insecticide exposure. The coupling duration itself, which is relatively long in this species, did not affect fertility, probably because the minimum threshold of time for sperm transfer is quickly achieved, thus securing fertilization. Long coupling duration may actually prove detrimental by increasing the mating couple vulnerability to predation in general, including intraguild predation (Felix and Soares 2004, Ahmad 2005, Spíndola et al. 2013).

Reduced latency to coupling led to higher fertility in *E. connexa*, which was affected by sublethal lambda-cyhalothrin exposure and varied between predator populations. The pyrethroid exposure led to a reduced peak of progeny production in the pyrethroid resistant population and reduced the period of progeny production in the susceptible population. More importantly though was the fertility difference between the predator populations, with the susceptible population exhibiting a higher peak of fertility and longer reproductive period leading to a higher progeny production (and rate of population growth) than the resistant population. These differences observed with lambda-cyhalothrin exposure are the likely result of delaying and impairing the coupling as a reflex of the fast neurotoxic activity of this compound (Sunderland 2010, Casida and Durkin 2013). In contrast, the population differences in mating behavior and reproductive output are probable consequences of the insecticide selection (or lack thereof) and the distinct genetic background of both populations.

Insecticide resistance is frequently associated with fitness costs with resistant individuals at a disadvantage under insecticide-free environments because of constitutive expression of their protective mechanisms against insecticidal stress (Roush and McKenzie 1987, Mallet 1989, Renton 2013, Stratonovich et al. 2014). Thus, without such stress the expression of such mechanisms can come as a trade-off at the expense of fertility (Coustau et al. 2000, Foster et al. 2003, Guedes et al. 2006, Kliot and Ghanim, 2012). Although common enough, the expression of these fitness costs associated with insecticide resistance is not universal (Guedes et al. 2017a,b, Kliot and Ghanim 2012, Oliveira et al. 2007). Fitness cost associated with pyrethroid resistance was reported in the resistant population of lady beetle used in our study (Ferreira et al. 2013), but divergent strains originated from the original stock may not exhibit such cost (e.g., Rodrigues et al., 2016). Nonetheless, pyrethroid resistance did result a fitness cost in our present study exhibiting lower fertility than the susceptible population. Thus, although prevailing under fields with pyrethroid residues, resistant *E. connexa* will be replaced by the susceptible phenotype when pyrethroid use is interrupted long enough, as supported by Lira et al. (2016).

In summary, sublethal exposure to the pyrethroid lambda-cyhalothrin compromises mating and progeny production by both pyrethroid-susceptible and resistant populations of *E. connexa*. Reduced progeny production was apparently a consequence of latency to couple, which was longer for the pyrethroid resistant population. Nonetheless, the population effect was stronger than the effect of sublethal exposure with susceptible lady beetles exhibiting significantly higher fertility. Thus, the reproductive cost of pyrethroid resistance in this species potentially counterweights the benefits of its use in pest management programs in scenarios without intensive pyrethroid use.

Acknowledgment

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References

Ahmad, P., 2005. Mating behavior of an aphidophagous ladybird beetle, Propylea dissecta (Musant). Insect Sci. 12, 37–44.

Bielza, P., 2016. Insecticide resistance in natural enemies. In: Horowitz, A.R., Ishaaya, I. (Eds.), Advances in Insect Control and Resistance Management. Springer, Switzerland, pp. 313–339.

Casida, J. E., Durkin, K. A., 2013. Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. Annu. Rev. Entomol. 58, 99–117.

Cordeiro, E. M. G., Corrêa, A. S., Venzon, M., Guedes, R. N. C., 2010. Insecticide survival and behavioral avoidance in the lacewings *Chrysoperla externa* and *Ceraeochrysa cubana*. Chemosphere 81, 1352–1357.

Costa, P. M. G., Torres, J. B., Rondelli, V. M., Lira, R., 2017. Field-evolved resistance to λ- cyhalothrin in the lady beetle *Eriopis connexa*. Bull. Entomol. Res. 18, 1–8.

Coustau, C., Chevillon, C., ffrench-Constant, R. H., 2000. Resistance to xenobiotics and parasites: can we count the cost? Trends Ecol. Evol. 15, 378–383.

Croft, B. A., Morse, J.G., 1979. Research advances on pesticide resistance in natural enemies. Entomophaga 24, 3–11.

Deguine, J. P., Gozé, E., LeClant, F., 2000. The consequences of late outbreaks of the aphid *Aphis gossypii* in cotton growing in Central Africa: towards a possible method for the prevention of cotton stickiness. Int. J. Pest Manag. 46, 86–89.

Felix, S., Soares, A. O., 2004. Intraguild predation between the aphidophagous ladybird Harmonia axyridis and *Coccinella undecimpunctata* (Coleoptera: Coccinelidae): the role of body weight. Eur. J. Entomol. 101, 237–242.

Ferreira, E. S., Rodrigues, A. R. S., Silva-torres, C. S. A., Torres, J. B., 2013. Life-history costs associated with resistance to lambda-cyhalothrin in the predatory ladybird beetle *Eriopis connexa*. Agric. For. Entomol. 15, 168–177.

Foster, S. P., Young, S., Williamson, M. S., Duce, I., Denholm, I., Devine, G. J., 2003. Analogous pleiotropic effects of insecticide resistance genotypes in peach-potato aphids and houseflies. Heredity 91, 98–106.

Guedes, N. M. P., Guedes, R. N. C., Campbell, J. F., Throne, J. E., 2017a. Mating behavior and reproductive output in insecticide-resistant and -susceptible strains of the maize weevil (*Sitophilus zeamais*). Ann. Appl. Biol. 170, 415–424.

Guedes, R. N. C., 2017. Insecticide resistance, control failure likelihood and the first Law of Geography. Pest Manag. Sci. 73, 479–484.

Guedes, R. N. C., Oliveira, E. E., Guedes, N. M. P., Ribeiro, B., Serrão, J. E., 2006. Cost and mitigation of insecticide resistance in the maize weevil, *Sitophilus zeamais*. Physiol. Entomol. 31, 30–38.

Guedes, R. N. C., Smagghe, G., Stark, J. D., Desneux, N., 2016. Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. Annu. Rev. Entomol. 61, 43–62.

Guedes, R. N. C., Walse, S. S., Throne, J. E., 2017b. Sublethal exposure, insecticide resistance, and community stress. Curr. Opin. Insect Sci. 21, 47–53. http://dx.doi.org/ 10.1016/j.cois.2017.04.010.

Hoy, M. A., 1990. Pesticide resistance in arthropod natural enemies: variability and selection responses. In: Roushm, R.T., Tabashinik, B.E. (Eds.), Pesticide Resistance in Arthropods. Chapman and Hall, New York, pp. 203–236.

IRAC [Insecticide Resistance Action Comnittee], 2017. Resistance Definition. http:// www.irac-online.org/about/resistance/, Accessed date: 29 September 2017.

Kidd, P. W., Rummel, D. R., 1997. Effects of insect predators and a pyrethroid insecticide on cotton aphid *Aphis gossypii* Glover population density. Southwest. Entomol. 22, 381–393.

Kliot, A., Ghanim, M., 2012. Fitness costs associated with insecticide resistance. Pest Manag. Sci. 68, 1431–1437.

Lima, D. B., Melo, J. W. S., Guedes, R. N. C., Siqueira, H. A. A., Pallini, A., Gondim Jr., M.G.C., 2013. Survival and behavioural response to acaricides og the coconut mite predator *Neoseiulus baraki*. Exp. Appl. Acarol. 60, 381–393.

Lira, R., Rodrigues, A. R. S., Torres, J. B., 2016. Fitness advantage in heterozygous ladybird beetle *Eriopis connexa* (Germar) resistant to lambda-cyhalothrin. Neotrop. Entomol. 45, 573–579.

MAPA [Ministério da Agricultura, Pecuária e Abstecimento], 2017. AGROFIT - Sistema de Agrotóxicos Fitossanitários. http://extranet.agricultura.gov.br/agrofit_cons/ principal_agrofit_cons, Accessed date: 25 September 2017.

Mallet, J., 1989. The evolution of insecticide resistance: have the insects won? Trends Ecol. Evol. 4, 336–340.

Oliveira, E. E., Guedes, R. N. C., Tótola, M. R., De Marco, P., 2007. Competition between insecticide-susceptible and -resistant populations of the maize weevil, *Sitophilus zeamais*. Chemosphere 69, 17–24.

Penman, D. R., Chapman, R. B., 1988. Pesticide-induced mite outbreaks: pyrethroids and spider mites. Exp. Appl. Acarol. 4, 265–276.

Renton, M., 2013. Shifting focus from the population to the individual as a way forward in understanding, predicting and managing the complexities of evolution of resistance to pesticides. Pest Manag. Sci. 69, 171–175.

Rodrigues, A. R. S., Siqueira, H. A. A., Torres, J. B., 2014. Enzymes mediating resistance to lambda-cyhalothrin in *Eriopis connexa* (Coleoptera: Coccinellidae). Pestic. Biochem. Physiol. 110, 36–43.

Rodrigues, A. R. S., Spindola, A. F., Torres, J. B., Siqueira, H. A. A., Colares, F., 2013a. Response of different populations of seven lady beetle species to lambda-cyhalothrin with record of resistance. Ecotoxicol. Environ. Saf. 96, 53–60.

Rodrigues, A. R. S., Torres, J. B., Siqueira, H. A. A., Lacerda, D. P. A., 2013b. Inheritance of lambda-cyhalothrin resistance in the predator lady beetle *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae). Biol. Contr. 64, 217–224.

Rodrigues, A. S., Botina, L., Nascimento, C. P., Gontijo, L. M., Torres, J. B., Guedes, R. N. C., 2016. Ontogenic behavioral consistency, individual variation and fitness consequences among lady beetles. Behav. Process. 131, 32–39.

Roush, R. T., McKenzie, J. A., 1987. Ecological genetics of insecticide resistance. Annu. Rev. Entomol. 32, 361–380.

Santos, D. S., Rodrigues, A. R. S., Torres, J. B., Lira, R., 2016. Performance of *Eriopis connexa* (Coleoptera: Coccinellidae) resistant to lambda-cyhalothrin after extended recovery from knockdown. Neotrop. Entomol. 45, 718–724.

Sawicki, R. M., 1987. Definition, detection and documentation of insecticide resistance. In: Ford, M.G., Holloman, D.W., Khampau, B.P.S., Sawicki, R.M. (Eds.), Combating resistace to xenobiotics; biological and chemical approaches. Ellis Horwood, Chichester, UK, pp. 105–117.

Spíndola, A. F., Silva-Torres, C. S. A., Rodrigues, A. R. S., Torres, J. B., 2013. Survival and behavioural responses of the predatory ladybird beetle, *Eriopis connexa* populations susceptible and resistant to a pyrethroid insecticide. Bull. Entomol. Res. 485–494.

Stratonovich, P., Elias, J., Denholm, I., Slater, R., Semenov, M. A., 2014. Na individualbased model of the evolution of pesticide resistance in heterogeneous environments: control of *Meligethes aeneus* population in oilseed rape crops. PLoS One 9, 1–24.

Sunderland, D. M., 2010. Sodium channels. In: Gilbert, L.I., Gill, S.S. (Eds.), Insect Pharmacology. Academic, New York, pp. 1–24.

Torres, A. J. B., Rodrigues, A. R. S., Barros, E. M., Santos, D. S., 2015. Lambda-cyhalothrin resistance in the lady beetle *Eriopis connexa* (Coleoptera : Coccinellidae) confers tolerance to other pyrethroids. J. Econ. Entomol. 108, 60–68.

Whalon, M. E., Mota-Sanchez, D., Hollingworth, R. M., 2008. Analysis of global pesticide resistance in arthropods. In: Whalon, R.M., Mota-Sanchez, D., Hollingworth, R.M. (Eds.), Global Pesticide Resistance in Arthropods. CABI, Wallingford, UK, pp. 5–31.

ARTIGO 3

Exposure on Pyrethroid-Susceptible and -Resistant Lady Beetles (*Eriopis connexa* (Coleoptera: Coccinelidae))

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Abstract

Sublethal insecticide exposure may affect foraging of insects, including natural enemies, although the subject is usually neglected. The lady beetle *Eriopis connexa* (Germar, 1824) (Coleoptera: Coccinelidae) is an important predator of aphids with existing pyrethroid-resistant populations that are undergoing scrutiny for potential use in pest management systems characterized by frequent insecticide use. However, the potential effect of sublethal pyrethroid exposure on this predator's foraging activity has not yet been assessed and may compromise its use in biological control. Therefore, our objective was to assess the effect of sublethal lambda-cyhalothrin exposure on three components of the prey foraging activity (i.e., walking, and prey searching and handling), in both pyrethroid-susceptible and -resistant adults of *E. connexa*. Both lady beetle populations exhibited similar walking patterns without insecticide exposure in non-contaminated arenas, but in partially contaminated arenas walking differed between strains, such that the resistant insects exhibited greater walking activity. Behavioral avoidance expressed as repellence to lambda-cyhalothrin was not observed for either the susceptible or resistant populations of *E. connexa*, but the insecticide caused avoidance by means of inducing irritability in 40% of the individuals, irrespective of the strain. Insects remained in the insecticide-contaminated portion of the arena for extended periods resulting in greater exposure. Although lambda-cyhalothrin exposure did not affect prey searching by susceptible lady beetles, prey searching was extended for exposed resistant predators. In contrast, prey handling was not affected by population or by lambda-cyhalothrin exposure. Thus, sublethal exposure to the insecticide in conjunction with the insect resistance profile can affect prey foraging with pyrethroid-exposed resistant predators exhibiting longer prey searching time associated with higher walking activity reducing its predatory performance.

Keywords: Biological control, biocontrol agent, sublethal effect, insecticide resistance.

Introduction

It can be difficult to integrate biological control under intensive agriculture production where insecticides are also used for pest management (Croft and Brown 1975, Croft 1990, Desneux et al. 2007). Compatibility of biological control agents with insecticides may be achieved by using insecticides that are more selective in favor of natural enemies or by using populations of natural enemies that have resistance to the insecticide. Selectivity is usually determined based on survival of natural enemies to exposure to the insecticide, with less attention given to potential sublethal and indirect effects of pesticides on natural enemies (Guedes et al. 2016, 2017). The occurrence of insecticide-resistant populations of natural enemies is relatively rare (Croft and Morse 1979, Hoy 1990, Bielza 2016), but the detection of insecticide resistance among lady beetles suggests that there might be opportunities in their conservation for aphid management in field crops (Rodrigues et al. 2013a,b; Costa et al. 2017; Torres et al. 2015). This has been reported previously in different regions (Ruberson et al. 2007, Kumral et al. 2011, Tang et al. 2014, Barbosa et al. 2017).

The lady beetle species *Eriopis connexa* (Germar, 1824) (Coleoptera: Coccinelidae) is the coccinelid predator exhibiting the highest levels of insecticide resistance, particularly pyrethroid resistance, in different crop systems encouraging the use of its resistant populations in pest management programs in the Neotropics (Rodrigues et al. 2013a,b). Pyrethroid resistance in *E. connexa* appears to be an autosomal and incompletely dominant trait, with enhanced esterase activity as the main underlying mechanism (Rodrigues et al. 2013a, 2014; Torres et al. 2015). Pyrethroid resistance is associated with fitness costs in *E. connexa*, but this can be mitigated by maintaining heterozygosity within the population (Lira et al. 2016). Despite these studies, the effects of sublethal pyrethroid exposure in pyrethroid-susceptible and -resistance populations of lady beetles, and particularly of *E. connexa*, remain neglected although important.

The importance of sublethal exposure on lady beetles is due to its rather ubiquitous occurrence since insecticide field rates are determined targeting pest species, not natural enemies. Thus, the predators are usually exposed to sublethal rates of the applied insecticide, which quickly undergoes natural (environmental) degradation further decreasing its field residue levels and favoring sublethal exposure (Guedes et al. 2016). In addition, peculiar behavioral traits of non-targeted species may also minimize insecticide exposure favoring their escape, but may also lead to divergent responses depending on the species and population involved, which is seldom considered (Desneux et al. 2007, Guedes et al. 2017). Thus, it is important to assess natural enemy responses following sublethal insecticide exposure; behavioral responses are particularly relevant as they may either enhance or minimize the insecticidal effect, while serving as an early sign of nontargeted exposure (Hellou 2011, Cutler 2013, Guedes and Cutler 2014, Cutler and Guedes 2017). Such studies among insecticide-resistant populations of natural enemies may be particularly important, given their potential use in pest management (Guedes et al. 2017).

Predator foraging on prey entails a set of behavioral traits important for biological control. This has implications for use of pyrethroid-resistant lady beetles in management of aphids in field crops (Cloyd and Bethke 2011, He et al. 2012). Neurotoxic insecticides like pyrethroids likely affect walking and interfere with prey searching and handling by predators due to their modulation of voltage-gated Na^+ channels in axons of excitatory neurons (Haynes 1988, Desneux et al. 2004, Banks and Stark 2011, Sunderland 2010, Casida and Durkin 2013). Therefore, sublethal exposure to pyrethroid insecticides may potentially affect prey foraging by both susceptible and resistant *E. connexa*. We hypothesized that similar level of exposure to lambda-cyhalothrin, one of the most widely used pyrethroids in field crops in Brazil (MAPA 2017), was more likely to affect the susceptible rather than the resistant insects due to their vulnerability to the insecticide. On the other hand, reduced prey foraging performance (i.e., extended prey searching and handling) could also occur because of fitness costs associated with insecticide resistance.

Materials and Methods

Insects

Insecticide-susceptible and -resistant strains of *E. connexa* were obtained from the Entomology Unit of the Federal Rural University of Pernambuco in Recife (State of Pernambuco, Brazil). They were field-collected by 2009 and have since been maintained in the laboratory. Resistance to lambda-cyhalothrin was maintained using methods previously described with periodic introduction of field insects and bioassays to ascertain of their resistance, what was also performed for the susceptible population (Rodrigues et al. 2013a, 2014; Torres et al. 2015). The resistance remained around 40-fold in our laboratory where both resistant and susceptible beetles were held separately from one another. Beetles were reared on a diet of eggs of the Mediterranean flour moth, *Ephestia* (=*Anagasta*) *kuehniella* (Zeller) (Lepidoptera: Pyralidae), provided ad libitum, throughout their development. Adult lady beetles were provided green peach aphids, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), every other day, and also received 10% honey solution to enhance reproduction (D'Ávila et al. 2018). Insect colonies were maintained, and the bioassays were performed under controlled environmental conditions (25 ± 1°C, 70 ± 10% RH, 12:12 [L:D]) h.

Walking Bioassay on Nontreated Arena

Nonexposed adults from both lady beetle strains were subjected to bioassays that examined their walking in nontreated arenas following Rodrigues et al. (2016). Briefly, individual insects were released in the center of Petri dish arenas (9-cm diameter) that had the bottom covered with filter paper (Whatman no. 1). The inner walls of each dish were coated with Teflon PTFE to prevent insect escape. The insect walking pattern was digitally recorded for 10 min via an automated video tracking system equipped with a CCD camera and associated software (ViewPoint Life Sciences, Montreal, Canada). The following parameters were recorded: distance walked (cm), walking velocity (cm/s), and resting time (s). Twenty-four insects of each strain and exposure condition (i.e., exposed or not to lambdacyhalothrin) were used in this bioassay.

Walking Bioassay on Half-Treated Arena

In addition to the walking parameters reported previously, insecticide behavioral avoidance through repellence and irritability was recorded through short-term (acute) response bioassays with nonexposed lady beetles. This was achieved through walking bioassays on half-treated arenas using the same methods described previously but with only half of the arena containing dried residue of lambda-cyhalothrin (50 g a.i./liter, encapsulated suspension; Syngenta, São Paulo, SP, Brazil). Two filter papers were used: one that received 1 ml water and one that received 1 ml of lambda-cyhalothrin (26.72 µg a.i./ml). Each was folded in half and glued to half of the arena using water-based white (synthetic) glue resin (Cordeiro et al. 2010, Freitas et al. 2017). As above, 24 adult insects (5–7 d old) from both the insecticide-susceptible and -resistance strains were used. Distance walked, walking velocity, and resting time were recorded in each half of the arena. In addition, lambda-cyhalothrin repellence and irritability were recorded as the number of insects spending less than 1 s in the insecticide-contaminated half of the arena, and the number of insects remaining less than 50% of the time on such half, respectively.

Insecticide Exposure

Adults of the lady beetle *E. connexa* (5–7 d after emergence) were exposed to the maximum label rate of lambda-cyhalothrin (i.e., 26.72 µg a.i./ml) for either 45 min or 48 h for the population, if susceptible or resistant, respectively. The differential exposure was determined through time-mortality bioassays previously carried out to determine the threshold of no observable effect time for each strain, which would allow similar levels of sublethal exposure to both strains (D'Ávila et al. 2018). Insecticide exposure was performed as described. This involved exposing batches of 10 adult predators in 250-ml glass jars, whose inner walls were coated with air-dried residues of lambda-cyhalothrin. The commercial formulation of this pyrethroid (50 g a.i./liter, encapsulated suspension; Syngenta, São Paulo, SP, Brazil) was applied as 2-ml suspension in water into glass jars maintained under rotation in a heavy-duty rotator (Roto-Torque model 7637, ColeParmer, Vernon Hills, IL) under laboratory conditions until drying. This procedure ensured even coverage of the inner walls of the glass jar with insecticide. The top inner walls of each jar were also coated with Teflon PTFE (DuPont, Wilmington, DE) to prevent insect escape. Lambda-cyhalothrin was applied at the concentration of 300 ng a.i./cm^2 (corresponding to 600-ml formulation/ha, and 30 g a.i./ha), which is the maximum label rate for this pyrethroid used in Brazilian field crops (MAPA 2017). Right after exposure, the insects were used in the desired bioassays described in the following section. Controls consisted of exposure only to water. No insect mortality was observed during the experiments.

Bioassay of Prey Searching and Handling

Predator searching and prey handling were assessed in lambda-cyhalothrin exposed and nonexposed adult predators from both strains using the same video tracking system and Petri dish arenas as previously described for the walking bioassays. However, in the prey searching and handling a third instar green peach aphid was glued to the center of the filter paper covering the bottom of Petri dish arena using white glue, and the adult predator was released at the edge of the arena. Each predator was recorded until the prey was consumed or up to 20 min, length of time large enough for the intended observation, as determined in preliminary investigation. Distance walked, walking velocity, resting time, total searching time to reach the prey (i.g., searching time), and time spent attacking and consuming the prey (handling time) were recorded. Adult *E. connexa* were starved for 48 h before bioassays were initiated. Fourteen replicates (i.e., predators) were used for the pyrethroid-resistant population and 24 were used for the susceptible population in each of the insecticide exposure conditions (i.e., exposed and nonexposed to lambda-cyhalothrin). Among these insects, 10 out of the 14 resistant predators preyed on the aphids, while 15 out of 24 susceptible predators preyed on the aphids within the 20-min period of observation. Only the data from bioassays in which predation took place were considered in our analyses.

Statistical Analyses

The results of the walking bioassays on fully nontreated arenas were subjected to analyses of variance to compare the walking patterns of pyrethroid-susceptible and -resistant populations (PROC GLM; SAS 9.4; SAS Institute, Cary, NC). The walking parameters on halftreated arenas were subjected to paired t-tests to compare behavior on treated and nontreated halves of the arenas (PROC TTEST, SAS), while the population differences were tested using Fisher's F test (PROC GLM; SAS 9.4). Prey searching and handling data were subjected to two-way analyses of variance (two strains × two exposure conditions) followed by Tukey's HSD test, when appropriate (PROC GLM; SAS 9.4). Normality and homoscedasticity assumptions were tested (PROC UNIVARIATE; SAS 9.4), and only searching time required data transformation (to log10). Correlations between searching and handling times with walking parameters were also tested using the procedure PROC CORR from SAS (SAS 9.4, SAS Institute). The results of lambda-cyhalothrin repellence and irritability were subjected to the nonparametric Mann–Whitman U test ($P < 0.05$).

Results

Walking Behavior - Nontreated Arenas

The pyrethroid-susceptible and -resistant strains did not exhibit significant differences on walking behavior on fully nontreated arenas ($F_{1,46} \leq 0.33$, $P \geq 0.57$), showing respectively similar distance walked (521.12 ± 73.57 vs 547.61 ± 67.03 cm), walking velocity (2.26 ± 0.21 vs 2.26 ± 0.19 cm/s), and resting time (170.65 ± 25.37 vs 151.97 ± 20.33 cm).

Walking Behavior - Half-Treated Arenas

Contrary to observations on nontreated arenas, when the adult lady beetles were released on partially treated arenas there were significant effects of strain or arena portion on walking behavior (Fig. 1). Repellency effects of lambda-cyhalothrin were negligible, and irritability was detected in 40% of the insects ($P < 0.05$), irrespective of the strain ($P \geq 0.81$). However, differences between strains were significant for resting time, distance walked, and walking velocity ($F_{1,94} \geq 6.98$, $P \leq 0.01$), regardless of the portion of the arena (paired $t_{23} \leq 0.85$; $P \geq 0.41$), with the pyrethroid-resistant insects always exhibiting greater walking activity, irrespective of lambda-cyhalothrin exposure (Fig. 2A–C). In contrast, time spent in each portion of the area varied significantly only for exposure (paired $t_{23} \leq 2.05$; $P \geq 0.04$), not strain ($F_{1,94} = 0.06$, $P = 0.81$), with the predators remaining for a longer duration on the insecticide-contaminated portion of the arenas (Fig. 2D).

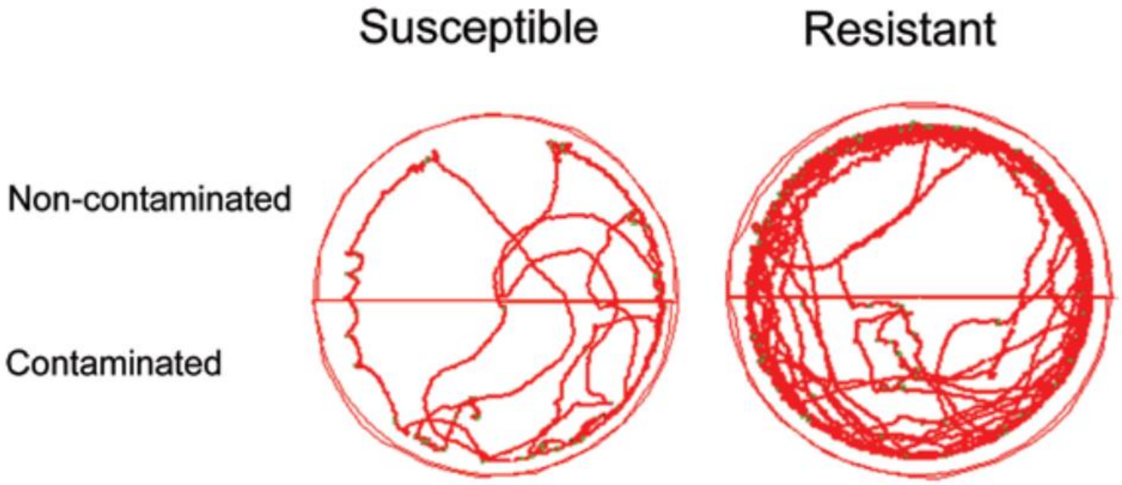

Fig. 1. Representative tracks showing the walking movements of pyrethroid-susceptible and -resistant lady beetles of the species *E. connexa* on walking arenas half-contaminated with the pyrethroid lambda-cyhalothrin. The red tracks indicate increased speed (>0.6 cm/s), and green tracks indicate reduced (initial) speed (<0.6 cm/s).

Prey Searching and Handling

Prey searching varied significantly between strains and exposure conditions ($F_{3,46} = 2.83$, $P = 0.04$) with a significant strain-exposure interaction ($F_{1,46} = 7.79$, $P = 0.008$), as illustrated by the representative tracks obtained in the respective bioassays (Fig. 3). While the lambda-cyhalothrin exposed resistant lady beetles spent the most time searching, exposed susceptible and unexposed resistant predators provided the lowest searching times (Fig. 4). In contrast, strain and insecticide exposure had no significant effect on handling time (370.27 ± 51.87 s) ($F_{3,45} = 1.76$, $P = 0.17$). Correlation analyses between prey searching and walking parameters indicated that the distance walked and resting time were significantly correlated with searching time ($r \geq 0.65$, $P < 0.001$), which was negatively correlated with walking velocity ($r = -0.40$, $P = 0.04$), indicating that the predator walking behavior is determinant of searching for prey, regardless of the strain and insecticide-exposure condition.

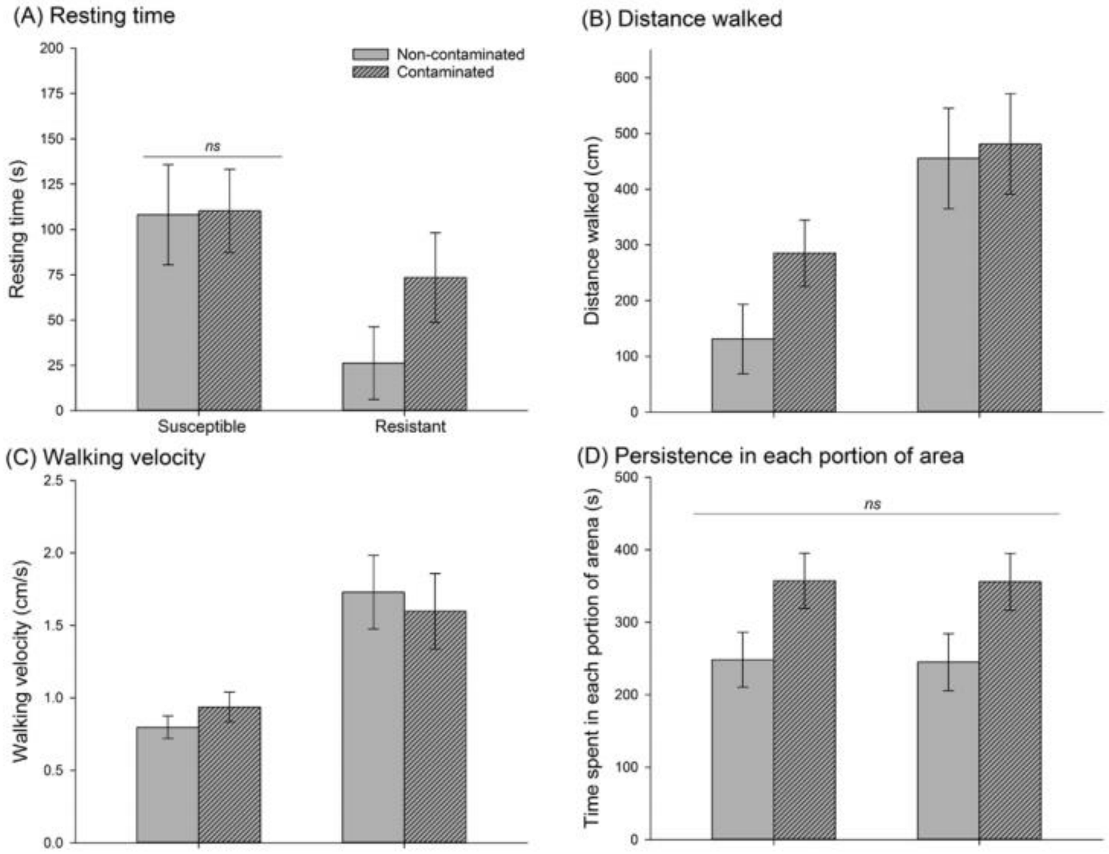

Fig. 2. Resting time (A), distance walked (B), walking velocity (C), and time spent in each portion (D) of arenas half-contaminated with the pyrethroid lambda-cyhalothrin by pyrethroid-susceptible and -resistant lady beetles of the species *E. connexa*. Vertical bars represent standard errors of means. Bars linked by a horizontal line with an asterisk are significantly different by Fisher's F test ($P < 0.05$), if comparing strains, or paired t-test ($P < 0.05$), if comparing halves of the arena. Horizontal lines with an "ns" are not significantly different.

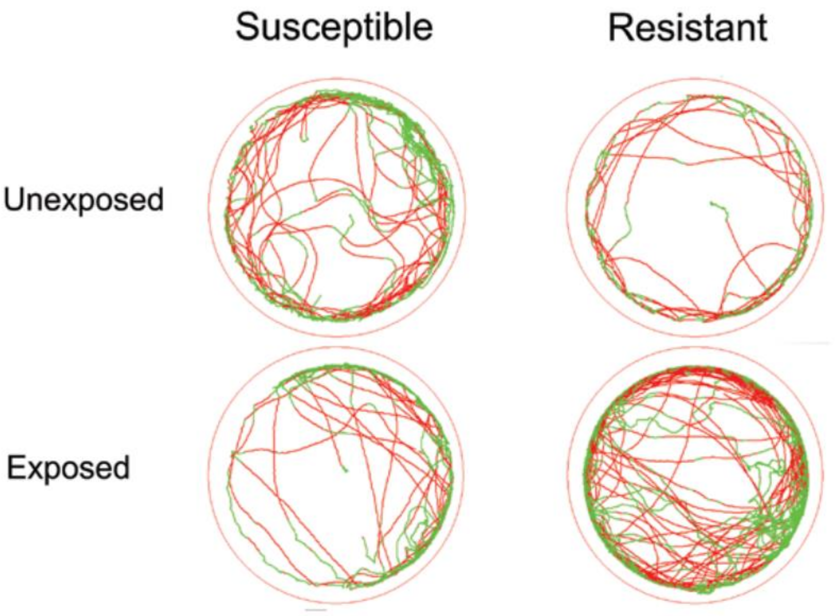

Fig. 3. Representative tracks showing the walking movements during prey searching by pyrethroid-susceptible and -resistant *E. connexa*, either exposed or not to the pyrethroid lambda-cyhalothrin. Red tracks indicate increased speed (>1.5 cm/s), and green tracks indicate eventual reduced speed (<1.5 cm/s).

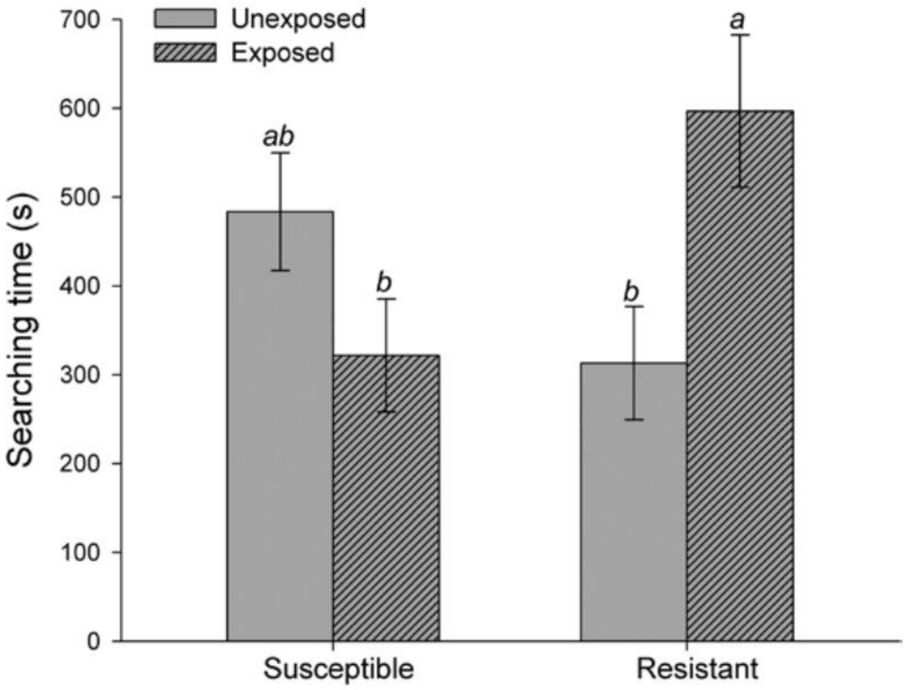

Fig. 4. Searching time (± SE) during prey foraging by pyrethroid-susceptible and –resistant *E. connexa*, either exposed or not to the pyrethroid lambdacyhalothrin. Bars with the same letter are not significantly different by Tukey's HSD test ($P < 0.05$).

Discussion

Exposure to lambda-cyhalothrin can affect prey foraging by pyrethroid-susceptible and -resistant lady beetles used as aphid biocontrol agents in field crops (Cloyd and Bethke 2011, He et al. 2012), and the neurotoxic activity of this compound can compromise insect activity and behavior (Haynes 1988, Desneux et al. 2007, Sunderland 2010, Casida and Durkin 2013). We expected that exposure to sublethal concentrations of lambda-cyhalothrin would exhibit stronger effect on our pyrethroid-susceptible strain of the lady beetle *E. connexa* relative to the resistant strain, due to its high vulnerability to this insecticide (Rodrigues et al. 2013a,b, Torres et al. 2015). However, we also recognized that prey foraging could be compromised in the resistant strain due to the fitness costs associated with insecticide resistance (Guedes et al. 2017). Our results with pyrethroid-susceptible and -resistant *E. connexa* indicate that sublethal insecticide exposure does affect prey foraging by this biocontrol agent. Furthermore, pyrethroid-resistant predators exhibited more extended searching time for prey capture when exposed to the insecticide relative to the susceptible predators.

Walking activity and prey searching and handling are key components of prey foraging, and these three end points were assessed following susceptible and resistant *E. connexa* exposure to sublethal concentrations of lambda-cyhalothrin. For walking activity, nonexposed insects from both strains exhibited similar behavior on noncontaminated surface, which is consistent with results of an earlier study (Spíndola et al. 2013). In contrast, when the predators were subjected to arenas half-contaminated with lambda-cyhalothrin, they remained for longer on the insecticide-contaminated portion of the arena, and 40% of the individuals exhibited irritability to this insecticide, regardless of the strain. It is notable that the pyrethroid-resistant predators exhibited greater walking activity than the susceptible ones. This could potentially lead to more efficient prey searching and handling, which would be beneficial in the field.

Prey handling was not influenced by strain or sublethal insecticide exposure, unlike prey searching. One might expect greater walking activity leading to faster and more efficient prey searching, but the opposite was observed, irrespective of *E. connexa* strain and insecticide exposure condition. Longer prey searching times were associated with increased walking distances, slower walking, and longer resting time. Thus, longer distances walked were done slowly such that searching of *E. connexa* was compromised rather than improved. Greater walking distances and search times were more prevalent among pyrethroid-resistant predators exposed to lambda-cyhalothrin. This is likely due to the mode of action of the insecticide as pyrethorids lead to initial hyperactivity subsequently impairing coordination as a result of its modulation of voltagegated Na^+ in axons of excitatory neurons (Haynes 1988, Desneux et al. 2007, Sunderland 2010, Casida and Durkin 2013). Therefore, searching time was likely longer due to the deficient locomotory coordination of the exposed insects favoring longer distances walked with lower velocities and more interruptions. This seems particularly acute in the resistance strain exposed to the pyrethroid, a potential consequence of altered voltage-gated Na^+ channels in the axon membrane in insects from this strain, if this mechanism shows any relevance in this case. Previous studies with other insect species, including the lady beetle Harmonia axyridis (Palias) (Coleoptera: Coccinellidae), also reported impaired activity with pyrethroid exposure (Roger et al. 1994, 1995; Provost et al. 2003, 2005).

Our findings have potential practical consequences for the intended use of these predators in integrated pest management programs aimed at aphid control in field crops. Although irritability of lambda-cyhalothrin to *E. connexa* could reduce potential exposure to contaminated surfaces, the predator was not repelled from lambda-cyhalothrin-treated surfaces, in contrast to reports with other insect species (e.g., Guedes et al. 2009, Cordeiro et al. 2010, Vélez et al. 2017). This means that exposure would still occur, with potential for adverse impacts on the predator. Nonetheless, irritability was mild among the *E. connexa* adults reaching less than half of the strain, and it was further minimized by the predators remaining for longer in the contaminated surfaces. Thus, walking activity did not reduce insecticide exposure among individuals of this predatory species. More importantly though, sublethal lambda-cyhalothrin exposure impaired prey searching by pyrethroid-resistant *E. connexa* reducing its aphid predatory potential as a biocontrol agent, besides potentially enhancing the exposure of these predators to intraguild predation (Felix and Soares 2004).

In summary, the pyrethroid-susceptible and -resistant strains of the lady beetle *E. connexa* exhibited similar walking behavior in noncontaminated surfaces and similar behaviors when subjected to partially contaminated arenas. Mild irritability to the pyrethroid was accompanied by greater residency of the predators on the contaminated portion of the arena, without reducing insecticide exposure. Insect population and lambda-cyhalothrin exposure did not affect prey handling, but exposed pyrethroid-resistant predators walked longer distances and more slowly with longer resting times, leading to extended searching times. The longer searching time reduces predatory performance and potentially increases vulnerability to intraguild predation. If these effects occur in the field, increased releases of the predator or reduced insecticide use may be needed to achieve satisfactory biocontrol of aphids, what deserves further attention particularly in field assessments.

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References

Banks, J. E., and J. D. Stark. 2011. Effects of a nicotinic insecticide, imidacloprid, and vegetation diversity on movement of a common predator, *Coccinella septempunctata*. Biopestic. Int. 7: 113–122.

Bielza, P. 2016. Insecticide resistance in natural enemies, pp. 313–339. In A. R. Horowitz, and I. Ishaaya (eds.), Advances in insect control and resistance management. Springer, Switzerland.

Casida, J. E., and K. A. Durkin. 2013. Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. Annu. Rev. Entomol. 58: 99–117.

Cloyd, R. A., and J. A. Bethke. 2011. Impact of neonicotinoid insecticides on natural enemies in greenhouse and interiorscape environments. Pest Manag. Sci. 67: 3–9.

Cordeiro, E. M., A. S. Corrêa, M. Venzon, and R. N. Guedes. 2010. Insecticide survival and behavioral avoidance in the lacewings *Chrysoperla externa* and *Ceraeochrysa cubana*. Chemosphere. 81: 1352–1357.

Costa, P. M. G., J. B. Torres, V. M. Rondelli, and R. Lira. 2017. Field-evolved resistance to λ-cyhalothrin in the lady beetle *Eriopis connexa*. Bull. Entomol. Res. doi: 10.1017/S0007485317000888

Croft, B. A. 1990. Arthropod biological control agents and pesticides. John Wiley and Sons Inc, New York.

Croft, B. A., and A. W. Brown. 1975. Responses of arthropod natural enemies to insecticides. Annu. Rev. Entomol. 20: 285–335.

Croft, B. A., and J. G. Morse. 1979. Research advances on pesticide resistance in natural enemies. Entomophaga. 24: 3–11.

Cutler, G. C. 2013. Insects, insecticides and hormesis: evidence and considerations for study. Dose Response. 11: 154–177.

Cutler, G. C., and R. N. C. Guedes. 2017. Occurrence and significance of insecticide-induced hormesis in insects, pp. 101–119. In S. O. Duve, P.

Kudsk, and K. Solomon (eds.), Pesticide dose: effects on the environment and target and non-target organisms. ACS, Washington.

D'Ávila, V. A., W. F. Barbosa, L. C. Reis, B. S. A. Gallardo, J. B. Torres, and R. N. C. Guedes. 2018. Lambda-cyhalothrin exposure, mating behavior and reproductive output of pyrethroid-susceptible and resistant lady beetles (*Eriopis connexa*). Crop Protection [accepted]. doi: 10.1016/j.cropro.2018.01.009

Desneux, N., H. Rafalimanana, and L. Kaiser. 2004. Dose–response relationship in lethal and behavioural effects of different insecticides on the parasitic wasp Aphidius ervi. Chemosphere. 54: 619–627.

Desneux, N., A. Decourtye, and J. M. Delpuech. 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52: 81–106.

Felix, S., and A. O. Soares. 2004. Intraguild predation between the aphidophagous ladybird beetles *Harmonia axyridis* and *Coccinella undecimpunctata* (Coleoptera: Coccinellidae): the role of body weight. Eur. J. Entomol. 101: 237–242.

Freitas, C. D., L. M. Gontijo, R. N. C. Guedes, and M. Chediak. 2017. Survival and locomotory behavior of earwigs after exposure to reduced-risk insecticides. J. Econ. Entomol. 110: 1576–1582.

Guedes, N. M. P., R. N. C. Guedes, G. H. Ferreira, and L. B. Silva. 2009. Flight take-off and walking behavior of insecticide-susceptible and –resistant strains of *Sitophilus zeamais* exposed to deltamethrin. Bull. Entomol. Res. 99: 393–400.

Guedes, R. N. C., and G. C. Cutler. 2014. Insecticide-induced hormesis and arthropod pest management. Pest Manag. Sci. 70: 690–697.

Guedes, R. N. C., G. Smagghe, J. D. Stark, and N. Desneux. 2016. Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. Annu. Rev. Entomol. 61: 43–62.

Guedes, R. N. C., S. S. Walse, and J. E. Throne. 2017. Sublethal exposure, insecticide resistance, and community stress. Curr. Opin. Insect Sci. 21: 47–53.

Haynes, K. F. 1988. Sublethal effects of neurotoxic insecticides on insect behavior. Annu. Rev. Entomol. 33: 149–168.

He, Y., J. Zhao, Y. Zheng, N. Desneux, and K. Wu. 2012. Lethal effect of imidacloprid on the coccinellid predator *Serangium japonicum* and sublethal effects on predator voracity and on functional response to the whitefly *Bemisia tabaci*. Ecotoxicology. 21: 1291–1300.

Hellou, J. 2011. Behavioural ecotoxicology, an “early warning” signal to assess environmental quality. Environ. Sci. Pollut. Res. Int. 18: 1–11.

Hoy, M. A., 1990. Pesticide resistance in arthropod natural enemies: variability and selection responses, pp. 203–236. In R. T. Roush, and B. E. Tabachnik (eds.), Pesticide resistance in arthropods. Chapman and Hall, New York.

Kumral, N. A., N. S. Gencer, H. Susurluk, and C. Yalcin. 2011. A comparative evaluation of the susceptibility to insecticides and detoxifying enzyme activities in *Stethorus gilvifrons* (Coleoptera: Coccinellidae) and *Panonychus ulmi* (Acarina: Tetranychidae). Int. J. Acarol. 37: 255–268.

Lira, R., A. R. Rodrigues, and J. B. Torres. 2016. Fitness advantage in heterozygous ladybird beetle *Eriopis connexa* (Germar) resistant to lambdacyhalothrin. Neotrop. Entomol. 45: 573–579.

MAPA [Ministério da Agricultura, Pecuária e Abastecimento]. 2017. AGROFIT – Sistema de Agrotóxicos Fitossanitários. http://extranet.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. (Accessed 26 November 2017).

Provost, C., D. Coderre, E. Lucas, and N. J. Bostanian. 2003. Impact of lambda-cyhalothrin on intraguild predation among three mite predators. Environ. Entomol. 32: 256–263.

Provost, C., D. Coderre, E. Lucas, G. Chouinard, and N. J. Bostanian. 2005. Impact of intraguild predation and lambda-cyhalothrin on predation efficacy of three acarophagous predators. Pest Manag. Sci. 61: 532–538.

Rodrigues, A. R., A. F. Spindola, J. B. Torres, H. A. Siqueira, and F. Colares. 2013a. Response of different populations of seven lady beetle species to lambda-cyhalothrin with record of resistance. Ecotoxicol. Environ. Saf. 96: 53–60.

Rodrigues, A. R. S., J. B. Torres, H. A. A. Siqueira, and D. P. A. Larcerda. 2013b. Inheritance of lambda-cyhalothrin resistance in the predator lady beetle *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae). Biol. Control. 64: 217–224.

Rodrigues, A. R. S., H. A. A. Siqueira, and J. B. Torres. 2014. Enzymes mediating resistance to lambda-cyhalothrin in *Eriopis connexa* (Coleoptera: Coccinellidae). Pestic. Biochem. Physiol. 110: 36–43.

Rodrigues, A. S., L. Botina, C. P. Nascimento, L. M. Gontijo, J. B. Torres, and R. N. Guedes. 2016. Ontogenic behavioral consistency, individual variation and fitness consequences among lady beetles. Behav. Process. 131: 32–39.

Roger, C., D. Coderre, and C. Vincent. 1994. Mortality and predation efficiency of *Coleomegilla maculata* Lengi (Coleoptera: Coccinellidae) following pesticide applications. J. Econ. Entomol. 87: 583–588.

Roger, C., C. Vincent, and D. Coderre. 1995. Mortality and predation efficiency of *Coleomegilla maculata* lengi Timb. (Col., Coccinellidae) following application of neem extracts (*Azadirachta indica* A. Juss., Meliaceae). J. Appl. Entomol. 119: 439–443.

Ruberson, J. R., P. Roberts, and J. P. Michaud. 2007. Pyrethroid resistance in Georgia populations of the predator *Hippodamia convergens* (Coleoptera: Coccinellidae). In Proceedings of Beltwide Cotton Conference. Vol. 1, pp. 361–365.

Soderlund, D. M. 2010. Sodium channels, pp. 1–24. In L. I. Gilbert, and S. S. Gill (eds.), Insect pharmacology. Academic Press, New York.

Spíndola, A. F., C. S. Silva-Torres, A. R. Rodrigues, and J. B. Torres. 2013. Survival and behavioural responses of the predatory ladybird beetle, *Eriopis connexa* populations susceptible and resistant to a pyrethroid insecticide. Bull. Entomol. Res. 103: 485–494.

Tang, L. D., X. M. Wang, F. L. Jin, B. L. Qiu, J. H. Wu, and S. X. Ren. 2014. De novo sequencing-based transcriptome and digital gene expression analysis reveals insecticide resistance-relevant genes in *Propylaea japonica* (Thunberg) (Coleoptea: Coccinellidae). PLoS One. 9: e100946.

Torres, J. B., A. R. Rodrigues, E. M. Barros, and D. S. Santos. 2015. Lambda-cyhalothrin resistance in the lady beetle *Eriopis connexa* (Coleoptera: Coccinellidae) confers tolerance to other pyrethroids. J. Econ. Entomol. 108: 60–68.

Vélez, M., W. F. Barbosa, J. Quintero, M. Chediak, and R. N. C. Guedes. 2017. Deltamethrin- and spinosad-mediated survival, activity and avoidance of the grain weevils *Sitophilus granarius* and *S. zeamais*. J. Stored Prod. Res. 74: 56–65.

Considerações finais

Nossos resultados sugerem que:

i. O inseticida lambda-cialotrina apresentou maior seletividade ao inimigo natural *Aphidius colemani* quando comparado aos inseticidas spinosad e imidacloprid, demonstrando assim que bioinsecticidas de risco reduzido podem ser mais tóxicos para os agentes de controle biológico do que inseticidas convencionais.

ii. Adultos de *Eriopis connexa* resistentes a lambda-cialotrina apresentam custo adaptativo associado à reprodução quando comparados a indivíduos da linhagem suscetível, o que pode contrabalancear os benefícios advindos da resistência a inseticidas nestes organismos.

iii. A exposição de *E. connexa* ao lambda-cialotrina altera o comportamento reprodutivo de ambas linhagens e resulta numa redução do pico de produção de progênie na linhagem resistente e da duração do período de produção para linhagem suscetível.

iv. Não foi detectado efeito significativo do custo adaptativo em relação ao caminhamento e manipulação de presa por adultos de *E. connexa* resistentes e suscetíveis a piretróides. Entretanto a exposição à lambda-cialotrina afetou significativamente o caminhamento e a busca pela presa por parte de insetos resistentes. Dessa maneira, essa consequência pode reduzir o desempenho predatório desses indivíduos, o que acarretaria numa demanda de liberação de uma maior quantidade de coccinelídes resistentes para suprir essa adversidade.