ASSOCIAÇÃO DA JOANINHA *Eriopis connexa* (GERMAR) (COLEOPTERA: COCCINELLIDAE) COM INSETICIDAS VISANDO AO CONTROLE DE PRAGAS DAS BRÁSSICAS

por

ROGÉRIO LIRA

(Sob Orientação do Professor Jorge Braz Torres - UFRPE)

RESUMO

A traça-das-crucíferas e os pulgões são pragas-chave das brássicas, frequentemente requerendo pulverizações inseticidas para o controle. A utilização de inseticidas seletivos, complementado com inimigos naturais resistentes a inseticidas, oferece a oportunidade de integrar inseticidas e inimigos naturais no controle dessas pragas. O desempenho da joaninha predadora, *Eriopis connexa* (Germar) (Coleopotera: Coccinellidae), mediante sua exposição a inseticidas recomendados para o controle da traça *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) e do pulgão *Lipaphis pseudobrassicae* (Davis) (Hemiptera: Aphididae) foi avaliado. Curvas de concentração-mortalidade foram determinadas para três inseticidas registrados para brássicas e índices de seletividade diferencial foram calculados para larvas da traça e adultos de joaninhas. O desempenho e a sobrevivência de larvas e adultos da joaninha expostas a diferentes inseticidas combinado com o consumo da presa não alvo do inseticida pela joaninha foram determinados. Após conhecer a seletividade dos inseticidas testados, foi avaliado o controle do pulgão e da traça em plantas confinadas em gaiolas, em campo, com pulverização e liberação da joaninha. Os resultados mostram que os inseticidas *Bacillus thuringiensis*, ciantraniliprole, clorantraniliprole, deltametrina, clorfenapir, espinosade, azadiractina e espiromesifeno são compatíveis com larvas e adultos de *E. connexa*. A exposição de larvas e adultos da joaninha expostas em sequência a duas e cinco aplicações dos inseticidas deltamentrina, pimetrozina, espinosade, ciantraniliprole, metomil, clorfenapir, clorantraniliprole ou espiromisefeno, não afetou o seu desempenho permitindo aplicações compatíveis. Os inseticidas recomendados para o controle da traça ou dos pulgões não afetaram significativamente o consumo dessas pragas. Os resultados de campo não caracterizaram efeito aditivo da liberação de *E. connexa* às aplicações de inseticidas no controle da traça e pulgões. O número de joaninhas e o momento de sua liberação precisam ser melhor estudados para viabilizar o controle integrado dessas pragas.

PALAVRAS-CHAVE: Seletividade de inseticidas, traça-das-crucíferas, pulgão, resistência a inseticidas.

COMBINATION OF THE LADY BEETLE *Eriopis connexa* (GERMAR) (COLEOPTERA: COCCINELLIDAE) WITH INSECTICIDES IN THE CONTROL OF BRASSICAS' PESTS

by

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(Under the Supervision of Professor Jorge Braz Torres – UFRPE)

ABSTRACT

The diamondback moth and aphids are key pest species of brassicas' crops, which frequently requires insecticide applications to their control. The use of selective insecticides, complemented with insecticide-resistant natural enemies, offers the opportunity to integrate insecticides and natural enemies in pest control. Thus, the performance of the predatory lady beetle, *Eriopis connexa* (Germs) (Coleopotera: Coccinellidae), was evaluated through its exposure to insecticides recommended against the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), and the turnip aphid, *Lipaphis pseudobrassicae* (Davis) (Hemiptera: Aphididae). Concentration-mortality curves were determined for three insecticides registered for brassicas and differential selectivity indices were calculated for diamondback moth larvae and adults of the lady beetle. After to know the selectivity of the insecticides tested, was evaluated the control of aphids and moths in plants confined in cages, in the field, with spraying and release of the lady beetle. The results show that the insecticides *Bacillus thuringiensis*, cyantraniliprole, chlorantraniliprole, deltamethrin, chlorfenapyr, spinosad, azadirachtin and spiromesifen are compatible with larvae and adults of *E. connexa*. The exposure of lady beetle larvae and adults exposed in sequence to two and five applications of the insecticides deltamethrin, pymetrozine, spinosad, cyantraniliprole, methomyl, chlorfenapyr, chlorantraniliprole or spiromisefen, did not affect their performance allowing compatible applications. The recommended insecticides for diamondback moth or aphid control did not affect significantly the consumption of these pests. The results of field did not characterize an additive effect of the release of *E. connexa* to insecticide applications in the control of diamondback moth and aphids. The number of lady beetle to be released and the release timing need to be determined for a better application of both, lady beetle release and insecticide application methods.

KEY WORDS: Selectivity of insecticides, diamondback moth, turnip aphid, insecticide resistance.

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ROGÉRIO LIRA

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“Em seu coração o homem planeja o seu caminho, mas o Senhor determina os seus passos.”

Provérbios 16:9

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CAPÍTULO 1

INTRODUÇÃO

A variação na dinâmica de espécies pragas nos agroecossistema pode demandar a adoção de medidas de controle, no entanto nem sempre contra todas as espécies simultaneamente. Isto porque múltiplas interações ecológicas podem ocorrer entre esses organismos como herbivoria, competição por recursos, onivoria, predação, predação intraguilda, parasitismo e hiperparasitismo, seja por via direta ou indireta (Janssen *et al.* 1999, Prasad & Snyder 2006, Evans 2008, Messelink *et al.* 2012). Essas interações ecológicas podem ser profundamente afetadas, dependendo da tática de controle implementada, a exemplo do que acontece com a aplicação de inseticidas não seletivos. Por outro lado, a aplicação de inseticidas seletivos a uma dada espécie alvo pode favorecer o manejo de pragas (Desneux *et al.* 2007, Fytrou *et al.* 2017). O principal resultado é a conservação dos inimigos naturais presentes que podem atuar sobre espécies pragas não alvo dos inseticidas aplicados, assim, evitando que essas atinjam densidades populacionais equivalentes ao nível de controle. Desta maneira, há necessidade de conhecimento das interações entre pragas, inimigos naturais e inseticidas recomendados para compor uma proposta de manejo de inseticidas, visando beneficiar o manejo das espécies de pragas no agroecoessistema.

As brássicas, *Brassica* spp. (couve, repolho, brócolis, dentre outras), estão sujeitas a perdas quantitativas e qualitativas de produção devido às infestações por insetos pragas. Dependendo da densidade populacional das pragas podem ocorrer injúrias diretas e indiretas (desfolha, doenças e desenvolvimento de fumagina) na cultura, o que resulta na redução de produtividade e qualidade. Os insetos pragas mais comuns citados na literatura em cultivos de brássicas no Brasil incluem: moscas-minadoras, *Liriomyza* spp. (Diptera: Agromyzidae); pulgões, *Lipaphis pseudobrassicae*

(Davis), *Brevicoryne brassicae* (L.) e *Myzus persicae* (Sulzer) (Hemiptera: Aphididae); mosca-branca, *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae); crisomelídeos, *Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae); e lagartas, *Ascia monuste orseis* (Godart) (Lepidoptera: Pieridae), *Agrotis ipsilon* (Hufnagel), *Spodoptera eridania* (Cramer), *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), *Hellula phidilealis* (Walker) (Lepidoptera: Pyralidae), e a traça-das-crucíferas, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Melo 2012, Holtz *et al.* 2015, AGROFIT 2022).

Dentre as espécies supracitadas, a traça-das-crucíferas é a mais importante em todo o mundo, devido às perdas ocasionadas e aos custos de controle envolvidos que estão estimados entre 4 e 5 bilhões de dólares anualmente (Zalucki *et al.* 2012). Além disso, a traça é o inseto praga com o maior número de casos de resistência a inseticidas, sendo 980 casos registrados (Mota-Sanchez & Wise 2021). Isto se torna um entrave para os produtores de brássicas pelas falhas de controle e o aumento no uso de inseticidas, comumente tornando o ambiente de baixa qualidade para os agentes de controle biológico. Há menção que o principal fator do *status* de praga da *P. xylostella*, em grande parte do mundo, é a ausência da ação de inimigos naturais (Lim 1986). A falta de inimigos naturais atuando na regulação populacional da traça-das-crucíferas é considerada um resultado do uso de inseticidas sintéticos de amplo espectro, prática intensificada a partir da década 1940, quando ainda não havia registro da traça-das-crucíferas como praga-chave (Talekar & Shelton 1993).

Existem três tipos de controle biológico: Controle biológico clássico, que consiste da importação de inimigos naturais visando o controle de pragas exóticas ou nativas; controle biológico natural, que atende um preceito básico do controle biológico, a conservação dos inimigos naturais através da manipulação do ambiente para sua atuação natural sobre as espécies pragas; e controle biológico aplicado, que trata da liberação de inimigos naturais a partir de uma criação massal visando a redução da população da praga abaixo do nível de dano (Parra *et al.* 2002). A literatura é abundante em registros de inimigos naturais de *P. xylostella*. Por exemplo, os parasitoides são representados por mais de 135 espécies (Delvare 2004), das quais cerca de 60 são de importância agronômica (Lim 1986, Talekar & Shelton 1993). Além dos parasitoides, os entomopatógenos também se mostram relevantes no manejo da traça-das-crucíferas (Sarfraz *et al.* 2005), já havendo produtos comerciais à base da bactéria *Bacillus* e do fungo *Beauveria* (Lacey *et al.* 2015, Li *et al.* 2016, AGROFIT 2022). Artrópodes predadores como aranhas, formigas, moscas, hemípteros, crisopídeos, dermápteros e coleópteros são citados como inimigos naturais de *P. xylostella* (CABI 2017, Silva *et al.* 2017). No Agreste de Pernambuco, diversos desses inimigos naturais ocorrem atuando sobre ovos, lagartas e pupas de *P. xylostella* (Silva-Torres *et al.* 2010).

Os predadores generalistas possuem destaque como agentes de controle biológico, por terem maior capacidade de sobrevivência quando a densidade da praga está baixa, como aquelas abaixo do nível de controle, pois podem sobreviver no agroecossistema se alimentando de várias espécies de presas (Whitcomb 1981, Symondson *et al.* 2002). Dentre os predadores, as joaninhas são importantes agentes de controle biológico de pulgões, ácaros, tripes, ovos e pequenas larvas de lepidópteros (Evans 2009, Giorgi *et al.* 2009). As espécies de joaninhas com registro de predação das fases imaturas da traça-das-crucíferas incluem: *Cycloneda sanguinea* (L.), *Hippodamia convergens* (Guérin-Meneville), *Coleomegilla maculata (*DeGeer), *Harmonia axyridis* (Pallas), *Coccinella septempunctata* L., *Propylaea japonica* (Thunberg) e *Eriopis connexa* (Germar) (Coleopotera: Coccinellidae) (Alam 1990, Ferry *et al.* 2003, CABI 2017, Lira *et al.* 2019). No entanto, existe uma dificuldade na utilização desses agentes de controle biológico no manejo de *P. xylostella*, não apenas por desconhecimento do comportamento de predação, mas também por fatores de interação com outros insetos (predadores ou presas), e pelo uso intensivo de inseticidas, muitos deles não seletivos.

Além da complexidade da cadeia trófica para o estabelecimento do inimigo natural no agroecossistema, outro desafio é a associação do inimigo natural com o controle químico, onde o inimigo natural teria que sobreviver aos inseticidas utilizados para o controle das pragas. No caso em estudo, inseticidas recomendados para o controle de pragas das brássicas, em especial, aqueles recomendados contra a praga-chave, *P. xylostella*. As pulverizações direcionadas para o controle da traça-das-crucíferas visam ao menos 80% de controle (Zalucki *et al.* 2012). Nesse contexto, pressupõe-se que até 20% da população da praga escape ao efeito do inseticida aplicado, seja por estarem abrigadas numa parte da planta onde o inseticida não atinge (Zago *et al.* 2013), ou por ela apresentar alelos de tolerância, ou mesmo, resistência ao inseticida aplicado (Kwon *et al.* 2004). Assim, para um manejo adequado desta praga, a atuação dos inimigos naturais auxiliaria no controle da população e, em especial, dos indivíduos remanescentes no caso de uma integração de controle. No entanto, devido ao intenso uso de inseticidas, os inimigos naturais contribuirão satisfatoriamente se o inseticida aplicado for seletivo à praga alvo, ou quando o inimigo natural apresentar resistência ao inseticida utilizado. Neste contexto, assim como a praga resistente, o inimigo natural sobreviverá às aplicações do inseticida, como já determinado para a joaninha predadora *E. connexa* (Spíndola *et al.* 2013, Lira *et al.* 2019).

A seletividade pode ser alcançada através de práticas de aplicação do inseticida que visem minimizar a exposição do inimigo natural, assim chamada seletividade ecológica (Hull & Beers 1985) ou através da diferença de toxicidade entre a praga e o inimigo natural, onde a praga é mais suscetível aos efeitos do inseticida quando comparada ao inimigo natural, sendo caracterizada como seletividade fisiológica (Ripper *et al*. 1951, Newsom *et al.* 1976). A seletividade fisiológica pode ser obtida mediante seleção natural ou artificial de populações de inimigos naturais para resistência a inseticidas e, assim, podendo ser utilizado em ação conjunta os métodos químico e biológico de controle das pragas (Torres 2012).

Atualmente o desenvolvimento de novas moléculas para o controle de pragas tem focado em inseticidas mais seletivos às pragas (Sparks 2013). Entre os inseticidas para o controle de pragas das brássicas no Brasil, registrados e passível de recomendação são 117 formulações comerciais com 44 ingredientes ativos (AGROFIT 2022). Assim, trabalhos realizados relatam diferença na toxicidade de pragas e inimigos naturais para produtos como *Bt*'s (*Btk* e *Bti*), diamidas (clorantraniliprole, flubendiamida, ciantraniliprole), espinosinas (espinosade e espinetoram), pyridine azometina (pimetrozine), piridinecarboxamida (flonicamida), piriproxifen e buprofenzina, o que permite a recomendação desses no manejo de pulgões, mosca-branca e lagartas (Torres *et al.* 2002, Medina *et al.* 2003, Williams *et al.* 2003, Brugger *et al.* 2010, Gentz *et al.* 2010, Jensen *et al.* 2011, Garzón *et al.* 2015, Vivian *et al.* 2016, Kim *et al.* 2018). Esta seletividade fisiológica pode ser estendida para produtos não seletivos quando o inimigo natural desenvolve resistência, sobrevivendo as concentrações recomendadas. Este é o caso de joaninhas predadoras e inseticidas normalmente não seletivos como piretroides e fosforados. Predadores comumente encontrados em brássicas como as joaninhas *E. connexa*, apresenta populações resistentes a piretroide (Rodrigues *et al.* 2013, Costa *et al.* 2018), e *H. convergens* resistência a piretroide e fosforados (Barbosa *et al.* 2016), bem como crisopídeos como *Chrysoperla carnea* (Steph) a piretroides e fosforados (Pathan *et al*. 2008), entre outros.

A resistência é uma característica pré-adaptativa, genética e hereditária (Dobzhansky 1951), definida como a capacidade desenvolvida, em uma dada população de insetos, em tolerar doses de produtos tóxicos que seriam letais para certos indivíduos da espécie (Croft *et al.* 1988), que na prática é caracteriza pela falha de controle quando empregando a concentração recomendada do produto comercial. A resistência advém de mecanismos envolvidos como alterações de aminoácido no sítio alvo, ação de enzimas destoxificantes, redução na penetração e mudança comportamental (Sarfraz *et al*. 2005, Ahmad *et al*. 2006, Zago *et al.* 2014). Contudo, os mecanismos mais comuns são o aumento na atividade de esterases, de glutationa *S*-transferase e de monooxigenases dependentes de citocromo P450 (Li *et al*. 2007, Bass & Field 2011), o que caracteriza uma resistência metabólica.

A hipótese da joaninha predadora *E. connexa*, resistente à lambda-cialotrina (Rodrigues *et al.* 2013), bem como tolerante a outros piretroides (Torres *et al.* 2015), ser utilizada em combinação com piretroide no controle da traça-das-crucíferas foi testada por Lira *et al.* (2019). De acordo com estes autores, a população de *E. connexa* resistente a piretroides não sofreu alteração no comportamento de predação em arenas tratadas com a dose de bula de deltametrina (7,5 mg de i.a/L) para controle de pragas das brássicas. Além disso, a presença do pulgão *L. pseudobrassicae* (presa preferencial da joaninha), não limitou a predação das lagartas de *P xylostella*, onde o consumo em 24h foi de 60 pulgões e 2 a 3 lagartas de *P. xylostella*, em média, mesmo na condição de abundância da presa preferida. Sob tais circunstâncias, o consumo de pulgões já resultaria na integração dos métodos biológico e químico como proposto. A partir dos resultados obtidos por Lira *et al.* (2019), estudos para averiguar a contribuição da conservação desta joaninha resistente a piretroides em controlar lagartas de *P. xylostella*, em especial aquelas que escapam de uma aplicação de inseticida, foram realizados com outros inseticidas e sequência de utilização dos inseticidas. Assim, o objetivo do presente trabalho foi estudar os principais inseticidas recomendados contra as pragas das brássicas e determinar curvas concentração-mortalidade para *P. xylostella* e *E. connexa*, população resistente e suscetível a piretroides, para os inseticidas que ocasionaram mortalidade da joaninha na máxima dose recomendada, consequentemente, estimou índices de seletividade diferencial e o coeficiente de risco. Ainda, estudar a sobrevivência e comportamento de predação das joaninhas expostas ao resíduo de inseticidas nas doses recomendadas de campo para esses inseticidas. Além disso, estudamos também a contribuição do uso integrado de *E. connexa* e inseticidas no controle de pragas das brássicas com foco em pulgões, presa preferencial da joaninha e da traça-das-crucíferas. Nesse contexto, acredita-se que o controle da traça-das-crucíferas determinará o sucesso do manejo integrado de todas as demais pragas.

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CHAPTER 2

PREDATION PERFORMANCE AND SURVIVAL OF SUSCEPTIBLE AND PYRETHROID-RESISTANT *Eriopis connexa* (GERMAR) (COLEOPTERA: COCCINELLIDAE) TO INSECTICIDES USED IN BRASSICA CROPS[1]

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ABSTRACT - Selective insecticides and insecticide-resistant natural enemies are components of chemical and biological methods that can be compatible in an integrated pest management program. Many insecticides that are labeled for treatment against insects in *Brassica* crops have lost their efficacy because of the development of resistance. However, natural enemies can provide an important role in regulating the population of these pests. We assessed the mortality of *Plutella xylostella* (L.) and *Lipaphis pseudobrassicae* (Davis) against 10 insecticides labeled to treat insect pests in *Brassica* crops. In addition, we compared the survival and predation performances of two populations of the predator *Eriopis connexa* (Germar), one susceptible (EcFM) and another resistant (EcViR) to pyrethroids, both exposed to 10 selected insecticides. Lethal concentrations (LCs) were determined for insecticides causing high mortality of adult *E. connexa* applied at the maximum labeled field rate, allowing the determination of differential selectivity index (DSI) and the risk quotient (RQ). Survival of both *E. connexa* populations was >80% when exposed to the insecticides, except for EcFM exposed to indoxacarb and methomyl. *Bacillus thuringiensis*, cyantraniliprole, chlorfenapyr, and spinosad caused high mortality of *P. xylostella* larvae, but neither affected *E. connexa* survival nor its predation upon *L. pseudobrassicae*. Cyantraniliprole, chlorfenapyr, deltamethrin, and methomyl caused high mortality of *L. pseudobrassicae*, but did not affect *E. connexa* survival nor its predation upon *P. xylostella* larvae. This study demonstrates that the insecticides *B. thuringiensis*, cyantraniliprole, chlorantraniliprole, deltamethrin, chlorfenapyr, spinosad, azadiracthin, and spiromesifen are compatible with adult *E. connexa* within an integrated pest control program in *Brassica* crops and would be considered to preserving this natural enemy.

KEY WORDS: Biological control, chemical control, physiological selectivity, diamondback moth

DESEMPENHO DE PREDAÇÃO E SOBREVIVÊNCIA DE *Eriopis connexa* (GERMAR) (COLEOPTERA: COCCINELLIDAE) SUSCETÍVEL E RESISTENTE A PIRETROIDES AOS INSETICIDAS USADOS EM CULTURAS DAS BRÁSSICAS

RESUMO - Inseticidas seletivos e inimigos naturais resistentes a inseticidas são componentes de métodos químicos e biológicos que podem ser compatíveis num programa de manejo integrado de pragas. Muitos inseticidas que são recomendados contra insetos em brássicas perderam sua eficácia devido ao desenvolvimento de resistência. No entanto, inimigos naturais podem desempenhar um papel importante na regulação da população dessas pragas. Avaliamos a mortalidade de *Plutella xylostella* (L.) e *Lipaphis pseudobrassicae* (Davis) contra 10 inseticidas recomendados para pragas em campos de brássicas. Além disso, comparamos o desempenho de sobrevivência e predação de duas populações de *Eriopis connexa* (Germar), uma suscetível (EcFM) e outra resistente (EcViR) a piretroides, ambas expostas a dez inseticidas selecionados. Concentrações letais (CLs) foram determinadas para os inseticidas que causaram mortalidade significante na dose máxima recomendada em adultos de *E. connexa*, permitindo a determinação do índice de seletividade diferencial (DSI) e do coeficiente de risco (RQ). A sobrevivência de ambas as populações de *E. connexa* foi >80% quando expostas aos inseticidas, exceto para EcFM exposta a indoxacarbe e metomil. *Bacillus thuringiensis*, ciantraniliprole, clorfenapir e espinosade causaram alta mortalidade em larvas de *P. xylostella*, mas não afetaram a sobrevivência de *E. connexa* nem sua predação sobre *L. pseudobrassicae*. Ciantraniliprole, clorfenapir, deltametrina e metomil causaram alta mortalidade em *L. pseudobrassicae*, mas não afetaram a sobrevivência nem a predação de larvas de *P. xylostella* por *E. connexa*. Este estudo demonstra que os inseticidas *B. thuringiensis*, ciantraniliprole, clorantraniliprole, deltametrina, clorfenapir, espinosade, azadiractina e espiromesifeno são compatíveis com *E. connexa* dentro de um programa de controle integrado de pragas em lavouras de brássicas e seriam considerados para preservar esse inimigo natural.

PALAVRAS-CHAVE: Controle biológico, controle químico, seletividade fisiológica, traça-das-crucíferas

Introduction

Somehow, the recommendation of selective insecticides is based in part their compatibility with natural enemies of key pest species, a desirable outcome for integrated pest management (IPM) and insecticide resistance management (IRM). Natural enemies that survive applications of insecticides in crops may delay the selection of insecticide resistance population for target pests by preying on individuals that would carry resistant alleles for future generations, and also prevent secondary pest outbreaks (Gould *et al.* 1991, Chilcutt & Tabashnik 1999, Liu *et al.* 2014). Natural enemies surviving in such a scenario would benefit *Brassica* ecosystems that are attacked by the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), and several other species of key and secondary pests. The diamondback moth is ranked as the number one insect pest species with respect to the number of recorded cases (980) of insecticide resistance (Mota-Sanchez and Wise 2021). Therefore, any control method that is capable of diminishing this resistance status should be pursued. Besides the diamondback moth, various aphid species are often sprayed with insecticides in fields of *Brassica* crops, which may negatively impact the population of natural enemies (Bacci *et al.* 2009, Silva-Torres *et al*. 2011, Nunes *et al.* 2019). However, there are selective insecticides that may reduce these adverse impacts (Singh *et al*. 2016) and favor biological control organisms.

Because selective insecticides can act against a pest species or group of pest species without affecting others, insecticide selectivity can be understood as a synonym of insecticide specificity (Torres & Bueno 2018). For instance, *Bacillus thuringiensis*, chlorantraniliprole, spinosad, and indoxacarb insecticides are registered and recommended to control defoliators (mainly against lepidopteran larvae) infesting *Brassica* crops in Brazil (AGROFIT 2022), whereas cyantraniliprole, spiromesifen, and pymetrozine target species of sap-sucking pests (aphids and whiteflies). Aside from the specific ones, some non-selective insecticides including azadirachtin, chlorfenapyr, deltamethrin, and methomyl are also among the most used insecticides in fields of *Brassica* crops. The specificity of an insecticide for a target species is driven by its chemical properties and the inability of the target insect to detoxifying it (Winteringham 1969). However, some insects develop physiological mechanisms that allow them to survive exposure to xenobiotics, which characterizes the so called "physiological selectivity" (Winteringham 1969, Croft 1990). In addition to pests, natural enemies can also boost their enzymatic activity to break down insecticides, and the successful individuals can develop into resistant populations. For example, studies have shown a metabolic role on resistance to insecticides by the predators *Stethorus gilvifrons* (Coleoptera: Coccinellidae) (Kumral *et al.* 2011), *Chrysoperla carnea* (Neuroptera: Chrysopidae) (Mansoor *et al.* 2017), and *Eriopis connexa* Germar (Coleoptera: Coccinellidae) (Rodrigues *et al.* 2014, Rodrigues *et al.* 2020).

The aphidophagous beetle *E. connexa* is a key predator in many agroecosystems, including *Brassica* crops (Harterreiten *et al.* 2012, Rodrigues *et al*. 2013, Fidelis *et al*. 2018). Although aphids are the preferential prey of *E. connexa,* it also consumes spider mites, thrips, whiteflies, psyllids, lepidopteran eggs, and young lepidopteran larvae (Evans 2009), including those *P. xylostella* (Lira *et al.* 2019, Nascimento *et al.* 2021). In Brazil, a population of *E. connexa* was reported as resistant to different pyrethroids (Rodrigues *et al.* 2013, Costa *et al.* 2018) and its predation behavior on the turnip aphid (*Lipaphis pseudobrassicae* (Hemiptera: Aphididae)) and *P. xylostella* was not affected after exposure to deltamethrin at the maximum labeled rate for *Brassica* crops (Lira *et al.* 2019). Furthermore, this pyrethroid-resistant *E. connexa* population has exhibited low to moderate susceptibility to other insecticides including pymetrozine, chlorantraniliprole (Barros *et al.* 2018), and spinetoram (Costa *et al*. 2020). Collectively, these results identify this *E. connexa* population as a good candidate for integrating management programs in *Brassica* crops, where biological and chemical control methods could be harmoniously combined to better regulate pest populations.

To establish compatibility among insecticides and natural enemies, researchers often use the differential selectivity index (DSI) and the risk quotient (RQ); both approaches are based on the mortality of the natural enemy and the pest species subjected to standardized exposure procedures (Preetha *et al*. 2009). The DSI approach requires knowledge about the mortality response of the natural enemy and the pest species as a function of different insecticide concentrations rated from the lethal concentration of the population. RQ requires knowledge about the LC_{50} calculated for the natural enemy and the labeled field rate of the insecticide against the target pest. Therefore, DSI represents physiological selectivity (Winteringham 1969, Mullin & Croft 1985), whereas RQ is an indicator of the ecological risk imposed on the natural enemy by the applied insecticide (Campbell *et al*. 2000, Peterson 2006, Preetha *et al*. 2009). The determination of these indices subsidizes a recommendation of insecticides less toxic to natural enemies compared to the target pests.

Aiming to combine biological and chemical control methods of *Brassica* crop pests, the present study assessed the survival of adult *E. connexa* from two populations, one susceptible and another resistant to pyrethroids, when exposed to dried residues of 10 insecticides labeled for use against *Brassica* insect pests. The insecticides that caused significant mortality of adult lady beetles at their maximum label rate (LR) were then used to estimate insecticide lethal concentrations (LCs) for *E. connexa*. We also determined the mortality of *L. pseudobrassicae* and larvae of *P. xylostella*, and the insecticides that caused low mortality of these pests were used in the predation behavior bioassay with *E. connexa*. Finally, based on the LCs for adult *E. connexa* and *P. xylostela* larvae, we estimated the DSI and RQ, which may guide choices of low-impact insecticides that target chewing- and sucking pests of brassicas.

Material and Methods

All experiments were performed in the laboratory at 25 ± 2 °C, 60 ± 12% relativity humidity, and a 12:12h L:D regime.

Lipaphis pseudobrassicae. The turnip aphid was collected from collard plants (*Brassica oleracea* var. *acephala*), which were grown without insecticides in an experimental field at the 'Universidade Federal Rural de Pernambuco' (UFRPE). Collard plants were cultivated in 5-L pots filled with a mixture of soil and humus (2:1), plus 10 g of fertilizer (N:P:K, formula 4:14:8). These plants were irrigated once a day.

Plutella xylostella. A diamondback moth colony was established from a pyrethorid-resistant population maintained at the Laboratory of Insect-Toxic Interactions at UFRPE, following the rearing methodology described by Santos *et al*. (2011). Briefly, diamondback moth larvae were reared in a plastic container (20 × 12 × 10 cm in L × W × H) with an open in the lid that was covered with mesh screen for ventilation. Collard leaves free of insecticide residues were provided as larval food. Diamondback moth larvae that pupated on the underside of the collard leaves were daily transferred to vials (1.2 × 8 cm in diam × H) that were sealed with polyvinyl chloride film (PVC), where they incubated until adult emergence. Adult moths were transferred to oviposition cages made from transparent 1-L plastic pots with lids (15 cm in diam) covered with organdy fabric. Each oviposition cage was lined with moistened filter paper onto which 5 cm diameter collard leaf discs were provided as an oviposition substrate. Moths were fed a 20% honey:water solution on cotton wool placed on a 2 cm diam dish at the bottom of the cage.

Eriopis connexa. A population of pyrethoid-susceptible *E. connexa* (hereafter EcFM) was collected from cotton fields in Frei Miguelinho, Pernambuco State, Brazil. It was annually renewed with specimens collected during the cotton cropping season and maintained in the laboratory without insecticide exposure. A population of pyrethoid-resistant *E. connexa* (hereafter EcViR) was originally collected in cabbage (*Brassica oleracea* L.) fields in Viçosa, Minas Gerais State, Brazil (Rodrigues *et al*. 2013); resistant beetles collected in cabbage fields from other locations were added to the colony after they were screened for resistance (Costa *et al*. 2018). The EcViR population was maintained under selective pressure by periodical exposure to lambda-cyhalothrin at technical grade. By the time of the experiment started, the adult EcViR population was tested with topical application of 5μg insect^{-1} of the lambda-cyhalothrin with survival greater than 90%; the EcFM individuals had 0% survival when tested with this same treatment.

Adult beetles from both populations were reared in 500 mL plastic containers with pieces of paper towel as a substrate for oviposition. The eggs were removed daily and placed into 80 mL plastic pots until hatching. Thereafter, a pair of larvae was transferred to another pot until pupation. Both larvae and adult lady beetles were fed frozen *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) (Mediterranean flour moth) eggs, and adult beetles were also provided psyllids *Isogonoceraia* sp. (Hemiptera: Psyllidae) and aphids *L. pseudobrassicae* to stimulate oviposition.

Insecticide Solutions. We tested 10 insecticides (Table 1) that are labeled in Brazil for managing key pests of *Brassica* crops; all of these in their commercial formulations were purchased from the local market. To facilitate adhesion of the insecticides on treated surfaces, the insecticides were diluted into an aqueous surfactant (Wil-Fix 30 g i.a. L^{-1}, Charmon Destyl Indústria Química Ltda, Campinas, SP, Brazil) solution at 0.1%, which alone served as control.

Susceptibility of *Lipaphis pseudobrassicae* and *Plutella xylostella* Larvae. By adapting the leaf dipping method number 18 from International Resistance Action Committee (IRAC 2010), we determined the mortality of turnip aphids and diamondback moth larvae exposed to dried residues of 10 insecticides on collard leaf disc surfaces. After dipping the leaf discs into the insecticide [at maximum label rate (LR)] or control solutions for 10-20 seconds, the discs (8cm diameter) were left to air-dry for about 2 h over a paper towel on a laboratory bench. Each treated disc was transferred to a clean glass Petri dish (80 mm diam) lined with moistened filter paper of the same diameter.

Collard leaves infested with the turnip aphid were harvested from an experimental field and were left to wither for 3-4 h and stimulate aphid dispersal. Both late instar nymphs and apterous adults were collected with a fine hairbrush and transferred to the treated leaf discs. With respect to the diamondback moth, larvae were 3-day-old when they were used in the experiment. Mortality, determined by the inability of the insects to walk after being softly touched on their abdomen, was tallied 24 h after confinement. This bioassay followed a completely randomized design set up with 11 treatments (10 insecticides plus control) and three replications, each one with 15–20 insects (aphids or DBM larvae).

Susceptibility of *Eriopis connexa* to Insecticide Label Rates. The susceptibility of *E. connexa* to insecticides was assessed following the same insecticide exposure procedure described in the aforementioned experiment. Briefly, collard leaf discs were dipped for 10-20 seconds into insecticide at maximum LR and 10-fold the LR for those that did not cause mortality at maximum LR, and control solutions, and were left to air-dry for about 2 h. They were then transferred to clean glass Petri dishes. Adult beetles (5-8 days old) from both EcFM and EcViR populations were transferred to petri dishes and provided free-pesticide frozen moth eggs *ad libitum* over the leaf disc surfaces. Mortality, determined by the inability of the lady beetle to turn upright and walk after being placed on its dorsum, was assessed 24 h after exposure to insecticide residues. This bioassay followed a completely randomized factorial design (2 × 11), considering two beetle populations (EcFM and EcViR) and 11 treatments (10 insecticides plus control). Each treatment was run with three replications with 15-20 adult beetles per replication.

Susceptibility of *Eriopis connexa* to Indoxacarb. Indoxacarb is considered a low impact insecticide to some natural enemies (Tillman & Mulrooney 2000, Michaud & Grant 2003). Because the bioactivation of indoxacarb is faster after ingestion and slower after topical treatment, it is toxically metabolized fast in chewing insects, such as lepidopteran and coleopteran larvae, and becomes highly toxic (Pluschkell *et al.* 1998, Wing *et al*. 2000, Liu *et al*. 2002). Thus, to confirm the impact of indoxacarb previously observed on the previous experiment (Susceptibility of *Eriopis connexa* to insecticide field rates**)**, we splitted the contamination of *E. connexa* with indoxacarb into a factorial design regarding residual contact (on treated leaves) and ingestion (of treated prey). The bioassay followed a completely randomized factorial design (2 × 2), with four treatments: (i) treated leaves + treated moth eggs (*E. kuehniella*), (ii) untreated leaves + treated moth eggs, (iii) treated leaves + untreated moth eggs, (iv) untreated leaves + untreated moth eggs (control). Each treatment was run with six replications with five adult beetles per replication. Briefly, collard leaf discs were dipped for 10-20 seconds into insecticide (at maximum LR) and control solutions. Moreover, ≈32 mg of moth eggs (*E. kuehniella*) was treated with 50 µL of insecticide or control solutions. The treated and untreated leaves and moth eggs were left to air-dry for about 2 h, when they were transferred to clean glass Petri dishes. Adult beetles (5-8 days old) from the EcViR population were then transferred to the dishes, where they were fed free-insecticide or insecticide treated moth eggs provided on plastic lid over the leaf disc surfaces. Mortality, determined by the inability of the beetle to turn upright and walk after being placed on its dorsum, was assessed 48 h after confinement on the treatments.

Dose-mortality Responses of *Eriopis connexa* adults and *Plutella xylostella* Larvae. Based on results from the above-mentioned bioassay indoxacarb and methomyl (mortality on LR), along with chlorfenapyr (mortality on 10x LR) were chosen for an experiment to estimate their lethal concentrations (LCs) to adult *E. connexa* and larvae of the diamondback moth. Based on preliminary tests and following the leaf dipping method, five to seven insecticide concentrations were established to cause mortality near 0% and ≈100% of both EcFM and EcViR beetles [indoxacarb (0.188 to 6.0 mg a.i. L^{-1}), methomyl (4.031 to 129.0 mg a.i. L^{-1}) and chlorfenapyr (9.0 to 288.0 mg a.i. L^{-1})] and DBM larvae [Indoxacarb (0.093 to 3.0 mg a.i. L^{-1}), methomyl (5.375-

129.0 mg a.i. L^{-1}) and chlorfenapyr (0.036 to 1.2 mg a.i. L^{-1})]. Based on that, we calculated the lethal mean concentrations using Probit analysis (Finney 1971).

Predation Bioassay. An experiment on predation was set up to determined the consumption of *L. pseudobrassicae* and *P. xylostella* larvae by adult *E. connexa* from both EcFM and EcViR populations under insecticide exposure. Based on our previous bioassays, there were selected insecticides with low impact on the lady beetles and that caused mortality <80% of the turnip aphid (i.e., azadirachtin, *B. thuringiensis*, chlorantraniliprole, and spinosad) and the *P. xylostella* larvae (i.e., azadirachtin, methomyl, and chlorantraniliprole), respectively. Collard leaf discs were dipped into the insecticide solutions at maximum FR and left to air-dry prior introduction to clean glass Petri dishes, where they were infested with late instar nymphs of turnip aphid (n = 70–80 per replicate) or second instar *P. xylostella* larvae (n = 20 per replicate). To stimulate consumption, an adult *E. Connexa* was starved for 24 h prior being released into the dish. The number of consumed prey was tallied 24 h later. This experiment followed a completely randomized design with five treatments involving aphis (four insecticides plus control), and four treatments involving diamondback moth larvae (three insecticides plus control). Each treatment was carried out with 10 replications, each one represented by an adult beetle.

Data Analysis. Insects exposed to control leaf discs exhibited 100% survival; therefore, there was no need for data correction for natural mortality. The survival percentage of adult *E. connexa*, and *P. xylostella* larvae and turnip aphid mortalities fit the normality (Shapiro-Wilk's test) and homogeneity (Bartlett's test) criteria for analysis of variance (ANOVA). The survival data of adult *E. connexa* were submitted to two-way ANOVA considering the beetle populations and insecticides as main treatment factors. The mortality of *P. xylostella* larvae was analyzed by one-way ANOVA with the insecticides as treatments. Both analyses were performed with the Proc GLM of SAS (SAS Institute 2002). Further, the average survival of adult *E. connexa* between populations and across the tested insecticides, as well as the mortality of *P. xylostella* larvae among the insecticides were separated by Bonferroni's test ($\alpha = 0.05$) to avoid type I error when comparing more than two means.

Data mortality from adult *E. connexa* and *P. xylostella* larvae exposed to different concentrations of insecticides were submitted to Probit's analysis using the Proc Probit of SAS (SAS Institute 2002). The lethal concentrations (LCs) to kill 50% and 90% of the assayed population were calculated with their respective 95% fiducial limits. The LCs were used to estimate the DSI and the RQ as follow: DSI = (LC_{90} *E. connexa* adults / LC_{90} *P. xylostella* larvae), and RQ = [Label rate (g a.i. ha^{-1}) / LC_{50} *E. connexa* (mg a.i. L^{-1})] (Preetha *et al.* 2009). A resulting DSI greater than 1 (DSI > 1.0) indicates that the insecticide is more toxic to the target pest species than to the natural enemy (Bacci *et al*. 2001). A RQ < 50 represents a harmless insecticide; a RQ value of 50 to 2500 represents a slightly-to-moderately toxic insecticide, and a RQ > 2500 represents a harmfull insecticide (Preetha *et al.* 2009).

Differences in prey (*L. pseudobrassicae* or *P. xylostella* larvae) consumption by adult *E. connexa* were assessed through a two-way ANOVA considering the beetle populations and insecticides as main treatment factors. The number of consumed *L. pseudobrassicae* or *P. xylostella* larvae fit to the ANOVA criteria for normality and homogeneity without transformation, and means were compared across insecticides using the Tukey HSD`s test ($\alpha = 0.05$).

Results

Susceptibility of *Lipaphis pseudobrassicae* and *Plutella xylostella* Larvae. The mortality of *P. xylostella* larvae ($F_{9, 20} = 314.82$, $P < 0.0001$) and *L. pseudobrassicae* ($F_{9, 20} = 41.07$, $P < 0.0001$) varied significantly across the tested insecticides (Fig. 1). *Bacillus thuringiensis,* cyantraniliprole, chlorfenapyr, indoxacarb and spinosad caused high mortality of diamondback moth larvae (>90%), while azadirachtin, chlorantraniliprole and methomyl caused mortality varying from 15 to 40%, but deltamethrin and spiromesifen did not have effect on mortality (Fig. 1). With respect to *L. pseudobrassicae*, cyantraniliprole, chlorfenapyr, deltamethrin and methomyl caused significantly higher mortality (>95%) of the aphids compared to azadirachtin, chlorantraniliprole, spinosad, and spiromesifen (about 40-50%), but *B. thuringiensis* and indoxacarb had no effect on aphid mortality (Fig. 1).

Susceptibility of *Eriopis connexa* to Insecticide Field Rates. Survival of *E. connexa* varied significantly between the pyrethroid-resistant and susceptible populations ($F_{1,\ 40}$ = 33.33, $P <$ 0.0001) and across the tested insecticides ($F_{9,\ 40}$ = 195.52, $P <$0.0001), mainly for indoxacarb and methomyl. Also, there was a significant interaction of these factors ($F_{9,\ 40}$ = 12.41, $P < 0.0001$). Irrespective of the tested populations, azadirachtin, *B. thuringiensis*, chlorantraniliprole, chlorfenapyr, spinosad and spiromesifen were innocuous to *E. connexa* (Fig. 2). Cyantraniliprole, deltamethrin and methomyl were also harmless to the EcViR population, but survival of these three treatments ranged from 65-95% for the EcFM population (Fig. 2). In contrast, indoxacarb was harmful to both EcFM and EcViR populations (survival ≤ 20%; Fig. 2).

Susceptibility of *Eriopis connexa* to Indoxacarb. Survival of *E. connexa* varied between exposure to indoxacarb-residue regarding treated and untreated leaves ($F_{1,\ 20}$ = 193.60, $P < 0.0001$), across the treated or untreated moth eggs ($F_{1,\ 20}$ = 10.00, $P <$0.0001), and there was a significant interaction of these factors ($F_{1,\ 20}$ = 19.60, $P < 0.0001$). Unlike the untreated or treated food, the residual treatment was harmful to *E. connexa* with only 3.3% and 10.0% of survival, respectively. Moreover, beetles caged on untreated leaf (without dried residues) but fed treated prey resulted also in reduced survival (60.0%) compared to the survival in the control (100%).

Dose-mortality Responses of *Eriopis connexa* and *Plutella xylostella* Larvae. The mortality data from both populations of *E. connexa* (EcFM and EcViR) and from *P. xylostella* larvae exposed to chlorfenapyr, indoxacarb and methomyl fit the Probit model ($P > 0.05$), thus allowing the calculation of lethal concentrations and their respective fiducial limits (Table 2). Chlorfenapyr was the most toxic insecticide to *P. xylostella* larvae resulting in DSI_{90} of 74- and 162-fold less toxic to the EcViR and EcFM populations, respectively. Methomyl was near 2-fold more toxic to *P. xylsotella* larvae compared to *E. connexa*, and yielded a DSI > 1 (Table 2). On the other hand, indoxacarb was more toxic to *E. connexa* compared to *P. xylostella* resulting in DSI < 1 (Table 2). When comparing the risk of contamination in a treated field (RQ), the EcViR population seems to be more affected by chlorfenapyr than the EcFM population, but the opposite was observed from the indoxacarb and methomyl treatments (Table 2).

Predation Bioassay. The consumption of *L. pseudobrassicae* by adult *E. connexa* was neither affected by the beetle population ($F_{1, 99} = 1.13$, $P = 0.2907$), nor by the insecticide treatments ($F_{4, 99} = 2.43$, $P = 0.0537$). However, consumption varied as a function of the interaction between these factors ($F_{4. 99} = 3.66$, $P < 0.0083$). Azadirachtin significantly reduced aphid consumption by the EcFM population compared to EcViR, while the other treatments did not affect prey consumption between beetle populations (Fig. 3A). The mean number of aphids consumed across all treatments varied from 43.8 to 53.0, and from 37.5 to 53.9 aphids by EcViR and EcFM beetles, respectively (Fig. 3A).

Likewise, consumption of *P. xylostella* larvae by *E. connexa* was neither affected by the studied beetle populations ($F_{1, 72} = 1.15$, $P = 0.2869$), nor by the insecticides ($F_{3, 72} = 2.10$, $P = 0.1080$); however, consumption varied as a function of the interaction between these factors ($F_{3, 72} = 3.46$, $P = 0.0206$). Azadirachtin significantly reduced the larval consumption by EcViR compared to EcFM; conversely, the methomyl treatment resulted in a reduction in feeding by the EcFM population (Fig. 3B). On the other hand, both populations consumed similar *P. xylostella* larvae in the chlorantraniliprole and control treatments with average consumption of 10 to 15 larvae (Fig. 3B).

Discussion

Many variables underlie insecticide selection for pest control in *Brassica* crops, but the efficacy of the material against the target pest while conserving natural enemies is paramount considering the challenges to control diamondback moth larvae and aphids simultaneously. Despite the 10 studied insecticides that are labeled for recommendation against these pest species in *Brassica* crops, only cyantraniliprole, chlorfenapyr, deltamethrin and methomyl at maximum LR provided control (mortality >80%) of *L. pseudobrassicae*. On the other hand, *B. thuringiensis*, cyantraniliprole, chlorfenapyr, indoxacarb and spinosad were effective against *P. xylostella* larvae. These findings reinforce the need to preserve natural enemies in fields of *Brassica* as a complementary control strategy. Following this rationale, the present study revealed that azadirachtin, *B. thuringiensis*, chlorantraniliprole, chlorfenapyr, spinosad and spiromesifen were compatible to both EcViR and EcFM populations of *E. connexa*. In contrast, cyantraniliprole, deltamethrin and methomyl were compatible to the pyrethroid-resistant *E. connexa* population, while exhibiting slight to moderate toxicity to the pyrethroid-susceptible population. Conversely, indoxacarb was highly toxic to both lady beetle populations, although causing greater mortality of the susceptible one. Perhaps the susceptible population may lack the boosted levels of detoxifying enzymes that are found in the resistant population (Rodrigues *et al.* 2014, Rodrigues *et al*. 2020), which may have driven such different responses from exposure to the latter insecticides. This study may contribute to the improvement of insecticide resistance management of *Brassica* crop pests and the conservation of natural enemies.

Among the insecticides in our study, only azadirachtin, chlorfenapyr, deltamethrin, and methomyl are labeled for control of both lepidopteran larvae and aphid. Nevertheless, our studies support that cyantraniliprole and chlorfenapyr could be used simultaneously against *P. xylostella* and *L. pseudobrassicae*. Cyantraniliprole is a diamide, like chlorantraniliprole, labeled against lepidopteran larvae, but with improved systemic properties becoming active against sap-sucking species, such as whiteflies and aphids (Barry *et al.* 2015). Cyantraniliprole caused 91% and 100% mortality of *P. xylostella* larvae and *L. pseudobrassicae*, respectively. These results agree with those reported with *Myzus persicae* (Sulzer) and *Aphis gossypii* Glover (Hemiptera: Aphididae) (Foster *et al*. 2011). In the current study, the exposure of *P. xylostella* larvae and *L. pseudobrassicae* to chlorfenapyr resulted in 90% and 100% of mortality, respectively. Resistance of *P. xylostella* to chlorfenapyr has been reported in northeastern Brazil in Pernambuco state (Lima Neto *et al*. 2021), and elsewhere (19 cases) (Mota-Sanchez & Wise 2021). Toxicity of chlorfenapyr has also been recorded against other aphid species (Patil *et al*. 2018), and without records of resistance to date.

The insecticides *B. thuringiensis*, indoxacarb and spinosad that caused effective mortality of *P. xylostella* larvae did not affect *L. pseudobrassicae*. Low mortality of *L. pseudobrassicae* was observed with indoxacarb, and this result may be explained by its slow action against sucking insects. These data support the high mortality obtained with *P. xylostella* exposed to indoxacarb. Although spinosad caused 98% mortality of *P. xylostella* larvae, this insecticide resulted in only 41% mortality against *L. pseudobrassicae,* which provides an opportunity for *E. connexa* to contribute as a predator and help suppress aphid outbreak after applications of spinosad.

Deltamethrin and methomyl caused 100% and 40% mortality of *L. pseudobrassicae*, respectively, but low mortality of *P. xylostella* larvae. Methomyl is known to be toxic to aphids (Smaili *et al*. 2014), but there are cases of resistance to this insecticide by other species of aphids (Mota-Sanchez & Wise 2021). Populations of *P. xylostella* from northeastern Brazil have been characterized with low resistance levels to methomyl (Santos *et al.* 2011). These authors suggested monitoring for resistance selection due to the high frequency of methomyl applied in *Brassica* crops. Deltamethrin is a non-selective insecticide labeled for use against *P. xylostella* larvae, *L. pseudobrassicae,* and other aphids in *Brassica* crops. Although the mortality response of *P. xylostella* to the label dose of deltamethrin was undetectable, 37 cases of resistance to this insecticide have been reported (Mota-Sanchez & Wise 2021), including the studied population (Oliveira *et al*. 2011). Cases of aphid resistance to deltamethrin have already reported (Mota-

Sanchez & Wise 2021), although the population of the turnip aphid in our study exhibited high mortality when exposed to deltamethrin label dose.

The low mortality rate observed with azadirachtin was not suprising under the 24h-evaluation period to insects. Azadirachtin has a gradual effect over time (Schmutterer 1990). Insecticides formulated with azadirachtin cause *P. xylostella* larvae mortality dose- and time-dependent with better results in a longer period of evaluation, thus affecting developmental time, host consumption, and adult fertility (Verkerk & Wright 1993). Recent studies indicate that azadirachtin has an inhibitory effect on digestive enzymes in the intestines (Qin *et al.* 2021). Similar effects have been observed on aphids (Lowery & Isman 1994). With respect to chlorantraniliprole, the low rate of mortality may be associated with the exposure time or due to the sensitivity of ryanodine receptors being different between some types of insects (Qi & Casida 2013); besides, it is common to find cases of *P. xylostella* resistant to chlorantraniliprole (54 reported cases) (Mota-Sanchez & Wise 2021). Lastly, spiromesifen is an insecticide and acaricide that is highly active against whiteflies and spider mites (Nauen *et al*. 2002). Our recorded mortality of 51% for spiromesifen against *L. pseudobrassicae* in 24 hours is compatible with studies with *A. gossypii* and *Aphis craccivora* (Koch) (Hemiptera: Aphididae), with mortality increasing over time (Patil *et al*. 2018). Because there was no mortality to either population of *E. connexa*, the data reinforces the low impact of spiromesifen to natural enemies (Singh *et al.* 2016). Therefore, the conservation of natural enemies through the use of selective insecticides can prey on individuals remaining after spray applications.

The data support our hypothesis that some insecticides used against *P. xylostella* larvae, which are compatible in preserving *E. connexa* to further help control aphids. Our data support that azadirachtin, *B. thuringiensis*, cyantraniliprole, chlorantraniliprole, chlorfenapyr, deltamethrin, spinosad, and spiromesifen are all compatible to *E. connexa* at the label rate for spray brassica crops. The overall risk of azadirachtin to natural enemies has been classified as low (Lowery & Isman

1994), including *E. connexa* (Haramboure *et al.* 2010). These authors did not find negative impact of azadirachtin on egg hatching, developmental time, adult emergence, fecundity, and fertility of *E. connexa*. Cyantraniliprole, chlorantraniliprole and spinosad, can be considered when needing to control *P. xylostella* larvae and aphids, because they are harmless to natural enemies (Liu *et al.* 2016, Singh *et al.* 2016), including species of coccinellids (Patel *et al.* 2015, Barros *et al.* 2018, Kambrekar 2019). Interesting, adult from both populations of *E. connexa* survived the exposure to deltamethrin tested at label dose (6 g a.i. ha^{-1}). Lira *et al.* (2019) found a lower number of *P. xylostella* in the treatment after applying deltamethrin, and a pyrethroid-resistant population of *E. connexa* compared to the treatment with the insecticide alone; this reinforced a lack of acute toxicity from deltamethrin to *E. connexa* when used at the label dose for brassica pest control.

The survival of the natural enemy is a desired outcome when exposed to insecticides used to control pest species, but also the survivors need to promote control of the nontarget pest of the applied insecticide, and possibly pest species individuals surviving the insecticide application. Hence, the aphid and *P. xylostella* larvae predation by adults of *E. connexa* was similar to control (untreated), when exposed to insecticides offering low control of each pest species (Fig. 2). It is the expected outcome for natural enemies to help with failures of pest control, within an IPM and IRM programs.

Across the 10 tested insecticides, indoxacarb and methomyl, in this order would be least compatible for conservation of *E. connexa* because only pyrethroid-resistant beetles exhibited high survival to methomyl. Furthermore, the calculated indices DSI_{90} and RQ determined for both *E. connexa* and *P. xylostella* larvae, and the label dose, indicate that indoxacarb is harmful to both beetle populations regardless of exposure to residues or ingestion of contaminated food. Galvan *et al.* (2005) observed low survival of earlier larval stages and adults of the multicolored Asian lady beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), including reduction of female fecundity when exposed to indoxacarb. However, other studies have indicated that indoxacarb possesses a low impact on certain natural enemies, including lady beetle species (Tillman & Mulrooney 2000, Michaud & Grant 2003). Nevertheless, promoted low mortality of the aphid in 24 h. Thus, there could be negative consequences if used in the lepidopteran pest control in brassica fields, where aphid is present, due to the potential risk for triggering aphid outbreak by affecting the aphid predator. On the other hand, chlorfenapyr was more toxic to the *P. xylostella* larvae than it was to *E. connexa*, which characterizes physiological selectivity. Both, the DSI_{90} and the RQ calculated indices for chlorfenapyr support the harmless outcome for *E. connexa* when used at label dose for brassica pest control. Further, methomyl exhibited physiological selectivity based on the DSI_{90}, but in less extension than chlorfenapyr, and it was also harmless to the lady beetle regarding the RQ. Therefore, these insecticides have positive qualities if considered when needing the control of both *P. xylostella* larvae and turnip aphids simultaneously.

In such a situation, natural enemies of *P. xylostella* larvae and aphids are needed to accomplish successful control. Key insect pest resurgence and secondary pest outbreaks after non-selective insecticide application are correlated with the negative impact of the insecticide on natural enemies (Dutcher 2007, Torres & Bueno 2018). Because spinosad and chlorantraniliprole are considered as low impact on natural enemies, including *E. connexa* (Barros *et al.* 2018), this will benefit natural enemy conservation.

In this study, we assessed and successfully revealed the opportunity for integration of chemical and biological controls using the insecticides recommended against two key pest species of *Brassica* representing different feeding habits (chewing and sucking sap), and a key aphid predator that is commonly found in brassica crops (Harterreiten *et al.* 2012, Rodrigues *et al.* 2013, Fidelis *et al.* 2018). Among the tested insecticides, only indoxacarb was shown harmful to predator; whereas the other nine target lepidopteran larvae, aphids, and whiteflies were harmless to *E.*

connexa when used at their label rate for *Brassica* crops. Furthermore, mortality varied among the pesticides. For example, chlorantraniliprole, deltamethrin, azadirachtin, and methomyl did not cause mortality of *P. xylostella* larvae at the expected level; while, Bt-based product, cyantraniliprole, indoxacarb, chlorfenapyr, and spinosad caused mortality rate greater than 90%, and except for indoxacarb, all insecticides were compatible with *E. connexa*. Regarding aphid control, the insecticides chlorfenapyr, cyantraniliprole, deltamethrin, and methomyl caused aphid mortality greater than 90% and were harmless to *E. connexa*. Moreover, for insecticides that resulted in lower mortality for the target pest species, predation by *E. connexa* was not affected. Therefore, the application of these insecticides allows *E. connexa* to contribute to pest population control.

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Table 1. Insecticides, commercial formulations, chemical groups, mode of action (MoA), and the highest label field rates to spray brassicas' crops against *Plutella xylostella* larvae (AGROFIT 2020).

Insecticides	Trademark	Chemical group	MoA	Label rate (per 100 L of water)
Azadirachtin	Azamax 12EC	Tetranortriterpenoid	UNKNOW	300 mL
Bacillus thuringiensis	Xentari 540WG	Bacteria	11A	60 mL
Cyantraniliprole	Benevia 100SC	Anthranilic diamide	28	100 mL
Chlorantraniliprole	Prêmio 200SC	Anthranilic diamide	28	7,5 mL
Chlorfenapyr	Pirate 240SC	Pyrrole	13	100 mL
Deltamethrin	Decis 25EC	Pyrethroid	3A	30 mL
Indoxacarb	Rumo 300WG	Oxadiazina	22A	10 g
Methomyl	Lannate 215EC	Carbamate	1A	100 mL
Spinosad	Tracer 480SC	Spinosyn	5	100 mL
Spiromesifen	Oberon 240SC	Ketoenol	23	600 mL

Table 2. Toxicity of the insecticides that caused significant mortality to *Eriopis connexa* populations at maximum label rate or 10-fold the label rates for *Plutella xylostella*, differential selectivity index (DSI) and the risk quotient (RQ).

Insecticides	n	df	Slope ± SE	LC$_{50}$ (FL$_{95\%}$)[1]	LC$_{90}$ (FL$_{95\%}$)[1]	$\chi^{2(P)}$	DSI$_{90}$ (FL$_{95\%}$)[2]	RQ[3]
P. xylostella larvae								
Chlorfenapyr	120	4	1.57 ± 0.28	0.34 (0.23 – 0.54)	2.23 (1.16 – 8.0)	$6.83^{0.14}$	-	-
Indoxacarb	120	4	1.44 ± 0.26	0.54 (0.36 - 0.83)	4.16 (2.18 – 14.80)	$3.09^{0.54}$	-	-
Methomyl	120	4	2.47 ± 0.37	17.46 (12.95 - 22.89)	57.42 (40.82 - 99.11)	$1.24^{0.87}$	-	-
EcFM population								
Chlorfenapyr	120	4	3.44 ± 1.13	85.03 (62.40 – 121.42)	361.68 (223.56 – 839.04)	$1.49^{0.82}$	161.6 (75.02 – 348.27)	2.8
Indoxacarb	120	4	3.50 ± 0.55	0.69 (0.54 - 0.87)	1.62 (1.23 – 2.49)	$6.86^{0.14}$	0.48 (0.32 – 0.71)	114.6
Methomyl	120	5	2.91 ± 0.43	28.66 (22.12 – 57.80)	78.92 (58.97 - 124.36)	$3.52^{0.62}$	1.8 (0.97 – 1.95)	7.5
EcViR population								
Chlorfenapyr	120	4	2.27 ± 0.33	45.16 (33.57 – 60.59)	165.66 (112.76 – 307.92)	$2.47^{0.64}$	74.1 (40.43 – 135.58)	5.3
Indoxacarb	120	4	2.83 ± 0.43	0.86 (0.66 - 1.11)	2.44 (1.77 – 4.10)	$2.69^{0.60}$	0.7 (0.48 – 1.08)	92.8
Methomyl	120	5	2.76 ± 0.45	48.14 (37.56 - 62.33)	139.91 (99.12 - 256.28)	$6.40^{0.26}$	2.44 (1.62 – 3.67)	4.4

[1] LC represents mean lethal concentrations calculated to kill 50% or 90% of assayed individuals, and their respective fiducial limits at 95%. [2] DSI > 1.0 stands for harmless insecticides, and DSI < 1.0 stands for harmful insecticides for *E. connexa*; and [3] RQ < 50 stands for harmless insecticides; 50 < RQ < 2500 stands for slightly to moderately toxic insecticides; and RQ > 2500 stand for harmful insecticides considering the label rate of chlorfenapyr (240 g a.i. ha^{-1}), indoxacarb (80 g a.i. ha^{-1}), and methomyl (215 g a.i. ha^{-1}).

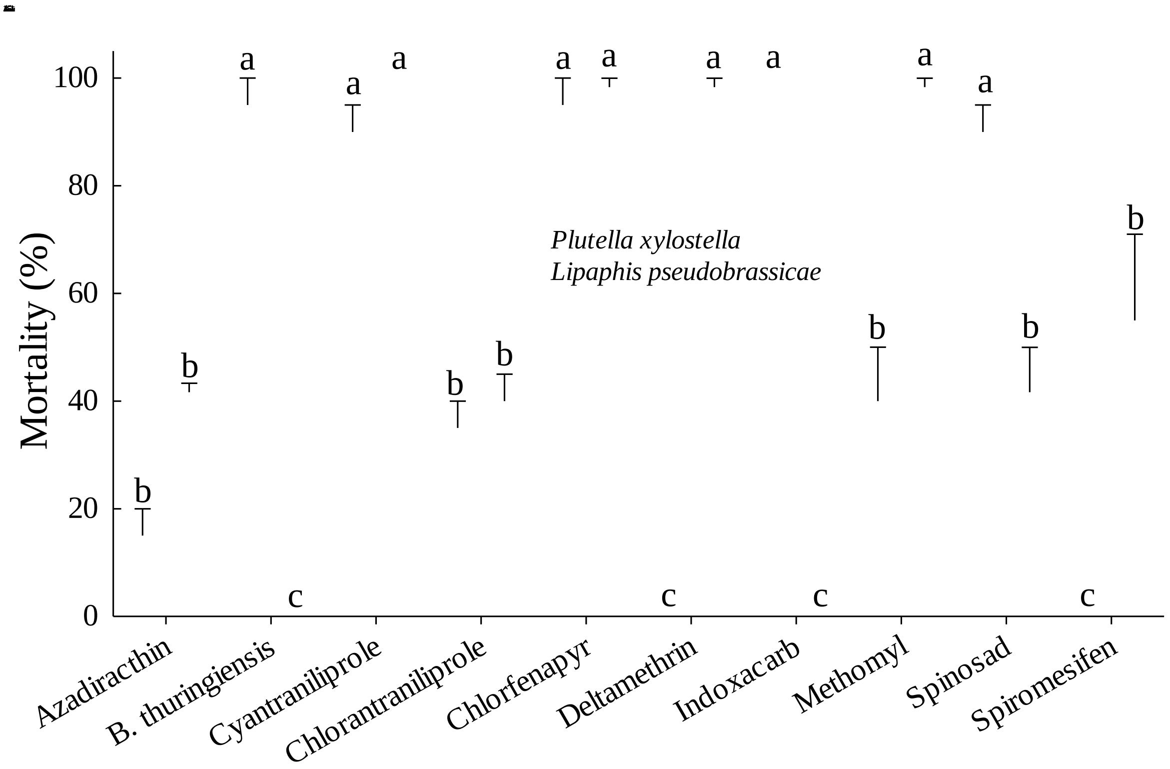

Figure 1. Mortality percentage (+SE) of *Plutella xylostella* 2nd-instar larvae and *Lipaphis pseudobrassicae* fed for 24h collard leaf discs containing dried-residues of different insecticides recommended after treatment with the highest label rates for spraying *Brassica* crops. Bars bearing different letters indicate significant differences among insecticides within a pest species according to the Bonferroni's test (α = 0.05).

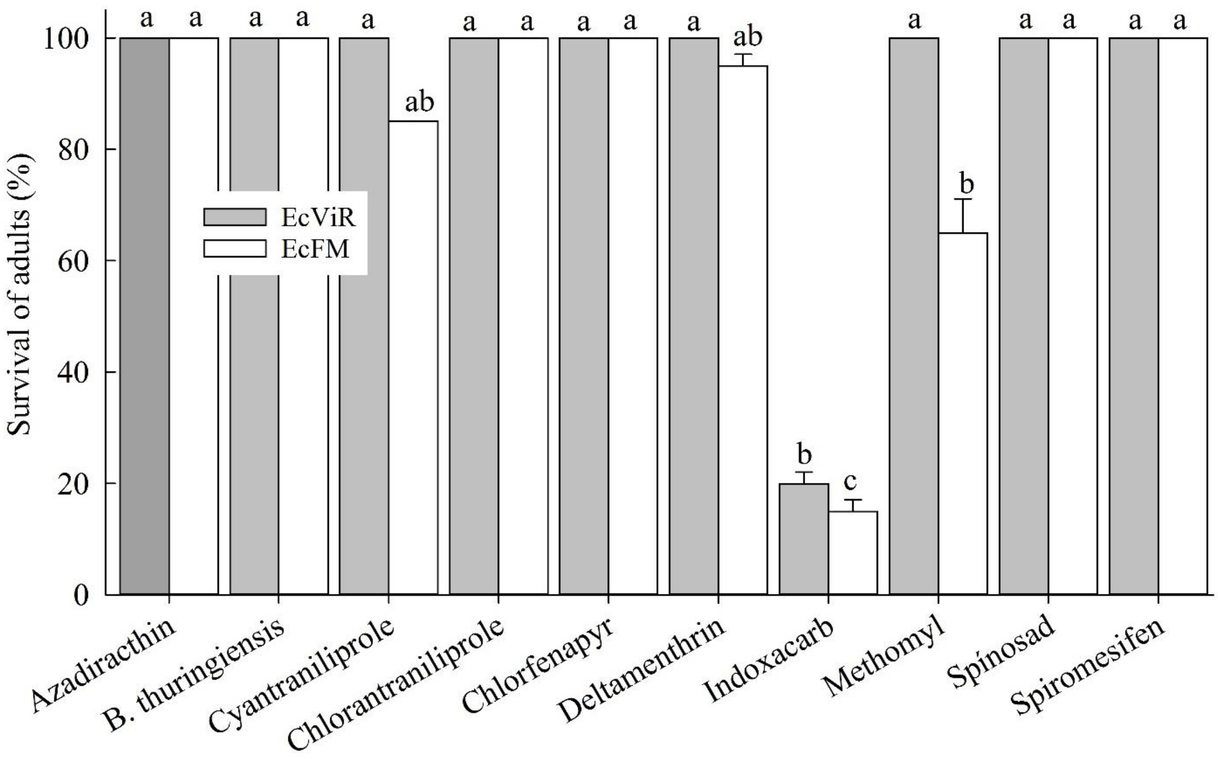

Figure 2. Survival of adults of the lady beetle, *Eriopis connexa*, susceptible (EcFM) and resistant (EcViR) to pyrethroids confined for 24h on collard leaf discs containing dried-residues of different insecticides recommended after treatment with the highest label rates to spray brassica's crop. Bars bearing different letters indicate significant differences among insecticides within a pest species accordinglg to the Bonferroni's test (α = 0.05).

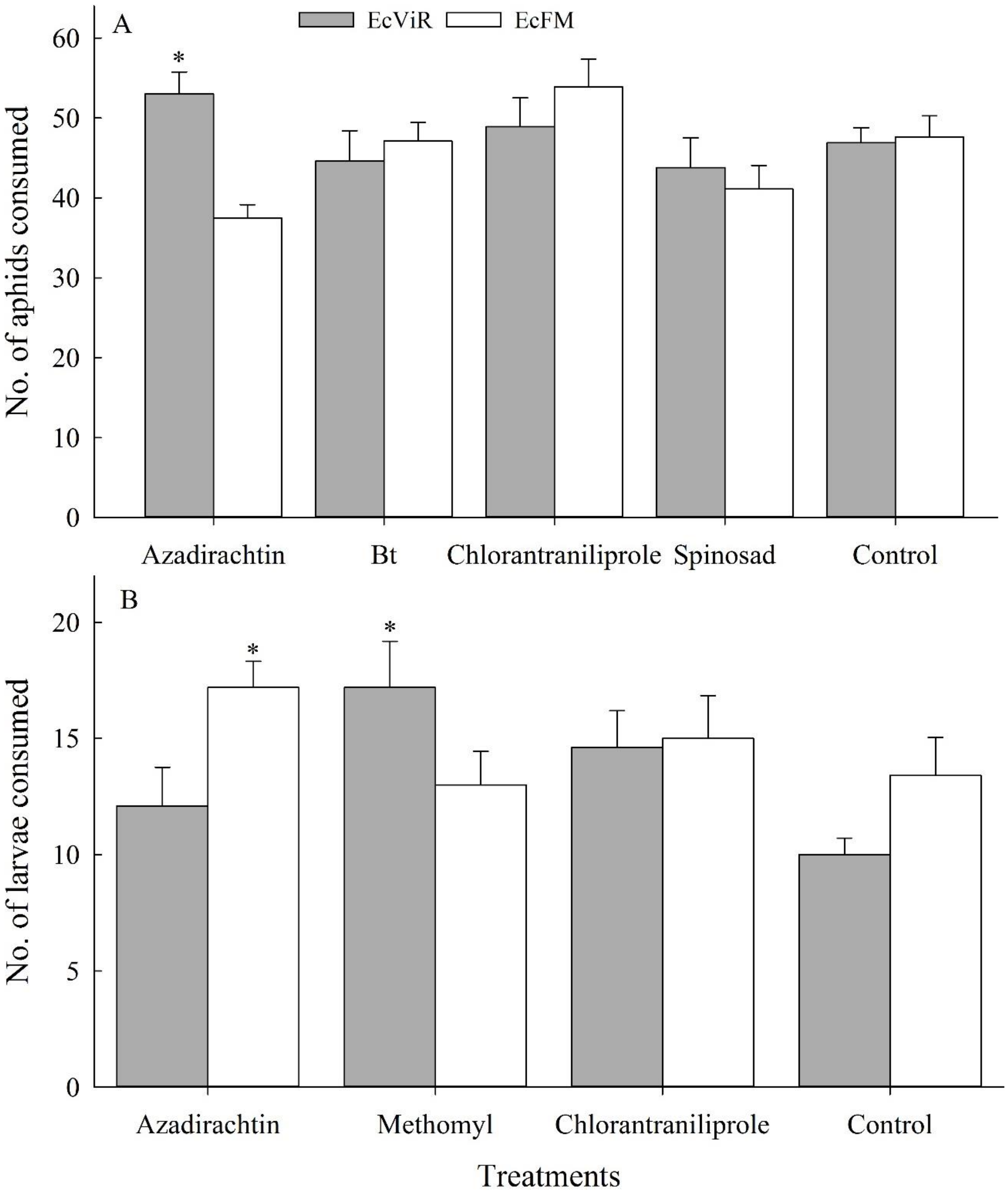

Figure 3. Average consumption (means + SE) of the brassica's pests, *Lipaphis pseudobrassicae* nymphs (A), and, *Plutella xylostella* 2nd-instar larvae (B), by adults of *Eriopis connexa* pyrethroid-resistant (EcViR) and -susceptible (EcFM) during 24h of simultaneous confinement on dried-residues of selected insecticides with survival of the respective target pest species over 20%. Asterisks (*) stand for difference in predation rate within the same treatment between EcViR and EcFM (Fisher's test α = 0.05). Note: Y-axis scales differ due to difference on predation rate.

CHAPTER 3

PERFORMANCE OF THE LADY BEETLE *Eriopis connexa* (GERMAR) (COLEOPTERA: COCCINELLIDAE) TO SEQUENTIAL EXPOSURE TO INSECTICIDES PREVAILED OVER ITS PYRETHROID RESISTANCE[1]

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[1] Lira, R. Peformance of the lady beetle *Eriopis connexa* to sequential exposure to insecticides prevailed over its pyrethroid resistance. To be submitted.

ABSTRACT – Insecticide resistance management and integrated pest management are supported by the conservation of biological control techniques to prevent pest resurgence and outbreaks of secondary pests. The lady beetle *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae) is among the natural enemies in agricultural systems of brassica crops and their populations may be disrupted by exposure to insecticides. Pyrethroid-resistant and pyrethroid-susceptible *E. connexa* larvae and adults were exposed to eight insecticides (cyantraniliprole, chlorantraniliprole, chlorfenapyr, deltamethrin, spinosad, spiromesifen, methomyl, and pymetrozine) that target different pests of brassica crops throughout the crop phenology. Evaluations were conducted on larval and adult survival across 30d, larval developmental time, fecundity and egg viability across 30d, and consumption of turnip aphid (*Lipaphis pseudobrassicae* Davis) or larvae of the diamondback moth (DBM, *Plutella xylostella* L.) 24h after exposure to the insecticides. In addition, the levels of aphids and DBM infestation in field cages were evaluated as a function of insecticide application and release of the lady beetles. Neither survival of larvae and adult beetles, nor fecundity, were affected by the insecticides. The pyrethroid-susceptible females exposed to residues of spinosad and methomyl consumed a greater number of aphids compared to the pyrethroid-resistant females. Likewise, the pyrethroid-susceptible beetles exposed to deltamethrin residues consumed more DBM larvae than pyrethroid-resistant beetles. There was no additive effect of the control of these pests when lady beetles were used alone or with insecticides. Results from this study are useful for the development of improved insecticide resistance management and integrated pest management in brassica crops.

KEY WORDS: Biological control, integrated control, chemical control, insecticide resistance, insecticide selectivity

DESEMPENHO DA JOANINHA *Eriopis connexa* (GERMAR) (COLEOPTERA: COCCINELLIDAE) À EXPOSIÇÃO SEQUENCIAL A INSETICIDAS PREVALECEU SOBRE SUA RESISTÊNCIA A PIRETROIDES

RESUMO - Os manejos de resistência a inseticidas e integrado de pragas são apoiados pela conservação do controle biológico para evitar o ressurgimento e surtos de pragas secundárias. A joaninha *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae) está entre os inimigos naturais nos agroecossistemas de brássicas e podem ser prejudicadas pela exposição a inseticidas. Larvas e adultos de *E. connexa* resistentes e suscetivéis a piretroides foram expostas a oito inseticidas (cianotraniliprole, clorantraniliprole, clorfenapir, deltametrina, espinosade, espiromesifeno, metomil e pimetrozina) recomendados para diferentes pragas de brássicas. Foram avaliadas a sobrevivência larval e adulta, tempo de desenvolvimento larval, fecundidade e viabilidade dos ovos ao longo de 30 dias, e consumo de pulgões (*Lipaphis pseudobrassicae* Davis) ou larvas da traça-das-crucíferas (*Plutella xylostella* L.) após exposição aos inseticidas em 24h. Além disso, os níveis de infestação de pulgões e da traça em plantas confinadas em gaiolas em campo foram avaliados em função da aplicação de inseticida e liberação das joaninhas. A sobrevivência de larvas e adultos da joaninha e sua fecundidade não foram afetadas pelos inseticidas. As fêmeas suscetíveis a piretroides expostas a resíduos de espinosade e metomil consumiram mais pulgões em comparação com as fêmeas resistentes. Igualmente, as joaninhas suscetivéis expostos a resíduos de deltametrina consumiram mais larvas da traça comparada as resistentes. Não houve efeito aditivo no controle dessas pragas quando as joaninhas foram utilizadas isoladamente ou com inseticidas. Os resultados deste estudo são úteis para o desenvolver um melhor manejo de resistência a inseticidas e integrado de pragas em brássicas.

PALAVRAS CHAVE: Controle biológico, controle integrado, controle químico, joaninha predadora, resistência de inimigos naturais

Introduction

Conservation of natural enemies in agricutural ecosystems is a basic tactic for a successful integrated pest management (IPM) (Naranjo *et al*. 2015, Torres & Bueno 2018). In the absence of natural enemies, pest resurgence and secondary pest outbreaks have been commonly reported after applications of non-selective insecticides (Bartlett 1968, Fritz *et al.* 2008, Hill *et al.* 2017). Therefore, conserving natural enemies by using selective insecticides, which impact only the target pest species, is a priority in IPM (Stanley & Preetha 2016, Torres & Bueno 2018). In addition, conservation of natural enemies plays a relevant role in insecticide resistance management (IRM) (Sparks & Nauen 2015, Blümel *et al.* 1999, Bueno *et al.* 2017) because natural enemies that survive applied insecticides may help delay resistance selection by killing the remaining pest species.

Although insecticide resistance represents a significant challenge to managing pest species, it may be advantageous in natural enemy populations by allowing them to survive after exposure to insecticide applications (Torres *et al.* 2015, Bielza 2016, Barbosa *et al*. 2016, Rodrigues *et al*. 2020). Similar to herbivores, natural enemies may become resistant to insecticides through insecticide detoxification by an enhanced enzymatic activity, differential insecticide intake through the tegument, and/or insensibility of target sites (Winteringham 1969, Gould 1984, Rodrigues *et al*. 2014). Furthermore, behavioral changes may also take place through repellency and irritability resulting in less contamination of the natural enemy with the applied insecticide (Cordeiro *et al.* 2010, Campos *et al.* 2011, Spíndola *et al.* 2013).

Pest control failure usually comes with direct increase in control costs and yield lost, among others (Forgash 1984, Grafius 1997). Insecticide resistance in the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a worldwide example of this problem. The life history traits of DBM combined with the abundant and diverse range of host plants, usually cultivated simultaneously, lead to multiple insecticide applications. Hence, DBM has been selected for resistance to several old and new marketed insecticides (Mota-Sanchez & Wise 2021). Under a conservative approach, an estimative of costs with DBM (control cost plus yield loss) is approximately 4 to 5 billion dollars each year (Zalucki *et al.* 2012).

In addition to the DBM, brassica IPM programs also need to consider aphids, which consists of a group of species that usually require applied chemical control (van Emden & Harrington 2007). The applied chemical control against DBM (a chewing pest) and aphids (sap-sucking pests) when infesting brassica fields simultaneously, may require non-selective insecticide applications. Therefore, considering the high frequency of insecticide applications in brassicas, and resistance selection in DBM and aphids, insecticide mode of action rotation is very important, or at least, alternation of insecticides modes of action over time (Onstad 2008, Sparks & Nauen 2015).

Conservation of natural enemies is a recommended tatic to help mitigate insecticide resistance (IRAC 2009). To attain this approuch, it is essential to know the impact of selected insecticides on target pests and natural enemies simultaneously. Previous studies have shown that some insecticides recommended against DBM [e.g., diamide (chlorantraniliprole), spinosyns (spinetoram and spinosade)]; aphids and whiteflies [e.g., pyiridine azomethine (pymetrozine), diamide (cyantraniliprole), cetoenol (spiromesifen)], and the entomopathogens (*Beauveria* and *Bacillus*), offer minor impact on aphid key predator, the lady beetle *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae) (Gusmão *et al.* 2000, Barros *et al*. 2018, Costa *et al.* 2020). Therefore, this lady beetle may play an important role for IPM and IRM in brassica crops and others as an aphid predator (Harterreiten-Souza *et al.* 2012, Soares *et al*. 2020). Furthermore, the lady beetle *E. connexa* has been characterized with natural populations selected for resistance to pyrethroids (Rodrigues *et al.* 2013, Costa *et al.* 2018), surviving field dosages of insecticide recommended against DBM larvae (Lira *et al.* 2019).

In this study, we tested the hypothesis that *E. connexa*, resistant to pyrethroids, would survive sequential exposures to eight specific insecticides that are commonly applied against DBM larvae and aphids in brassica fields. The experiments consisted of assays of biological aspects of immature and adult stages of the lady beetle, along with its predation capacity toward the turnip aphid, *Lipaphis pseudobrassicae* (Davis) (Hemiptera: Aphididae), and its alternative prey, DBM larvae. The use of insecticides and lady beetles to control aphids and DBM was tested using field cages. In this context, we hope to be able to define which insecticides in this study may be most compatible with the natural enemy when simultaneously targeting control of chewing (lepidopteran larvae) and sap-sucking pests (aphids and whiteflies). We expect to show that chemical and biological control may act additively to attain IPM and IRM, under the premise that besides direct control of the pests (IPM), surviving lady beetles will complement control of surviving pest individuals from an insecticide application, thus delaying resistance selection (IRM).

Material and Methods

All experiments were conducted at the Biological Control Laboratory and in experimental field plots at the Department of Agronomy of the 'Universidade Federal Rural de Pernambuco – UFRPE'.

Insects. The pyrethroid-resistant (EcViR) and -susceptible (EcFM) populations of *E. connexa* were reared separately according to Rodrigues *et al*. (2013), whereas rearing of the pest *P. xylostella* followed the methodology described by Lira *et al*. (2019). A population of pyrethroid-susceptible *E. connexa* was collected from cotton fields in Frei Miguelinho, Pernambuco State, Brazil and a population of pyrethroid-resistant *E. connexa* was originally collected from cabbage (*Brassica oleracea* L.) fields in Viçosa, Minas Gerais State, Brazil (Rodrigues *et al.* 2013), and supplememnted with other resistant individuals (Costa *et al.* 2018). A diamondback moth colony was established from a pyrethroid-resistant population maintained at the Laboratory of Insect-Toxic Interactions at UFRPE. Larvae and adult lady beetles were fed frozen *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs, and DBM larvae were fed organic collard leaves. Insect colonies and laboratory experiments were conducted under controlled conditions at 25 ± 1°C, 60-70% RH and 12:12h photoperiod (L:D) on the bench.

The turnip aphid population was collected from collard and cabbage plants cultivated in the field plots of the Plant Protection Division, Department of Agronomy at UFRPE.

Insecticide Solutions. We tested eight insecticides recommended against key brassicas` pests, all of them in their commercial formulations purchased from local market. Assuming application in 100 L of water per hectare, we prepared solutions of the insecticides at their highest label rates (LRs) (Table 1). To facilitate adhesion of the insecticides on treated surfaces, they were diluted into an aqueous surfactant (Wil-Fix 30 g i.a. L^{-1}, Charmon Destyl Indústria Química Ltda, Campinas, SP, Brazil) solution at 0.1%, which alone served as control.

Larval Exposure to Residues. This experiment determined the response of *E. connexa* larvae from both EcViR and EcFM populations exposed to dried residues of deltamethrin or chlorantraniliprole (simulation against lepidopterans), followed by exposure to pymetrozine (simulation against aphids). Following the leaf dipping method No. 18 (IRAC 2010), collard leaf discs (8cm diameter) were cut from organically grown plants and dipped into control (hereafter '-Ins') or insecticide (hereafter '+Ins') solutions for ≈30 seconds. The leaf discs were left to air-dry for about 2 h under laboratory conditions before transferring them to clean glass Petri dishes (80 mm diam) lined with moistened filter paper. Lady beetle larvae (5-days old) were confined with contaminated or non-contaminated leaf discs for 48 h, when the survivors were individually transferred to plastic containers where they were fed frozen moth eggs provided *ad libitum*. Five days after exposure to the first insecticide (deltamethrin or chlorantraniliprole), the surviving larvae were exposed to the second insecticide (pymetrozine) following the same methodology. Treated larvae were tracked for larval duration, tallied from the day of hatching until pupation; pupation time as the period from pupal formation until adult emergence; and immature survival, which was calculated as the percentage of larvae that yielded viable adults.

This bioassay followed a 2 × 2 factorial design [EcViR and EcFM populations exposed or not to insecticide residues], totaling four treatments (i.e., EcViR+Ins, EcViR-Ins, EcFM+Ins, and EcFM-Ins). For logistical reasons regarding larvae availability, this bioassay was performed in two experimental sets: *i*) larvae (= replicates) exposed to deltamethrin followed by pymetrozine, where the treatments EcViR+Ins, EcViR-Ins, EcFM+Ins, and EcFM-Ins comprised 74, 37, 64 and 40 replicates, respectively; and *ii*) larvae exposed to chlorantraniliprole followed by pymetrozine, where the treatments EcViR+Ins, EcViR-Ins, EcFM+Ins, and EcFM-Ins comprised 46, 46, 49 and 41 replicates, respectively.

Adult Exposure to Residues. Male and female beetles (5-days old) yielded from larvae within the same treatment in the prior bioassay (i.e., *i* or *ii*) were paired. Based on the leaf dipping method, they were exposed (for 48 h) to residues of a new insecticide each five days for 25 days, which resulted in five sequential exposures to insecticides with different modes of action and target pests. During 30 days since the first exposure, these couples were tracked daily for adult mortality, fecundity (= number of eggs laid), and egg viability (= percentage of hatching eggs). They were fed frozen moth eggs provided *ad libitum* or turnip aphid/DBM larvae (predation rate experiment). The newly hatched larvae were separated from unhatched eggs to avoid cannibalism.

This bioassay followed a 2 × 2 factorial design [two populations of *E. connexa* exposed or not to insecticide residues]. There were formed 12 and 15 pairs (= replicates) from adults emerged from larvae previously exposed to deltamethrin/pymetrozine and chlorantraniliprole/pymetrozine, respectively. The former pairs were sequentially exposed to residues of spinosad, cyantraniliprole, methomyl, chlorfenapyr, and chlorantraniliprole, whereas the latter ones were exposed to residues of deltamethrin, spinosad, spiromesifen, cyantraniliprole, and methomyl, and their respective controls (12 and 15 pairs).

Predation Rate of Female *Eriopis connexa* Subjected to Sequential Exposure to Insecticides. During the sequential exposures in the prior bioassay, we tested the hypothesis that the recommended insecticide against a target pest does not affect the consumption of an eventual non-target pest by the lady beetle. The expected result is the reduction of pest outbreaks, not targeted by the insecticide after their applications.

The consumption of the turnip aphid was quantified when female lady beetles were exposed to residues of the insecticides recommended against DBM, i.e., spinosad (day 5), methomyl (day 15) and chlorantraniliprole (day 25). Following the same rationale, the consumption of DBM larvae was quantified when female lady beetles were exposed to residues of insecticides recommended against aphids, i.e., deltamethrin (day 5), spiromesifen (day 15), and methomyl (day 25). To do so, collard leaf discs were dipped into the insecticide solution as previously described and were infested with 70-80 aphids (late instar nymphs of turnip aphid) or 15 DBM larvae (3-days old). Because the turnip aphids can produce 4-6 aphids per day (Capinera 2008), newly born aphids were not counted.

This bioassay followed a 2 × 2 factorial design with four treatments (two populations *E. connexa* exposed or not to insecticide residues), each treatment with 10 replicates represented by a female lady beetle from EcViR or EcFM population. To avoid male interference on prey consumption, they were transferred to another plate with their respective treatments. After 24 h of confinement, the number of missing turnip aphids or DBM larvae was recorded, as well as the number of dead female lady beetles. At this moment, the surviving females were again paired to their mates to diminish eventual impact on their reproductive traits.

Combination of Res *Eriopis connexa* and Insecticides on DBM and Turnip Aphid Suppression. In this experiment, we evaluated a possible additive effect of combining the lady beetle and some insecticides on suppression of DBM and turnip aphid under a field cage trial.

The experiment was carried out using collard plants cultivated in concrete pip plots (1.0 m in diam x 0.5 m in H) filled with soil. The plants were covered by cylindrical cages (1.0 m in diam x 1.2 m in H) made with an anti-aphid mesh held on place by a cylindrical iron structure. Throughout its height, the side of the cages had a longitudinal opening fixed with a 4 cm wide Velcro®, which allows us to access the plants inside. A week before setting up the experiments, the plots were sprayed with malathion (150 mL/100 L) to minimize the occurrence of opportunistic arthropods. Malathion is a non-selective insecticide with short residue on plants (Rolim *et al.* 2019). The plants were then infested with pieces of collard leaves containing turnip aphids (unaccounted), and after seven days they exhibited low population levels of aphid infestation. Once a week, during the evaluation period, each collard plant was infested with two second/third instar DBM larvae (= economic threshold cited by Massarolli *et al.* 2019) and four pupae, thus totaling 24 DBM specimens per plot.

This bioassay followed a completely randomized design consisting of four treatments: *i*) plants with aphids + DBM larvae and pupae + insecticide; *ii*) plants with aphids + DBM larvae and pupae + insecticide + EcViR lady beetles; *iii*) plants with aphids + DBM larvae and pupae + EcViR lady beetles; and *iv*) the control represented by plants with aphids + DBM larvae and pupae. Each treatment comprised six replicates, each one represented by a plot containing four caged collard plants.

When the turnip aphid or the DBM infestations reached the control level on treatments *i* and *ii*, plants were treated with selected insecticides. Plants were first treated with cyantraniliprole [against DBM larvae and whiteflies *Bemisia tabaci* (Hemiptera: Aleyrodidae), MEAM1], followed by a second and a third application with pymetrozine and spiromesifen, which targeted whiteflies and turnip aphids, respectively. Insecticide solutions were applied using a hand sprayer (Guarany^{TM}, Catanduva, SP) with pre-compression and adjustable jet with a capacity of 3 L. After ~2 h of the first insecticide application; four third-instar larvae and one lady beetle couple from the Res population were released into the cages.

The plants were assessed twice a week for three weeks to decide about insecticide application (based on the pest infestation level) and, 48 h after the first assessment, to record the pest infestation level and the number of lady beetle specimens. During the assessments, the whole plant was inspected. Aphid infestation was determined by the presence and size of the colony using a rating scale: 0 = no aphid; 1 = presence; 2 = low population; 3 = high population without black sooty mold; 4 = high population with black sooty mold; and 5 = plant death. Simultaneously, the number of alive DBM specimens (larvae, pupae or adults) and the number of alive lady beetles (larvae, pupae or adults) were recorded. At the end of the experiment (about 60 days), collard leaf production (commercial evaluation) was tallied considering the percentage of commercial leaves in ratio to the total leaves per plant. In addition, we determined the fresh mass of the commercial leaves to estimate the potential loss avoided in treatments involving insecticides and the lady beetle compared to the control treatment.

Data Analysis. Survival curves of larvae and adult lady beetles were estimated using the Kaplan-Meyer method, and pair-wise compared by the log-rank test using Proc LIFETEST. After fitting to normality (Shapiro-Wilk, Proc UNIVARIATE) and homogeneity of variance (Bartlet, Proc ANOVA) without requiring data transformation, the larval duration, pupation time, fecundity and egg viability were subjected to 2 × 2 factorial analysis of variance (EcViR and EcFM populations and +Ins and –Ins) using the Proc ANOVA.

The numbers of aphids and DBM larvae consumed per female lady beetle were submitted to ANOVA, using the procedure of repeated measurements in time, considering the four treatments (EcViR+Ins, EcViR-Ins, EcFM+Ins, and EcFM-Ins) and dates of exposure to the insecticides as time blocking factors. The population effect for each insecticide was submitted to Proc GLM. Data were transformed into square root (x + 0.5) to meet the assumptions of normality and homogeneity of variance. Comparisons of mean consumption across the treatments were performed for each evaluation date with Tukey HSD's test ($\alpha = 0.05$).

Data regarding the infestation scores of aphids on the collard plants were reduced to a numeric average per plant and replications varying from 0 to 5. The total number of DBM survivors, lady beetles, and the percentage and weight of commercial collard leaves were subjected to the Shapiro-Wilk's and Bartlet's tests for checking normality and homogeneity of variance. Count data were transformed by square root (x + 0.5), and leaf percentages were transformed by arsin (square root(x/100)) to meet ANOVA assumptions. The results were submitted to ANOVA using the procedure of repeated measures for pest and predator, while percentages and weights of commercial leaves were analyzed with two-way ANOVA, and the treatment means compared by Tukey HSD's test ($\alpha = 0.05$). All data analyses were conducted in SAS (SAS Institute 2002).

Results

Larval Exposure to Residues. The larval duration was not affected regardless of the population ($F_{1, 198} = 2.24$, $P = 0.1363$), and there was no interaction of population and insecticide residue exposure ($F_{1, 198} = 0.34$, $P = 0.5627$). However, this parameter varied between the populations as a function of larval exposure to deltamethrin followed by pymetrozine ($F_{1, 198} = 7.38$, $P = 0.0072$); EcFM-Ins larvae developed faster than EcViR+Ins ones, with the other treatments intermediate ($F_{1, 198} = 7.38$, $P = 0.0072$) (Table 2). Furthermore, pupation time did not vary as a function of the lady beetle population ($F_{1, 193}$ = 1.11, P = 0.2932), exposure to deltamethrin followed by pymetrozine ($F_{1, 193}$ = 0.01, P = 0.9177), or interaction of these factors ($F_{1, 193}$ =3.79, P = 0.0530). In this bioassay, there was no significant difference ($\chi 2$ = 3.83, df = 3, P = 0.28) with respect to immature survival, which ranged from 89.1-91.9%.

Similarly, the larval duration of insects exposed to chlorantraniliprole followed by pymetrozine was neither affected by the lady beetle population ($F_{1, 162}$ = 0.50, P = 0.4794), nor the exposure itself ($F_{1, 162}$ = 1.12, P = 0.2925) (Table 2). Likewise, pupation time did not vary as a function of lady beetle population ($F_{1, 158}$ = 0.09, P = 0.7690), insecticide exposure ($F_{1, 158}$ =1.04; P = 0.3103), or interaction of these factors ($F_{1, 158}$ = 0.37; P = 0.5418) (Table 2). There was no significant difference (χ^2 = 4.18, df = 3, P = 0.24) with respect to immature survival, which ranged from 84.8- 95.7%.

Adult Exposure to Residues. Regardless of population, the fecundity of female beetles that emerged from larvae exposed to deltamethrin followed by pymetrozine was not affected by subsequent exposures to spinosad, cyantraniliprole, methomyl, chlorfenapyr, and chlorantraniliprole ($F_{1, 44}$ = 0.86, P = 0.3581), and there was no interaction between population and exposure ($F_{1, 44}$ = 0.41, P = 0.5249). However, this parameter varied as a function of insecticide exposure ($F_{1, 44}$ = 4.42, P = 0.0413), with exposed female beetles laying ≈20.7% fewer eggs compared to those unexposed to the insecticides within 30 days of evaluation regardless of resistant status (Table 2). Furthermore, egg viability significantly differed between populations depending on insecticide exposure ($F_{1, 44}$ =10.10, P = 0.0027). Eggs from EcViR-Ins treatment exhibited greater viability than eggs laid by EcFM+Ins, with the others exhibiting intermediate viability (Table 2). The eggs viability did not result in significant effect from the sequence of insecticides ($F_{1, 44}$ =2.73, P = 0.1058), and the interaction of population and the sequence of insecticides applied ($F_{1, 44}$ =1.32, P = 0.2568). Adult survival along 30 days did not differ across the treatments (Log-rank: $\chi^2 = 4.59$, df = 3, P = 0.204), and ranged from 66.7% (EcFM+Ins) to 91.7% (EcViR+Ins, EcViR-Ins, and EcFM-Ins treatments), respectively.

With respect to females that emerged from larvae exposed to chlorantraniliprole followed by pymetrozine, their exposure to deltamethrin, spinosad, spiromesifen, cyantraniliprole and methomyl neither affected their fecundity (population factor: $F_{1, 54} = 2.73$, P = 0.1043; exposure factor: $F_{1, 54} = 2.62$, P = 0.1110; and the interaction of these factors: $F_{1, 54} = 1.89$, P = 0.1750), nor the egg viability (population factor: $F_{1, 54} = 0.05$, P = 0.8204; exposure factor: $F_{1, 54} = 1.79$, P = 0.1868; and the interaction of these factors: $F_{1, 54} = 0.18$, P = 0.6772). Adult survival along 30 days was not affected by the treatments (Log-rank: $\chi^2 = 0.70$, df = 3, P = 0.872), and ranged from 66.7% (EcViR-Ins) to 73.3% (EcViR+Ins) and 80.0% (EcFM-Ins and EcFM+Ins treatments), respectively.

Predation Rate of Female *Eriopis connexa* Subjected to Sequential Exposures to Insecticides. Predation toward the turnip aphid was not affected by the lady beetle population when confined with spinosad ($F_{3, 29} = 2.33$, P = 0.0952) or chlorantraniliprole ($F_{3, 29} = 1.71$, P = 0.1874) residues, it was affected by methomyl residues ($F_{3, 29} = 6.81$, P = 0.0013). In addition, turnip aphid predation was not variable over the time regarding the insecticide application (Wilks' lambda = 0.27, $F_{2, 28} = 37.16$, P < 0.0001), but not by the interaction of the populations and the insecticides (Wilks' lambda = 0.72, $F_{6, 56} = 1.66$, P = 0.1470) (Fig. 1A). Females from the EcFM population exposed for 24 h to spinosad and methomyl residues consumed ~ 56 to 76 aphids, which was greater than 44 and 65 aphids consumed by EcViR females, respectively (Fig. 1A). In contrast, chlorantraniliprole residues did not have an effect on the consumption of aphids by the lady beetles; they consumed on 44 to 69 aphids (Fig. 1A).

Lady beetle predation on DBM larvae was not affected when they were confined with deltamethrin ($F_{3, 27} = 3.17$, P = 0.0529), spiromesifen ($F_{3, 27} = 1.87$, P = 0.1755), and methomyl ($F_{3, 27} = 0.16$, P = 0.9210) residues. However, they were affected by the insecticides labeled for the control of aphids (Wilks' lambda = 0.54, $F_{2, 15}$ = 6.32, P = 0.0102). There was no interaction between beetle populations and insecticides (Wilks' lambda = 0.48, $F_{6, 30}$ = 2.20, P = 0.0710) (Fig. 1B). Female beetles from the EcFM+Ins treatment that were exposed to deltamethrin residues consumed significantly more DBM larvae (~ 12 larvae) than those from the EcViR-Ins treatment (~ 8.3 larvae), with the females in the other treatments consuming an intermediate number of DBM larvae (11.3 and 11.5 larvae) (Fig. 1B).

Combination of Res *Eriopis connexa* and Insecticides on DBM and Turnip Aphid Suppression. The densities of turnip aphid fluctuated over time (Wilks' lambda = 0.07, $F_{5, 11}$ = 6.32, P < 0.0001), and this parameter was affected by the time and the treatment factors (lady beetle release with and without insecticides) (Wilks' lambda = 0.02, $F_{15, 30}$ = 5.74, P < 0.0001). The aphid infestation was considered high on collard plants during the first evaluation (Fig. 2A). Aphid infestation increased significantly in the treatments without insecticides (Fig. 2A), where the classes varied from high population without black sooty mold to plant death. In contrast, plants from treatments with insecticides exhibited aphid infestation levels varying from no aphid to near low population.

The DBM infestation on collard plants varied as a function of time (Wilks' lambda = 0.17, $F_{4, 12}$ = 14.49, P = 0.0002), but not with the interaction of time and treatments (Wilks' lambda = 0.22, $F_{12, 32}$ = 2.05, P = 0.052). Despite our attempts to increase the DBM population through artificial infestations performed every week, their survival was low due to unfavorable weather and other uncontrolled factors. Therefore, the number of DBM specimens (larvae, pupae, and adults) was similar between +Ins and -Ins treatments and varied from 0 to 2 individuals per four plants (Fig. 2B).

The lady beetle densities did not vary over time (Wilks' lambda = 0.03, $F_{4, 2}$ = 14.20, P = 0.067), without significant interaction between time and treatments (Wilks' lambda = 0.60, $F_{4, 2}$ =

0.33, P = 0.840). Irrespective of insecticide application, the number of lady beetle specimens was similar between treatments and varied from 0 to 2.6 individuals per replication (Fig. 2C).

The percentage of commercial collard leaves was affected by treatments ($F_{3, 20}$ = 10.49, P = 0.0002). Similarly, the fresh leaf weight was affected by treatments ($F_{3, 20}$ = 18.12, P < 0.0001). The greatest percentage of commercial leaves (59.3% and 53.9%) and fresh weight of leaves (142.4 g and 108.9 g) were yielded from treatments with insecticides, regardless of the release of the lady beetles (Fig. 3).

Discussion

Both larvae and adult *E. connexa* from pyrethroid-resistant and pyrethroid-susceptible populations survived the sequential exposures to selected insecticides that are labeled for the control of chewing and sap-sucking pests of brassica crops. Sequential exposures to chlorantraniliprole, spiromesifen, and methomyl did not affect the predation rate either upon aphids or DBM larvae. Therefore, these findings support the hypothesis that a proper selection of insecticides can be compatible with certain natural enemies, even when it is needed in successive applications targeting different pest species while pursuing IPM and IRM. In fact, the outcome indicated that insecticide selectivity prevailed over the lady beetle resistance trait tested.

Treated larvae exhibited a degree of sensitivity to the sequence of insecticide exposures, but not enough to hamper their complete development to the adult stage. Pymetrozine followed by chlorantraniliprole exhibited no acute toxicity to *E. connexa*; this is an outcome also observed with other species of lady beetles (Cabral *et al*. 2008, Jansen *et al*. 2011, Almasi *et al*. 2013, Sabry *et al*. 2014, Barros *et al.* 2018). In contrast, deltamethrin has been reported to be highly toxic to lady beetles (Garzon *et al*. 2015, Galvan *et al.* 2005, Liu *et al.* 2012). Our results, however, showed that deltamethrin at the label rate against brassica pests was compatible with both larvae and adults of *E. connexa,* regardless of its status of pyrethroid resistance. This was an expected result to the pyrethroid-resistant beetles (Torres *et al.* 2015), but not for the susceptible ones. Despite the impact of sublethal effects on insect population growth, there are few studies simulating successive exposures of lady beetles to insecticides with different modes of action. Thus, knowledge of predation rate seems relevant to combining selective insecticides and predators, which may diminish negative impacts on prey attack.

The cumulative effect of multiple insecticide exposures for adult *E. connexa* after two and five insecticide exposures during the larval stage and 30 days of adulthood, respectively, was negligible. Spinosad, spiromesifen, cyantraniliprole, chlorantraniliprole, and chlorfenapyr are labeled as insecticides of reduced impact for lady beetles (Fogel *et al*. 2009, Youn *et al*. 2003, Singh *et al*. 2016, Liu *et al*. 2016), as is further supported by our findings and their recommendation within IPM and IRM for brassicas. Interesting that deltamethrin and methomyl, they are considered as non-selective insecticides (Garzon *et al*. 2015, Galvan *et al.* 2005, Liu *et al.* 2012, Liu *et al.* 2016), but there is a lack of data for their impact on lady beetles when applied in an insecticide mode of action rotation program. Multiple successive insecticide applications in the field usually reduce the natural enemy populations, especially when using non-selective materials (Bommarco *et al.* 2011, Saeed *et al.* 2017, Machado *et al.* 2019). In the field, however, even using selective insecticides, natural enemies may experience prey shortage in quality and quantity (Machado *et al.* 2019, Bordini *et al.* 2021), which highlights the importance of a judicious insecticide recommendation and application when the target pest population reaches the economic threshold (Torres & Bueno 2018). In this study, larvae and adult beetles received abundant prey, but the predator did not have the chance to escape the continuous contact with the insecticide residues. Therefore, the finding supports the compatibility of *E. connexa* to selected insecticides when used at the label rates against brassicas pests.

The application of insecticides with different modes of action, as in our test, is the basic tatic for IRM and needs to target different pest species. Therefore, our results will help brassicas growers in their decisions to select insecticides against aphids or DBM larvae such that judicious consideration can be given to IRM. Reduction in the abundance of natural enemies in the field under successive insecticide application can result from rotation and alternation with non-selective insecticides (Saeed *et al*. 2016, Naranjo *et al*. 2004, Bommarco *et al*. 2011, Machado *et al.* 2019). Thus, non-selective insecticides can reduce natural enemy abundance while planning insecticide applications with compatible insecticides will help IPM. For instance, the tatic of early applications of insect growth regulators in cotton fields resulted in the conservation of natural enemies depending on the subsequent insecticide selection (Naranjo *et al*. 2004).

Differences in predation rate on DBM larvae and aphids were observed when female lady beetles were exposed to deltamethrin, spinosad and methomyl residues, respectively. We expected greater consumption of aphids and DBM larvae by pyrethroid-resistant (EcViR) females, especially when they were exposed to residues of deltamethrin as compared with pyrethroid-susceptible (EcFM) females. Insecticides can interfere with foraging and predation behaviors of natural enemies (Cloyd & Bethke 2011, Haynes 1988, Desneux *et al*. 2007, Spíndola *et al*. 2013, D'Avila *et al*. 2018a), sometimes reducing prey consumption (Decourtye & Pham-Delegue 2002). However, previous results revealed that adult *E. connexa* females consume about 60 aphids per day (cotton aphids, Ferreira *et al*. 2013; or turnip aphids, Lira *et al*. 2019) or ≈10 DBM larvae per day (Lira *et al*. 2019). This consumption is within the predation rates found in our study for both turnip aphid and DBM larvae, regardless of the sequence of contact with insecticide residues.

The field cage study failed to show an additive control of brassica's pest with release of lady beetle and insecticide application at the studied conditions. The turnip aphid and DBM larva were equally suppressed by the insecticide applied in the absence and presence of the lady beetle. Despite recovering a few lady beetles by the end of the study, thus suggesting their maintenance after release, their number was low and could not suppress the aphid population alone. Lady beetles are key predators of aphids, with potential of restraining aphid population growth at the beginning (Dixon & Dixon 2000, Völkl *et al.* 2007, Franscesena *et al.* 2019), but after aphid populations become established, lady beetles alone are not able to suppress their population due to the shorter lifecycle of aphids in comparison to the beetles (Kindlmann *et al.* 2007). This is the first trial releasing this pyrethroid-resitant *E. connexa* population combined with a program of insecticide application required by the pest infestation (sucking or chewing) pest species. Therefore, there are many other variables to be solved before a recommendation, such as the time of release as function of aphid infestation, number of individuals to be released, and comparisons between larvae and adults considering the high dispseral of adults.

In summary, both lady beetle populations survived and produced offsprings under the successive exposures to the tested insecticides under laboratory and semi-field conditions. The tested schedule of two and five insecticide exposures during larval and adult stages with eight insecticides representing seven modes of action generated relevant information to cope the negative impact of the multiple required insecticide applications for IPM and IRM of brassicas pests. Furthermore, the application of insecticides targeting one pest species and the predator attacking a non-target pest of the applied insecticide simulate the field situation regarding the conservation of natural enemies and simultaneous usage of chemical control. This opens the opportunity to combine biological and chemical control as preconized in concert with the principles of IPM. The combination of environmentally friendly insecticides and natural enemies offers several benefits to reduce pest resurgence and secondary pest outbreaks, and delay of insecticide resistance evolution, especially in brassica fields, where DBM larvae and aphids are key insecticide-resistant pests.

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Table 1. Data on insecticides tested including chemical group and mode of action (MoA), recommended field rates from label by manufacturer for 100L dilution, and target pest species (AGROFIT 2020).

Insecticides	Chemical group (MoA)	Label rate (100L)	Target pests[1]
Cyantraniliprole 100SC	Anthranilic diamide (28)	12.5 mL	*Px*^{C}, *Bt*^{S}, *Mp*^{S}
Chlorantraniliprole 100SC	Anthranilic diamide (28)	7.5 mL	*Px*^{C}, *Tn*^{C}
Chlofenapyr 240SC	Pyrroles (13)	100.0 mL	*Am*^{C}, *Px*^{C}, *Bb*^{S}
Deltamethrin 25EC	Pyrethroid (3)	30.0 mL	*Am*^{C}, *Ai*^{C}, *Bb*^{C}, *Ds*^{C}, *Px*^{C}, *Tn*^{C}
Spinosade 380SC	Spinosyns (5)	12.5 mL	Am^{C}, Ai^{C}, Px^{C}, Tn^{C}, Hp^{C}
Spiromesifen 240 SC	Cetoenol (23)	600.0 mL	*Bt*^{S}
Methomyl 250EC	Carbamate (1A)	100.0 mL	*Px*^{C}, *Bb*^{S}, *Am*^{C}
Pymetrozine 500WG	Pyridine azomethine (9B)	50.0 g	*Bb*^{S}, *Mp*^{S}, *Bt*^{S}

[1] Superscript C and S stand for chewing and sucking sap pest species, respectively; *Am* = *Ascia monustes orseis*, *Ai* = *Agrotis ipsilon*, *Bb* = *Brevycorine brassicae*, *Bt* = *Bemisia tabaci*, *Hp* = *Hellula phidilealis*, *Mp* = *Myzus persicae*, *Px* = *Plutella xylostella*, *Tn* = *Trichoplusia ni*.

Table 2. Larval duration (days), pupation time (days), immature survival (%), fecundity (no. of eggs laid), and egg viability (% of hatched eggs) of *Eriopis connexa* from pyrethroid-resistant (EcViR) and -susceptible (EcFM) populations exposed (+Ins) or not (-Ins) to dried residues of different insecticides.

Treatments	n	Larval duration	Pupation time	Survival (%)	No. formed couples	Fecundity	Egg viability
Deltamethrin-pymetrozine*						Spinosad-cyantraniliprole-methomyl-chlorfenapyr-chlorantraniliprole*	
EcViR+Ins	74	11.9 ± 0.07a	3.8 ± 0.05	91.9	12	344.1 ± 46.49	55.8 ± 4.69ab
EcViR-Ins	37	11.7 ± 0.01ab	3.7 ± 0.01	97.3	12	409.2 ± 49.35	66.3 ± 3.32a
EcFM+Ins	64	11.8 ± 0.07ab	3.8 ± 0.06	89.1	12	356.9 ± 35.91	48.1 ± 3.21b
EcFM-Ins	40	11.5 ± 0.09b	3.9 ± 0.08	97.5	12	479.2 ± 45.46	50.0 ± 3.68b
Chlorantraniliprole-pymetrozine*						Deltamethrin-spinosad-spiromesifen-cyantraniliprole-methomyl*	
EcViR+Ins	46	12.9 ± 0.18	3.4 ± 0.08	84.8	15	117.6 ± 76.15	39.7 ± 21.04
EcViR-Ins	46	12.2 ± 0.18	3.6 ± 0.08	95.7	15	187.9 ± 102.65	48.2 ± 17.87
EcFM+Ins	49	12.3 ± 0.20	3.5 ± 0.09	85.4	15	189.8 ± 86.96	42.8 ± 17.87
EcFM-Ins	41	12.5 ± 0.17	3.5 ± 0.08	91.8	15	194.5 ± 95.55	47.3 ± 17.16

Means (± SE) followed by the same letter within the same column are statistically similar (Tukey HSD test α = 0.05). *Indicates a 5-day interval between insecticide exposure.

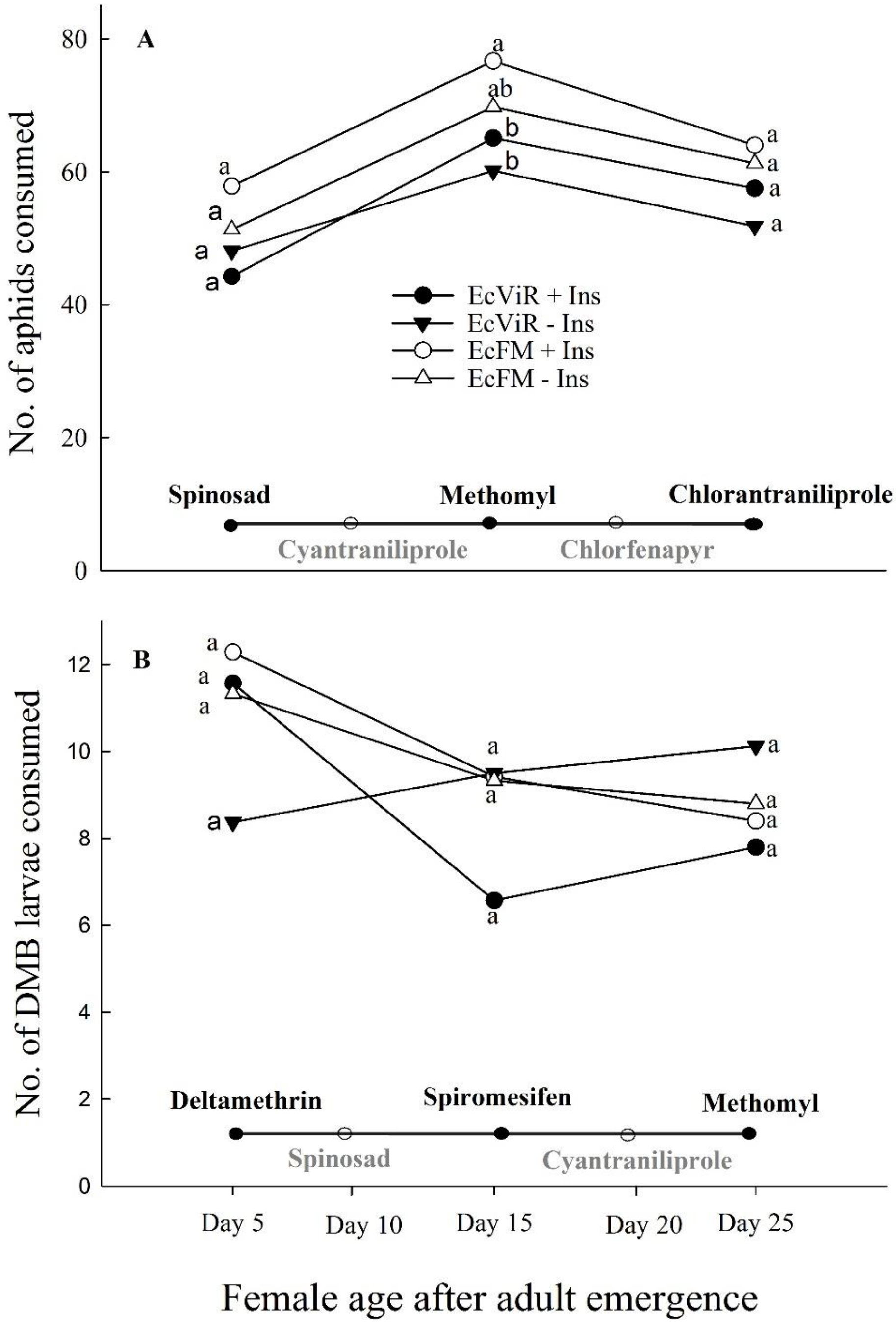

Figure 1. Mean number of turnip aphids (*Lypaphis pseudobrassicae*) or DBM (diamondback moth, *Plutella xylostella*) larvae consumed by adult female *Eriopis connexa* from pyrethroid-resistent (EcViR) and -susceptibile (EcFM) populations sequentially exposed to insecticides (+Ins), marked in bold, within 30 days of adult emergence. Note: Means indicated with the same letter are not significantly different by Tukey HSD's test ($P > 0.05$)

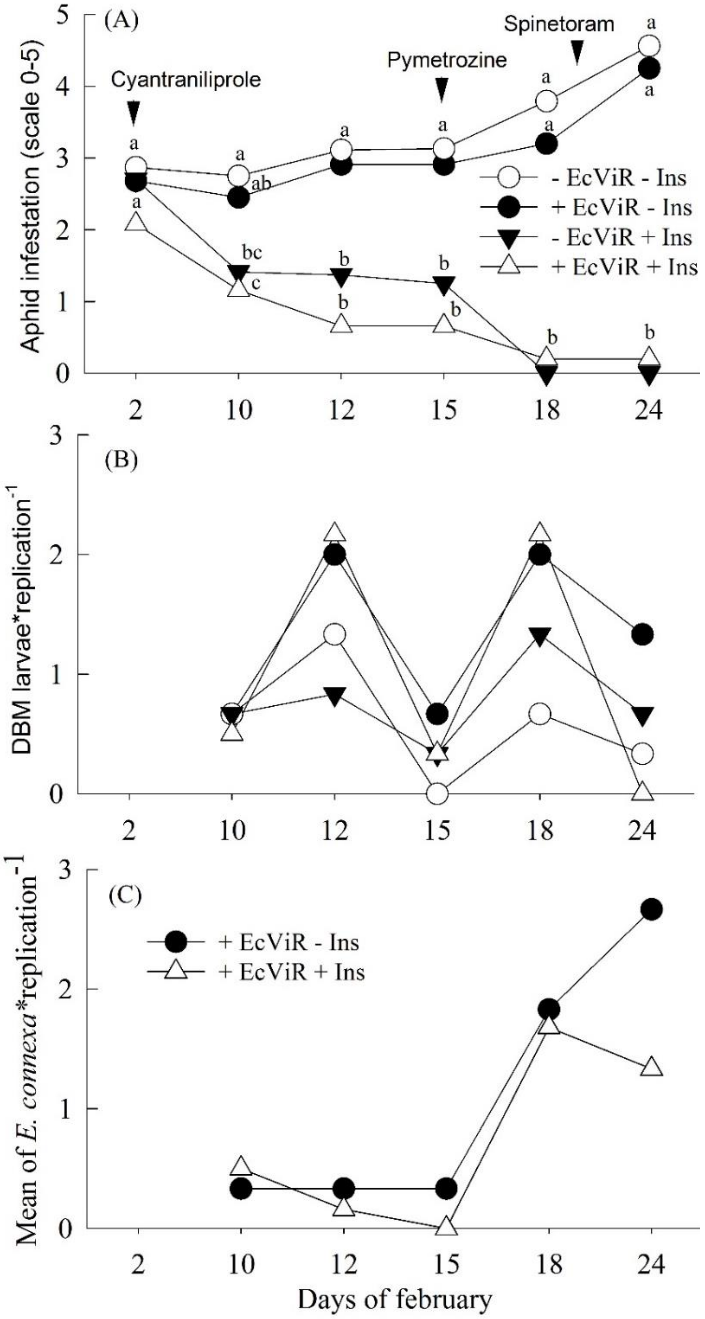

Figure 2. Abundance of aphids (scale 0 – 5, see Material and Methods section for detailed information), *Plutella xylostella* larvae (DBM), and *Eriopis connexa* from a pyrethroid-resistant population in the treatments with (+Ins) or without (-Ins) insecticide combination. Symbols bearing different letters indicate significant differences among treatments at each evaluation by Tukey HSD's test (α = 0.05).

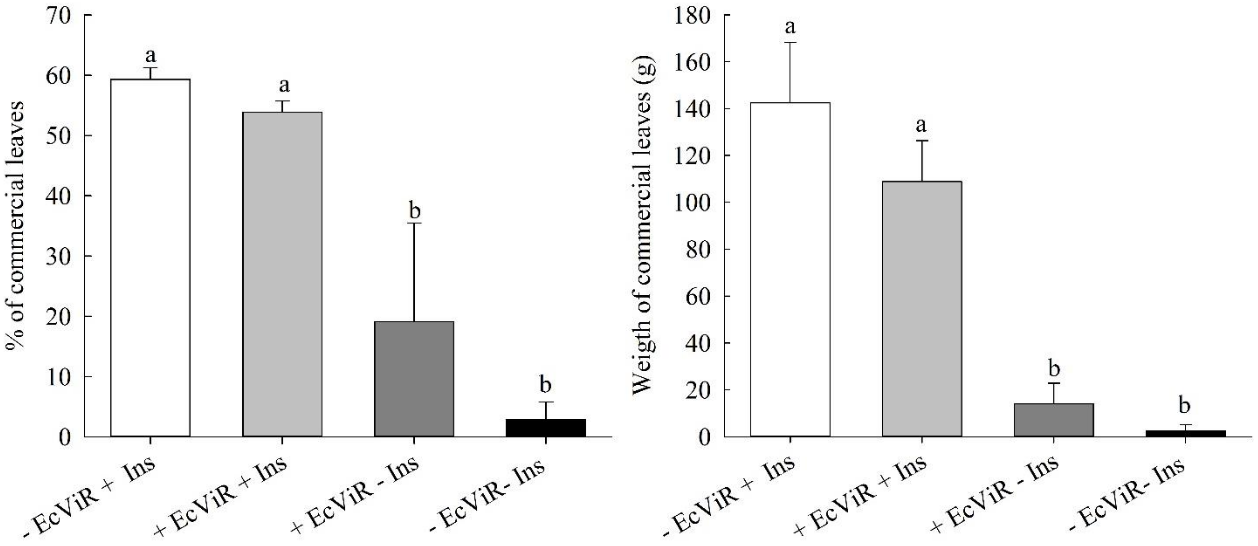

Figure 3. Yield variables of collard plants based on the percentage and fresh weight of commercial leaves in the treatments with (+Ins) and without (-Ins) insecticides combined with release (+ EcViR) or without (-EcViR) *Eriopis connexa* pyrethroid-resistant. Bars (+SE) bearing different letters indicate significant differences among means of treatments according to Tukey HSD’s test (α = 0.05).

CAPÍTULO 4

CONSIDERAÇÕES FINAIS

Um dos principais desafios do Manejo Integrado de Pragas (MIP) é a integração dos controles químico e biológico, devido à comum incompatibilidade entre eles. Isto porque ambos os agentes de controle biológico e os inseteicidas possuem o mesmo alvo (i.e., a praga) e tanto predadores quanto parasitoides são, suscetíveis aos inseticidas na sua maioria. O trabalho conduzido demonstra que ambos os métodos químico e biológico podem ser recomendados para o controle da praga-chave das brássicas, a traça-das-crucíferas (*Plutella xylostella*), bem como de pragas de similar importância como os pulgões. Com base nessas informações, é possível a preservação da joaninha predadora *E. connexa*, sem prejuízo de seu desempenho no consumo dessas pragas. Ambas as populações de *E. connexa*, suscetível e resistente a piretroides, produziram resultados similares referente à sobrevivência e predação, quando utilizadas com os inseticidas *Bacillus thuringiensis*, ciantraniliprole, clorantraniliprole, deltametrina, clorfenapir, espinosade, azadiractina e espiromesifeno, mesmo sob uma exposição sequencial desses ingredientes ativos. Tais produtos representam diferentes modos de ação, e mostraram ser compatíveis com *E. connexa*, não apenas a população com seletividade estendida via resistência aos piretroides. Os resultados deste estudo, a respeito da exposição sequencial é importante devido ao seu possível uso em programas de rotação de modo de ação para mitigar futuros problemas de resistência, mas também, visando à conservação da joaninha predadora.

Historicamente, o controle biológico de pragas tem focado em manter um inimigo natural para cada praga. Contudo, os agroecossistemas são compostos por várias espécies de pragas e inimigos naturais que devem ser consideradas no manejo integrado de pragas da cultura. Para contornar infestações com múltiplas espécies, é comum a utilização de inseticidas de amplo espectro. Alternativamente, é possível utilizar inseticidas seletivos e de menor impacto ambiental, enquanto outros controles atuam sobre as demais pragas, como a joaninha para o controle de pulgões. Apesar de não termos verificado um controle aditivo da liberação da joaninha predadora às aplicações de inseticidas na supressão do cresimento populacional do pulgão *L. pseudobrassicae*, a preservação da joaninha já é justificada pelo seu potencial em consumir pragas remanescentes de uma pulverização do inseticida, podendo ainda atuar sobre pragas secundárias. Futuros estudos poderão definir a relação do número de joaninhas e o momento mais adequado de liberação para retardar o crescimento populacional do pulgão, dependendo da infestação.