

COMPATIBILIDADE DE INSETICIDAS COM *Euborellia annulipes* (LUCAS)
(DERMAPTERA: ANISOLABIDIDAE) NO MANEJO DA TRAÇA-DAS-CRUCÍFERAS

por

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RESUMO

A infestação de pragas limita a produtividade das brássicas, com destaque para a traça-das-crucíferas *Plutella xylostella* L. (Lepidoptera: Plutellidae). O controle desta praga requer uso intensivo de táticas de controle, predominantemente o uso de inseticidas químicos. Este manejo tem resultado em casos de resistência, a todos os modos de ação de inseticidas recomendados para o seu controle. A integração de inimigos naturais para o manejo de *P. xylostella*, visa não somente reduzir o uso de inseticidas, mas também mitigar os frequentes casos de resistência. A tesourinha predadora *Euborellia annulipes* (Lucas) (Dermaptera: Anisolabididae), apresenta características interessantes para a sua conservação no agroecossistema das brássicas voltada ao manejo da traça-das-crucíferas por ser um predador generalista, que habita o solo e os espaços entre as folhas imbricadas e inflorescências das brássicas. Além disso, trata-se de inimigo natural facilmente criado em laboratório a um baixo custo, o que pode viabilizar a sua produção em larga escala para liberações inundativas no campo. O presente estudo avaliou a compatibilidade de inseticidas recomendados para o controle de *P. xylostella* em brássicas, com a tesourinha, visando a sua conservação para o manejo de pragas das brássicas. Bioensaios foram conduzidos para identificar efeitos letais e

subletais, predação e preferência alimentar de *E. annulipes*. A tesourinha apresenta alta sobrevivência quando expostas ao resíduo seco dos inseticidas azadiractina, ciantraniliprole, clorantraniliprole, deltametrina, espinosade, indoxacarbe, metomil e teflubenzurom. A predação de *P. xylostella* pela tesourinha não foi afetada pela exposição aos inseticidas testados. O inseticida ciantraniliprole ocasionou efeito subletal, prolongando o tempo de desenvolvimento e efeito letal com menor sobrevivência de ninfas. Além disso, houve um aumento no tempo para a primeira oviposição e redução no número de ovos depositados por fêmeas em contato com o ciantraniliprole. A associação de indoxacarbe e a tesourinha promoveu mortalidade superior a 90% de larvas e pupas de *P. xylostella* presentes na planta. Os resultados indicam a compatibilidade de *E. annulipes* com diversos inseticidas recomendados para o manejo de pragas das brássicas, o que permite a combinação desses inseticidas e a tesourinha para o manejo da traça-das-crucíferas e outras pragas.

PALAVRAS-CHAVE: Controle biológico, seletividade de inseticidas, controle integrado, comportamento de predação, *Plutella xylostella*.

INSECTICIDES COMPATIBILITY WITH *Euborellia annulipes* (LUCAS) (DERMAPTERA:
ANISOLABIDIDAE) FOR DIAMONDBACK MOTH MANAGEMENT

by

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ABSTRACT

Pest infestation limits the productivity of brassica crops, especially infestation of diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae). The DBM requires intensive control, predominantly through insecticide applications. This management has resulted in cases of DBM resistance to all insecticide modes of action recommended for its control. The integration of natural enemies for control of *P. xylostella* not only aims to reduce the use of insecticides but also to mitigate the frequent records of resistance. The ring-legged earwig, *Euborellia annulipes* (Lucas) (Dermaptera: Anisolabididae), has interesting features for conservation in the ecosystem of brassicas and for use in the management of the DBM. For example, it is a generalist predator that lives in the soil but also in brassicas' intertwined leaves and inflorescences. Furthermore, this natural enemy is easily reared in laboratories at a low cost, which could enable its large-scale production for release in brassica's fields. The present study evaluated the compatibility of insecticides recommended for *P. xylostella* control in brassicas with the ring-legged earwig, aiming at its conservation for the management of brassica pests. Bioassays were conducted to identify the lethal and sub-lethal effects, predation, and feeding preferences of *E. annulipes*. The earwig *E. annulipes* exhibited high survival when exposed to dry residues of the insecticides azadirachtin, cyantraniliprole, chlorantraniliprole, deltamethrin, spinosad, indoxacarb,

methomyl, and teflubenzuron. Exposure to the tested insecticides did not affect ring-legged earwig predation on *P. xylostella*. The insecticide cyantraniliprole caused a sublethal effect on developmental time and a lethal one, with lower survival of nymphs. In addition, earwig females exposed to dry residues of cyantraniliprole delayed the first egg batch's production and laid a lower number of eggs per batch. The association between indoxacarb and the ring-legged earwig released on caged Chinese cabbage plants promoted the mortality of more than 90% of DBM larvae and pupae. The results indicate the compatibility of *E. annulipes* with several insecticides recommended for brassica pest control, which allows the combination of these insecticides and the ring-legged earwig for the management of DBM and other brassica pests.

KEY WORDS: Biological control, insecticide selectivity, integrated control, predatory behavior, *Plutella xylostella*.

COMPATIBILIDADE DE INSETICIDAS COM TESOURINHA *Euborellia annulipes* (LUCAS)
(DERMAPTERA: ANISOLABIDIDAE) NO MANEJO DA TRAÇA-DAS-CRUCÍFERAS

por

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Tese apresentada ao Programa de Pós-Graduação em Entomologia, da Universidade Federal Rural de Pernambuco, como parte dos requisitos para obtenção do grau de Doutor em Entomologia.

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SUMÁRIO

CAPÍTULOS

1	INTRODUÇÃO	1
	RELEVÂNCIA DO ESTUDO.....	7
	LITERATURA CITADA.....	9
2	INSECTICIDE COMPATIBILITY WITH THE PREDATORY RING-LEGGED EARWIG <i>Euborellia annulipes</i> INCREASES MORTALITY OF DIAMONDBACK MOTH	14
	ABSTRACT.....	15
	RESUMO	16
	INTRODUCTION.....	17
	MATERIAL AND METHODS	18
	RESULTS.....	23
	DISCUSSION	24
	ACKNOWLEDGMENTS.....	30
	LITERATURE CITED	31
3	INDOXACARB, CYANATRANILIPROLE AND THE EARWIG, <i>Euborellia annulipes</i> (LUCAS) (DERMAPTERA: ANISOLABIDIDAE), AS OPTIONS FOR INTEGRATED DIAMONDBACK MOTH CONTROL.....	43
	ABSTRACT	44
	RESUMO	45

INTRODUCTION.....	46
MATERIAL AND METHODS	48
RESULTS.....	54
DISCUSSION	56
ACKNOWLEDGMENTS.....	60
LITERATURE CITED	60
4 CONSIDERAÇÕES FINAIS	70

CAPÍTULO 1

INTRODUÇÃO

O Brasil é um dos maiores produtores agrícolas do mundo (Hubbard *et al.* 2017), incluindo a produção de hortaliças. A produção de várias hortaliças como as espécies de brássicas visa atender o consumo interno. Brassicaceae é composta por cerca de 3700 espécies incluindo aquelas cultivadas para produção de óleo, para consumo de folhas, caules e flores e para o preparo de condimentos (Pedras & Yaya 2010). Assim, várias espécies são importantes, destacando-se aquelas do gênero *Brassica*, como: *Brassica napus* L. (canola), *Brassica oleracea* L. var. *italica* (brócolis), *Brassica rapa* L. *pekinensis* (nabo), *Brassica oleracea* L. var. *gemmifera* (couve-de-Bruxelas), *Brassica oleracea* L. var. *botrytis* (couve-flor), *Brassica oleracea* L. var. *acephala* (couve folha) e *Brassica oleracea* L. var. *capitata* (repolho), além de outras (Fahey 2003, Ahuja *et al.* 2009). Entre essas espécies, a couve-chinesa é amplamente consumida em diferentes regiões do mundo. A couve-chinesa é consumida predominantemente na China, mas também é consumida em outras regiões, como a Europa e o continente Americano, principalmente, devido ao seu sabor característico e adocicado (Zhang *et al.* 2021).

Um dos grandes desafios para o cultivo de brássicas é o manejo das diversas espécies de pragas que estão associadas ao seu cultivo. Entre as espécies pragas, existem aquelas especializadas tendo as brássicas como hospedeiro preferencial (Sibanda *et al.* 2000, Saeed *et al.* 2010), tornando o principal fator biótico limitante de produção (Kanrar *et al.* 2002). As principais pragas adaptaram-se, não sendo impactadas pelos compostos secundários, capazes de oferecer defesa contra a herbivoria e, portanto, são capazes de ocasionar redução no desenvolvimento e perda de produção. Entre as espécies pragas das brássicas destacam-se os pulgões. O pulgão-verde, *Myzus persicae*

Sulzer, e o pulgão-das-brássicas, *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae), são importantes em cultivos de brássicas de regiões de clima mais ameno, enquanto o pulgão-do-nabo, *Lipaphis pseudobrassicae* (Davis) (Hemiptera: Aphididae), se destaca tanto em climas amenos como em regiões mais quentes, e as três espécies estão entre as principais espécies de pulgões pragas no mundo (Blackman & Eastop 2007). De acordo com Melo (2017), esta última espécie tem-se tornado problema em várias regiões produtoras, inclusive Pernambuco.

Além dos pulgões, a traça-das-crucíferas, *Plutella xylostella* L. (Lepidoptera, Plutellidae), destaca-se como praga-chave para a cultura das brássicas em todas as regiões importantes de cultivo no mundo (<https://www.cabi.org/isc/datasheet/42318>). A traça-das-crucíferas é considerada uma praga cosmopolita, com ocorrência em 145 países, sendo responsável por ocasionar danos econômicos relevantes, podendo ocasionar perdas de até 90% ao atingir altas densidades populacionais (Niu *et al.* 2013, Saeed *et al.* 2019). Portanto, sendo a praga mais importante das brássicas mundialmente (Talekar & Shelton 1993, Saeed *et al.* 2019, Jaleel *et al.* 2019). Estima-se que os custos para a realização de seu manejo na ordem de 4 a 5 bilhões de dólares anualmente (Saeed *et al.* 2010, Zalucki *et al.* 2012). De acordo com Shelton & Nault (2004), o status de praga de importância global atribuído a *P. xylostella* se deve à: alta capacidade reprodutiva com duração de desenvolvimento relativamente curto e grande produção de descendentes resultando em várias gerações por ciclo de cultivo, diversidade hospedeira em Brassicaceae, perturbações no ecossistema que levam a perda de inimigos naturais devido a aplicações de inseticidas para o seu controle, e capacidade de desenvolver resistência a inseticidas.

Adultos da traça-das-crucíferas são pequenas mariposas de coloração parda, de hábito crepuscular, com os machos possuindo, mais destacado que em fêmeas uma, mancha escura e alongada na porção dorsal das asas anteriores formando o desenho de um diamante quando em repouso, sendo vulgarmente denominada de “diamondback moth” (Gallo *et al.* 2002). Os ovos

apresentam coloração amarelada, são depositados predominantemente na face inferior das folhas, isoladamente ou em grupos, ao longo das nervuras (Zago *et al.* 2010), com período de incubação variando de 3 a 4 dias. Após a eclosão, as larvas de primeiro ínstar minam as folhas se alimentando do parênquima, e que abandonam as minas no segundo ínstar, passando a alimentar da face abaxial das folhas até atingirem o quarto ínstar, quando passam a consumir todo o mesófilo foliar (De Bortoli *et al.* 2013). Ao final da fase larval, as larvas tecem casulos para se transformarem em pupas, as quais são de coloração clara inicialmente, mas se tornando escuras à medida que se aproximam da emergência do adulto. Os adultos mantêm-se protegidos sob as folhas durante o período diurno e voam ativamente no período crepuscular (Castelo Branco *et al.* 1997). O ciclo de vida de ovo a adulto é diretamente afetado pela temperatura, apresentando desenvolvimento máximo quando criadas a temperatura de 25 °C, com um período médio de desenvolvimento de 18 dias, e as fêmeas apresentando fecundidade de aproximadamente 288 ovos (De Bortoli *et al.* 2013, Saeed *et al.* 2019).

A traça-das-crucíferas tem um longo histórico de dificuldade de manejo, pois é uma das principais espécies com citações de desenvolvimento de resistência aos inseticidas sintéticos. Esta praga foi a primeira a ser citada possuindo resistência ao dicloro-difenil-tricloroetano (DDT), transcorridos um período de três anos após o início da utilização do DDT (Ankersmit 1953). De forma inédita, *P. xylostella* também foi a primeira espécie de inseto a apresentar resistência, em campo, ao inseticida biológico a base de *Bacillus thuringiensis* Berliner (Kirsch & Schmutterer 1988, Tabashnik *et al.* 1990), o que também impactou negativamente as perspectivas de produção de plantas de repolho *Bt*, resistente a lepidópteros. No início dos anos 2000, foi observado no Brasil, detecção de resistência em *P. xylostella* ao *B. thuringiensis*, em populações coletadas em diferentes regiões produtoras de repolho, indicando a perda de eficácia no controle da praga com produtos formulados à base de *Bt* (Castelo Branco *et al.* 2003). Além disso, o incremento no uso de inseticidas biológicos e sintéticos para o controle de *P. xylostella* resultou em pressão de seleção

para resistência a inseticidas nessa praga. Isso resultou na constatação de resistência em populações de *P. xylostella* a mais de 100 ingredientes ativos pertencentes a todas as classes de inseticidas sintéticos, incluindo aqueles pertencentes às classes de inseticidas mais recentes como espinosina, oxadiazina, fenilpirazol e diamidas (Mota-Sanchez & Wise 2022).

A dificuldade de controle e a rápida seleção para resistência em *P. xylostella* compõem o grande desafio para implementação do Manejo Integrado de Pragas (MIP) na cultura das brássicas (Steinbach *et al.* 2017). Apesar do controle da traça-das-crucíferas ser fundamentado nas aplicações inseticidas, são necessárias múltiplas estratégias visando suprimir as populações da praga e, simultaneamente, mitigar a seleção para resistência a inseticidas (Philips *et al.* 2014).

Visto como uma alternativa ao controle químico, ou mesmo, uma forma adicional ao controle químico para o controle da traça-das-crucíferas, a utilização do controle biológico depende da identificação de potenciais agentes de controle biológico que possam ser utilizados de forma integrada, em especial com os inseticidas frequentemente utilizados (Furlong *et al.* 2013). Diferentes agentes de controle biológico são identificados no agroecossistema das brássicas, realizando a predação ou parasitismo de *P. xylostella*. Em todo mundo, já foram registradas mais de 60 espécies de parasitoides em cultivos de brássicas. No Brasil, destacam-se *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae), espécies do gênero *Apanteles*, *Diadegema* e *Cotesia* (Monnerat *et al.* 2000, Ferreira *et al.* 2003, Silva-Torres *et al.* 2009, Machioro & Foerster 2016). Além dos parasitoides, diversos predadores têm sido identificados em áreas de cultivo de brássicas onde há infestação de *P. xylostella* como: *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) (Silva-Torres *et al.* 2009, Vacari *et al.* 2013), *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), *Hippodamia convergens* (Gérin-Méneville) (Coleoptera: Coccinellidae) e larvas de *Chrysoperla externa* Hagen (Neuroptera: Chrysopidae) (Vilchez 1997, 1996, Silva-Torres *et al.* 2009, Nunes *et al.* 2018). Dentre os inimigos naturais presentes em sistemas de cultivos de

brássicas, também são encontradas as tesourinhas (Dermaptera) (Ribeiro & Gontijo 2017), que apresentam potencial de realizar o controle da traça-das-crucíferas, devido a sua alta capacidade de predação, bem como sua voracidade (Moral *et al.* 2017).

Entre as tesourinhas, a espécie *Euborellia annulipes* (Lucas) (Dermaptera: Anisolabididae) apresenta ampla distribuição geográfica, sendo encontradas em cultivo de brássicas (Kocarek *et al.* 2015, Nunes *et al.* 2018, Rana *et al.* 2019). Esta espécie é encontrada habitando o solo, locais sob rochas e troncos, onde escavam aberturas utilizadas como abrigo e local para reprodução (Klostermeyer 1942). Além disso, *E. annulipes* apresenta hábito noturno de forrageamento e permanece nos locais de abrigo e reprodução durante o dia (Silva *et al.* 2018). Morfologicamente, *E. annulipes* apresenta coloração marrom-escura para preto, com a porção ventral do corpo de coloração castanha. Como característica notória, as pernas dessa espécie de tesourinha são de coloração amarelada com anéis escurecidos circundando a tibia e o fêmur, assemelhando-se a anéis. As antenas possuem números de segmentos distintos ao longo do desenvolvimento, apresentando 8, 11, 13, 14 e 16 segmentos nos cinco estádios de ninfas, respectivamente, e 16 segmentos na fase adulta. O dimorfismo sexual nesta espécie é observado através dos fórceps, com as fêmeas apresentando fórceps igualmente curvados, enquanto os machos apresentam o fórceps direito significativamente mais curvado que o esquerdo (Klostermeyer 1942).

A tesourinha *E. annulipes* apresenta alta longevidade (até 206 dias) apresentando um ciclo de vida ovo-adulto médio de 95 dias (Klostermeyer 1942). As fêmeas de *E. annulipes* exibem cuidado parental com os ovos e ninfas recém-eclodidas, protegendo-os do ataque de predadores e da contaminação por patógenos (Jacobs & Stigall 2019). Os ovos são organizados pela fêmea constantemente quando são retirados do local, promovendo durante esse processo a manipulação dos ovos com as mandíbulas através de movimentos circulares, onde tais movimentos resultam na limpeza dos ovos, mantendo-os protegidos do ataque de ácaros e fungos (Marucci 1955, Knable &

Grigrick 1971, Shepard *et al.* 1973). Em relação à biologia de *E. annulipes*, as fêmeas realizam diversas posturas durante a vida, onde uma única cópula é suficiente para várias posturas, que pode variar de 47 a 52 ovos cada (Klostermeyer 1942, Bharadwaj 1966).

O potencial de *E. annulipes* como agente de controle biológico se dá pelo hábito alimentar generalista, podendo não somente se alimentar da praga alvo quando abundante, mas também utilizar de outras presas para se manterem no agroecossistema e, este hábito generalista permite variação de alimento-presas para criação em laboratório. Estudos têm caracterizado a atividade de predação de *E. annulipes* em diferentes espécies de pragas como *Cosmopolites sordidus* German (Coleoptera: Curculionidae) na cultura da banana, onde é identificada a predação desta praga nas fases de ovo e larval até o quarto ínstar (Klostermeyer 1942, Koppenhöfer *et al.* 1992, Carval *et al.* 2016). Também, como predadora da lagarta-do-cartucho, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), nos diferentes estágios de desenvolvimento do predador e da praga; onde foi verificado que há um incremento no consumo médio diário de ovos da praga em função da idade da tesourinha, variando de 11 a 374 ovos consumidos no primeiro e quinto ínstars, respectivamente. Além disso, o consumo de *S. frugiperda* foi maior para lagartas de primeiro e segundo ínstars por ninfas de quarto e quinto ínstars de *E. annulipes*, durante 10 dias de consumo (Silva *et al.* 2009). A *E. annulipes* foi relatada como predadora de larvas da principal praga associada à cultura do algodão, o *Anthonomus grandis grandis* Boh. (Coleoptera: Curculionidae) (Ramalho & Wanderley 1996), predando larvas desta praga, presente no interior de botões florais caídos das plantas (Potin *et al.* 2022).

A presença de *E. annulipes* em sistemas de cultivo de brássicas levou ao desenvolvimento de estudos que avaliaram a capacidade de predação da tesourinha sobre *P. xylostella*. Nunes *et al.* (2018) avaliaram a capacidade de predação de ninfas e fêmeas adultas de *E. annulipes* sobre larvas de quarto ínstar e pupas da traça-das-crucíferas. Ninfas do terceiro ao quinto ínstar e fêmeas adultas

da tesourinha consumiram entre 7,5 e 45 larvas de *P. xylostella*, respectivamente, A resposta funcional de *E. annulipes* predando *P. xylostella* foi determinada como sendo do tipo II para larvas e pupas da praga, demonstrando um crescente consumo das presas até um limite (Nunes *et al.* 2019). Ainda, esses autores encontraram maior consumo de larva em relação a pupas, em situação com chance e sem chance de escolha.

Fatores abióticos são importantes na interação predador presa. Assim, Nunes *et al.* (2020) encontraram que a predação de *P. xylostella* por *E. annulipes* foi maior sob temperatura de 32 °C (T/T_h) e menor tempo de manipulação (T_h), que aquelas mantidas sob temperaturas de 18 e 25 °C. Esta variação na taxa de predação em função da temperatura resultou em diferente resposta funcional sendo do tipo III (sigmoide) nas temperaturas de 18 e 25 °C e tipo II (curvilínea) a 32 °C.

Relevância do Estudo

O cultivo de brássicas se destina principalmente ao consumo *in natura* ou pré-cozido, como couve folha, couve-flor, repolho, brócolis, couve-chinesa, entre outras. Esta variedade de utilização das brássicas requer produto de qualidade visual, fresco e macio, bem como sem resíduo de produtos químicos, embora esta última exigência deva ser considerada a qualquer produto agrícola. Assim, um dos empecilhos para a maximização da produção de brássicas atendendo a essas exigências são as infestações de pragas como da traça-das-crucíferas, *P. xylostella*, e de pulgões (Telekar & Shelton 1993, Zalucki *et al.* 2012, Sharma 2017). Somente relativo a traça-das-crucíferas, as perdas são estimadas de forma conservativa na ordem de U\$ 105 milhões anuais, sendo necessária a constante demanda por práticas de controle, com destaque para a utilização de inseticidas sintéticos (Zaluzcki *et al.* 2012, Holtz *et al.* 2015). Assim, problemas gerados pela utilização de inseticidas no cultivo de brássicas são comuns, incluindo a presença de resíduos tóxicos tanto para o ambiente quanto para humanos, desenvolvimento de resistência aos inseticidas utilizados (Li *et al.* 2012). Porém,

como preconizado no manejo integrado de pragas (MIP), estudos acerca da utilização de inimigos naturais de espécies-praga de forma integrada a outros métodos de controle geram a expectativa da redução do uso de inseticidas nos cultivos (Liu *et al.* 2014, Philips *et al.* 2014).

As tesourinhas predadoras são encontradas naturalmente em uma diversidade de sistemas agrícolas, incluindo os sistemas de cultivo de brássicas, ambientes estes que utilizam de forma contínua inseticidas sintéticos com diferentes ingredientes ativos (Kocarek *et al.* 2015, Ribeiro & Gontijo 2017). A tesourinha *E. annulipes* se abriga durante o período diurno e exibe atividade de predação predominantemente durante a noite. O forrageio por alimento na superfície do solo e folhagem das plantas cria possibilidade de contato com resíduo de inseticidas. A tesourinha além de forragear nas folhagens, ela pode se abrigar entre as folhas imbricadas (repolho, couve-chinesa, etc.) e flores de brássicas (couve-flor, brócolis), o que favorece o contato da tesourinha com as pragas a serem controladas (nas folhas) e, também, contato com inseticidas aplicados. Com a presença de *E. annulipes* em cultivos de brássicas, onde podem realizar a predação da traça-das-crucíferas e pulgões, pragas-chave, surge o questionamento dos impactos causados pelo uso dos inseticidas sobre o inimigo natural.

Diante do exposto, esta tese foi desenvolvida para demonstrar a compatibilidade de inseticidas, pertencentes a diferentes grupos, aplicados em dosagens recomendadas para o controle da traça-das-crucíferas, e a tesourinha *E. annulipes*. Além disso, evidenciar a capacidade de predação da tesourinha predadora sobre *P. xylostella* exposta a doses máximas recomendadas de inseticidas registrados para a cultura das brássicas, bem como a determinação da preferência alimentar de *E. annulipes* por diferentes estágios de *P. xylostella* quando em contato com inseticidas. Em uma segunda etapa do estudo foi empregado condição de confinamento para averiguar o controle de *P. xylostella* pelo inseticida que ocasiona a maior mortalidade da praga em

associação com a tesourinha *E. annulipes*, demonstrando, assim, a compatibilidade do inseticida e do inimigo natural.

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CAPÍTULO 2

INSECTICIDE COMPATIBILITY WITH THE PREDATORY RING-LEGGED EARWIG

Euborellia annulipes INCREASES MORTALITY OF DIAMONDBACK MOTH¹

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¹Morato, R.P. Insecticide compatibility with the predatory ring-legged earwig *Euborellia annulipes* increases mortality of diamondback moth. *Biocontrol Science and Technology*.

ABSTRACT - Brassica growers rely on insecticides to control the diamondback moth (DBM) *Plutella xylostella* (L.), resulting in cases of insecticide resistance and control failure. The ring-legged earwig *Euborellia annulipes* (Lucas) is found in brassica fields and may prey upon DBM eggs, larvae, and pupae. Therefore, preservation of *E. annulipes* in brassica crops could enhance DBM control and may help with DBM insecticide resistance management. The insecticides azadirachtin, chlorantraniliprole, cyantraniliprole, deltamethrin, indoxacarb, methomyl, spinosad, and teflubenzuron were evaluated to assess mortality of *E. annulipes* adults and DBM larvae when exposed to insecticide dry residues, and predation rate upon DBM larvae and pupae. *Euborellia annulipes* exhibited survival >98% to all tested insecticides when exposed to label rates used on brassica crops against DBM. The highest mortality of DBM larvae confined on insecticide dry residue was 61% after 24 h exposure. This mortality significantly increased up to 92% when *E. annulipes* was present in the same period. Female of *E. annulipes* showed a preference for DBM larvae over pupae irrespective of ratio availability and insecticide combinations. When only larvae or pupae were available, up to nine larvae or four DBM pupae were consumed within 24 h. At the label rate for spraying brassica crops, all tested insecticides were compatible with *E. annulipes* and the predator added significant mortality to DBM larvae in the presence of insecticide residue. The findings reveal opportunities for integrating *E. annulipes* as a biological control agent into management programs for DBM that cannot effectively rely on insecticides alone.

KEY WORDS: Insecticide toxicology, chemical control, integrated control, biological control conservation

COMPATIBILIDADE DE INSETICIDAS COM A TESOURINHA PREDADORA *Euborellia annulipes* AUMENTA A MORTALIDADE DA TRAÇA-DAS-CRUCÍFERAS

RESUMO – Produtores de brássicas dependem de inseticidas para o controle da traça-das-crucíferas, *Plutella xylostella* (L.), resultando em casos de resistência e falhas de controle. A tesourinha *Euborellia annulipes* (Lucas) é encontrada em campos de brássicas e podem consumir ovos, larvas e pupas de *P. xylostella*. Assim, a conservação de *E. annulipes* pode favorecer o controle de *P. xylostella* e, conseqüentemente, o manejo de resistência a inseticidas. Os inseticidas azadiractina, clorantraniliprole, ciantraniliprole, deltametrina, indoxacarbe, metomil, espinosade e teflubenzurom foram estudados quanto à mortalidade de adultos de *E. annulipes* e larvas de *P. xylostella*. Também se determinou a predação de larvas e pupas da traça na presença do inseticida. A sobrevivência de *E. annulipes* foi > 98% quando expostas ao resíduo seco de todos os inseticidas recomendados contra *P. xylostella*. Entre todos os inseticidas, a maior taxa de mortalidade de *P. xylostella* foi de 61%, após 24 h de exposição. Contudo, a mortalidade aumentou significativamente até 92% quando *E. annulipes* estava presente, no mesmo intervalo de 24h de exposição. Fêmeas de *E. annulipes* mostraram preferência por larvas da traça em relação a pupas, independente da disponibilidade dessas e combinação com inseticidas. Quando somente larvas ou pupas foram ofertadas, até nove larvas e quatro pupas da traça foram consumidas durante 24 h. Todos os inseticidas aplicados na dosagem para o controle de pragas das brássicas foram compatíveis com *E. annulipes*, permitindo efeito aditivo na mortalidade da praga com a presença do predador. Os resultados mostram oportunidade para a integração de *E. annulipes* e inseticidas para o controle das traça-das-crucíferas e, conseqüentemente, redução da dependência dos produtores por inseticidas.

PALAVRAS CHAVE: Toxicidade de inseticidas, controle químico, controle integrado, controle biológico conservativo

Introduction

Diamondback moth (DBM), *Plutella xylostella* (L.), is an important pest of brassica crops, being found in 145 countries with global management cost estimates of around 5 billion US\$ annually (Saeed *et al.* 2010, 2019, Zalucki *et al.* 2012, Jaleel *et al.* 2020, Machekano *et al.* 2020). Continuously availability of many cultivated and uncultivated brassica species helps maintain DBM populations, and key life-history traits (short life cycle, high fecundity, etc.) enhance its pest status and management challenges. Control strategies ranging from host plant resistance to behavioral methods have been developed against DBM (Talekar & Shelton, 1993, Furlong *et al.* 2013, Li *et al.* 2016), but insecticide applications remain the most used tactic against DBM. Heavy reliance on insecticides has not surprisingly resulted in development of resistance of DBM to all mode of action groups of insecticides, showing resistance to 111 different modes of action (Mota-Sanchez & Wise, 2022). Therefore, it is important that other pest management strategies complement insecticide applications as much as possible, to improve pest population suppression while mitigating insecticide resistance within an insecticide resistance management (IRM) program (Philips *et al.* 2014).

Earwigs are considered natural enemies of DBM (Ribeiro & Gontijo, 2017), with potential for augmentative biological control (Nunes *et al.* 2018). The ring-legged earwig, *Euborellia annulipes* (Lucas) is a species with wide geographic distribution (Kocarek *et al.* 2015). It occurs naturally in cultivated brassica fields and preys upon DBM larvae and pupae (Nunes *et al.* 2018, 2020). Ring-legged earwigs commonly live between leaves of cabbage, Chinese cabbage, flowers of broccolis and cauliflower, etc. These habitats bring them into contact with immature stages of DBM. They are easily reared in large numbers in the laboratory using a diet consisting of relatively low-cost ingredients, with an estimated cost for diet of about R\$ 4 (<US\$1) per 1000 earwig adults (Silva *et al.* 2009, Souza, 2021). Due to their generalist feeding behavior these predators also offer

opportunities to enhance biological control of other pest species of brassica crops such as aphids and lepidopteran larvae. In other crop ecosystems, ring-legged earwigs also prey upon fall armyworm (Ballal *et al.* 2021), larvae and pupae of boll weevil inside fallen cotton bolls (Ramalho & Wanderley 1996, Lemos *et al.* 1998), eggs and larvae of sugarcane borer (Tryon Jr., 1986, Ramamurthi & Solayappan 1980), and eggs and immature stages of banana weevil (Koppenhöfer *et al.* 1992). These attributes make ring-legged earwigs potentially a good option for conservation and augmentative biological control.

Frequent insecticide use can compromise the biology and predatory performance of natural enemy populations in agroecosystems, diminishing their contribution to pest population regulation (e.g., Desneux *et al.* 2007, Biondi *et al.* 2012a, El-Wakeil *et al.* 2013, Potin *et al.* 2022). Insecticide applications should attempt to optimize insecticidal action against the target pest, with minimum impact on populations of non-target species, including natural enemies (Bommarco *et al.* 2011, Li *et al.* 2016, Anjum & Wright 2016, Torres and Bueno 2018, Lira *et al.* 2019). In this study we aimed to examine the compatibility of ring-legged earwigs and insecticides used against DBM, predation rate of the predator to DBM exposed to insecticides as well the feeding preference to different development stages of DBM. Thus, we assessed the survival and predation rate of the ring-legged earwigs and hypothesized that insecticides registered against DBM would be compatible with them allowing the integration of biological and chemical control methods. Because the studied insecticides are also used in other crop ecosystems, the results will support conservation of the ring-legged earwig on a wide range of crops.

Material and Methods

All insect species were reared at Biological Control Lab and used in bioassays at a temperature of 25 ± 2 °C, 12:12 h (L:D) photoperiod, and relative humidity from 55 to 65% (DataLogger Hobo[®], Onset Computer, Bourne, MA, USA).

Insects rearing: *Euborellia annulipes* and *Plutella xylostella*. Earwigs used in the study originated from a colony maintained in the Laboratory of Biological Control of the Universidade Federal Rural de Pernambuco (UFRPE), Recife, Pernambuco State, Brazil. This colony was established from field-collected individuals in 2019 by Potin *et al.* (2022). Rearing procedures were detailed in Potin *et al.* (2022). Briefly, nymphs and adults were reared using transparent plastic containers (13 × 20 × 7 cm in H × L × W), which were kept on a shelf covered by a black curtain to avoid direct light over the 12h light period. In addition, 8–10 layers of double sheet toilet paper (Personal Vip, Bragança Paulista, São Paulo, Brazil) were added at the bottom of the containers as hiding and oviposition substrate. The paper sheets were moistened every two days with tap water and were replaced once a week. The insects were fed *ad libitum* with a dry diet prepared from chicken feed (35%), wheat bran (26%), yeast (22%), powdered milk (15%), and the antimicrobial Nipagin (2%) (Ueno Fine Chemicals Ind, Ueno, Japan) (Silva *et al.* 2009). To avoid contact of the diet with the moistened paper sheets, it was placed in plastic caps (10 × 3 mm in diam × H) and replaced when necessary.

Inside each rearing container, we placed ≈40 adults at a ratio of 4 females: 1 male (Silva *et al.* 2009). Earwig females exhibit parental care, therefore, to track the age of the individuals used in the bioassays, the egg batches were carefully transferred with the female to Petri dishes (8 × 1.5 cm in diam × H) and fed the same diet. The eggs were placed on 2×2 cm moistened paper sheets inside the dishes. Females were maintained with the egg batch and hatched nymphs for up to 3 days after eclosion. Next, females were returned to their original containers, while nymphs were transferred in groups of 50-60 individuals to new rearing containers with the same diet and reared to the adult stage.

A DBM population was established using pupae given by the Laboratory of Insect-Toxic Interactions of UFRPE. These insects were originated from various populations established after

collections made at Agreste region of Pernambuco State. DBM larvae were reared in a plastic container (20 × 12 × 10 cm L × W × H) with an opening in the lid that was covered with mesh screen for ventilation. Collard leaves [*Brassica oleracea* L. var. *acephala* (Brassicaceae)] harvested from plants cultivated without insecticide application were provided as larval food and replaced when needed. Pupae were transferred daily to 80-mL plastic pots until adult emergence. DBM adults were released inside rearing cages made of 3-L transparent plastic pots with a 10 cm diameter screw cap lid. The cage contained one 6-cm diameter opening on the side covered with anti-aphid mesh screen to allow ventilation and a 2-cm diameter opening on the bottom. An 8-cm diameter leaf disc cut from a fresh collard leaf used for oviposition was placed over a moistened filter paper of the same diameter laid inside the cap lid. Then, the cage was inverted over the cap lid holding the leaf disc and then, closed. Adults were released through the opening made on the cage bottom. The opening was closed with a cotton ball with the side of the ball left inside the cage moistened with a solution of 20% honey:water (v/v) as food for the adult moths. The collard discs were replaced every day and placed over a moistened towel paper sheet in the bottom of the rearing container for larvae. After hatch, larvae were fed by moving the old collard leaf holding the larvae to the top of a new collard leaf and cleaning the cage.

Chinese Cabbage Plants. Seeds of Chinese cabbage plants [*Brassica rapa* L. var. *pekinenses* (Brassicaceae)] were obtained from Isla (Porto Alegre, Rio Grande do Sul, Brazil). Plant seedlings were grown in Styrofoam™ trays filled with a 2:1 mixture of humus and soil. Seedlings at the 3-leaf stage were transplanted to 2 L plastic pots filled with sandy soil + humus (8:2) and 10 g of NPK fertilizer (10-10-10) per pot and watered daily. Potted plants were enclosed in cages in a greenhouse and when reached 5-6 fully expanded leaves (≈15 cm height), they were used in the bioassays.

Insecticides. Eight insecticides were tested using commercial formulations at the maximum label rate (Table 1) registered in Brazil to be used in brassica crops against *P. xylostella* (AGROFIT,

2022). Insecticides were diluted with tap water (pH 5.8-6.5 based on weekly measurements). The surfactant Wil Fix[®] (30 g/L, Charmon Destyl, Valinhos, São Paulo, Brazil) was also added to each mixture at 0.05% v/v. Water + surfactant controls were used in all experiments.

Experiment 1 - Residual Toxicity of Insecticides to the Earwig. This bioassay was conducted with 5-10 days old adult earwigs exposed to dried insecticide residues (Table 1) on leaf discs (8 cm diam) cut from leaves of Chinese cabbage plants grown without insecticide. Treated leaf discs were immersed in insecticide solution for 20s (IRAC 2014) and air-dried for 2 h inside an exhaust chamber Nalgon mod. 3700 (Nalgon Equipamentos Científicos, Itupeva, São Paulo, Brazil). Each treatment (insecticides and control) had 10 replications each consisting of a Petri dish lined with a collard leaf disc and five virgin females adult earwigs (total n = 50 insects per treatment). Also, a portion of diet sufficient to feed the adults for 48 h was offered on the leaf disc. Mortality was recorded after 48 h exposure. An insect was considered dead if unable to turn upright and walk after being placed on its dorsum. Next, 10 surviving females from each treatment were transferred to clean 1 L plastic pots containing toilet paper as oviposition substrate and artificial diet offered ad libitum as food, and males to allow mating. These females were monitored daily to determine the time elapsed from insecticide exposure to first oviposition, number of eggs laid, and egg viability.

Experiment 2 - Mortality of *Plutella xylostella* and Predation by *Euborellia annulipes* with Residual Insecticide Exposure. A factorial design (9 × 2) was used to evaluate DBM larval mortality and predation rate. Treatments were +/- insecticides (8 different insecticides were tested) and +/- earwig. Each replication consisted of ten 3rd-instar DBM larvae placed on leaf discs treated as previously described, with or without one adult female earwig. The number of living DBM larvae, the number of DBM larvae dead, and the number of DBM larvae consumed were recorded after 24 h.

Experiment 3 - Insecticide Control of DBM Larvae and Pupae (Prey Preference). Indoxacarb, chlorantraniliprole, and methomyl provided respectively a high, intermediate, and low DBM mortality among the tested insecticides in the aforementioned experiment. Then, they were selected for a follow-up experiment. Leaf discs preparation containing insecticide-dried residues, caging and prey release were as previously described. Different densities of DBM 3rd-instar larvae and 24 h-old pupae were established through six treatments according to the following larvae (L) and pupae (P) ratios: 10L:0P, 10L:1P, 10L:4P, 10L:8P, 10L:10P, 0L:10P. The number of larvae and pupae alive, dead, or consumed was tallied after 24 h.

Statistical Analysis. Percentage survival of adults, time to first oviposition, number of eggs produced, and number of hatched nymphs data were subjected to analysis of variance (ANOVA). Two-way ANOVA was used to analyze the effect of insecticide treatment and predation on number of dead earwigs, and number of dead, alive, or consumed DBM. Analyses were performed using the ExpDes package in RStudio, with treatment means separation done using Scott-Knott's test $\alpha = 0.05$ (RStudio Team, 2020).

Earwig predation preference was examined with polynomial regression analysis using SigmaStat[®] 14.0 (SigmaPlot 2017). We tested the hypothesis that earwigs preferred mobile larvae (L) more than immobile pupae (P). The proportion of larvae consumed (L_c) over pupae consumed (P_c) [$(L_c/(L_c + P_c))$] was considered the dependent variable (y) as a function of the ratio of prey availability ($L/(L+P)$) (= independent variable x). Preference was based on the linear coefficient of the fitted equation: significant coefficient >1.0 indicates positive preference for larvae; <1.0 indicates negative preference for larvae; and $= 1.0$ indicates absence of preference (Cock, 1978).

Results

Experiment 1 - Residual Toxicity of Insecticides to the Earwig. Survival of *E. annulipes* on Chinese cabbage foliage containing dry insecticide residues following treatment with maximum registered label rates against *P. xylostella* did not differ among treatments ($F_{8, 81} = 0.75$, $P = 0.65$) (Table 2). Survival was equal or superior than 98% for all treatments. Time elapsed from contact with insecticide residues to first oviposition of surviving female earwig varied among treatments ($F_{8, 81} = 3.13$, $P = 0.004$). Females had delayed oviposition of 8-9.1 days following contact with dry residues of azadirachtin, chlorantraniliprole, indoxacarb, and spinosad (Table 2), compared to other treatments, including the control, which varied from 4.6 (control) to 6.2 days (deltamethrin). The number of eggs laid per female earwig also varied among treatments ($F_{8, 81} = 2.71$, $P = 0.011$). Females exposed to azadirachtin, chlorantraniliprole, indoxacarb, and spinosad residues that had delayed oviposition produced larger egg batches (ca. 55.4 to 62.9 eggs) (Table 2). The percent egg hatch was similar across all treatments and greater than 80% in all treatments ($F_{8, 62} = 0.28$, $P = 0.97$).

Experiment 2 and 3 - Mortality of *Plutella xylostella* and Predation by *Euborellia annulipes* with Residual Insecticide Exposure. Mortality of DBM larvae on insecticide residues, within 24 h evaluation period, in the absence of the predator varied across treatments (Table 3). Mortality was greatest with indoxacarb (61%), and spinosad (49%), followed by chlorantraniliprole (23%). The other tested insecticides caused <10% mortality (Table 3, Fig. 1). The addition of a predatory earwig to the bioassay arena greatly increased DBM mortality to 66-92% across all treatments, with DBM consumption rates of 6.0-8.2 larvae per 24 h (Table 3). The effect of predator presence was much more pronounced with insecticides that had relatively low toxicity to DBM, as opposed to treatments like indoxacarb and spinosad, which had relatively higher rates of DBM mortality in the absence of *E. annulipes* (Fig. 1).

Earwigs consumed more DBM larvae than pupae irrespective of the availability ratio and the residues of chlorantraniliprole, indoxacarb, and methomyl. The relationship between the proportion of larvae consumed as a function of increased availability of pupae was always significant and positive (slopes > 1.0) (Fig. 2). Furthermore, the presence of pupae did not affect the number of larvae consumed (Fig. 3). Across all treatments, there was a high rate of larvae consumed regardless of presence or absence of pupae. Except in the case of indoxacarb, the predation rate upon larvae was greater in the presence of pupae. The predation rate upon pupae differed in the control ($F = 3.80$, $df = 3, 36$, $P = 0.018$), chlorantraniliprole ($F = 4.60$, $df = 3, 36$, $P = 0.008$), and indoxacarb ($F = 3.38$, $df = 3, 36$, $P = 0.028$) treatments, but not with methomyl ($F = 0.247$, $df = 3, 36$, $P = 0.86$). This difference, however, was due to greater predation on pupae in the absence of larvae.

Discussion

Adults of ring-legged earwigs survived and consumed diamondback larvae when confined with foliage containing dry residues of insecticides applied at the maximum label rate registered against the diamondback moth. The insecticides tested represented several different modes of action that collectively cover a broad spectrum of pest control. Insecticides such as deltamethrin, methomyl, indoxacarb, and spinosad are registered against different pest species of brassicas and many other crops. Thus, at tested label rates the results indicate compatibility with ring-legged earwig of broad-spectrum and selective insecticides used in this study (cyantraniliprole, chlorantraniliprole, azadirachtin, and teflubenzuron) and other studies (e.g., pymetrozine, spinetoram, cyromazine, triflumuron, and pyriproxyfen) (Barros *et al.* 2018, Potin *et al.* 2022), suggesting good potential within a brassica IPM and IRM.

The toxicity of spinosad to the earwig *Doru taeniatum* (Dohrn) was previously reported (Cisneros *et al.* 2002). When *Doru luteipes* (Scudder) was exposed to dry residues of spinosad on

a glass surface this insecticide was classified as moderately harmful (Redoan *et al.* 2013). Biondi *et al.* (2012b) found that in 88% of studies with spinosad and beneficial predatory insects this insecticide showed lethal effect to predatory insects. At the label maximum application rate recommended against cotton pests, dry residues of spinosad on a glass plate caused 100% mortality of *Chrysoperla externa* (Hagen), *Orius insidiosus* (Say), and *Podisus nigrispinus* (Dallas) (Barros *et al.* 2018). In a striking contrast, spinosad was not harmful to *E. annulipes* showing 100% survival in our study, and 97% survival of *E. annulipes* exposed to spinosad maximum application rates for cotton (Barros *et al.* 2018). Spinosad is also compatible with *D. luteipes*, based on the selectivity index obtained from toxicity ratios against fall armyworm and this earwig (Campos *et al.* 2011). Likewise, Torres *et al.* (1999) studying the selectivity indexes between fall armyworm, tomato leafminer, and the predatory stinkbug *P. nigrispinus*, found that indexes were favorable to the predator. Spinosad toxicity not surprisingly varies among species and type of exposure. Our findings indicate that spinosad is compatible with *E. annulipes* adults on brassica crops in Brazil, similar to previous results that demonstrated its compatibility to predators in cotton fields (Barros *et al.* 2018).

Despite high survival, some tested insecticides caused delays in *E. annulipes* oviposition and affected the number of eggs laid in the first egg batch. For instance, females exposed to spinosad generally took twice as long to lay the first egg batch relative to controls. However, *E. annulipes* females exposed to spinosad, indoxacarb, chlorantraniliprole, and azadirachtin laid higher number of eggs. This may be indicative of a biological tradeoff whereby insecticide exposure delayed oviposition but this cascaded subsequently to increased egg batch size. Alternatively, increased fecundity is commonly seen in insecticide-induced hormesis, whereby mild insecticide stress stimulates reproductive outputs, other life history traits, molecular processes, or ecological functions (Cutler, 2013, Cutler *et al.* 2022). Further studies should address physiological changes

in the females exposed to those insecticides in order to understand the causes and effects of oviposition delay but the increase in the number of eggs laid. Irrespective of the size of the first egg batch produced, *E. annulipes* females exhibited similar survival across all insecticides. Therefore, delaying egg production seems to be compensated by higher oviposition and similar female survival. Furthermore, the likelihood of time lag caused in predator numbers for biological control service, *E. annulipes* exhibit long developmental time (≈ 50 days) and adult stage (100 to 200 days, Nunes *et al.* 2022) with overlapping generations, which would minimize the oviposition delay.

Some of the tested insecticides may affect oogenesis, such as those classified as growth regulators, e.g., azadirachtin and teflubenzuron. Nevertheless, azadirachtin exposure at label rate for spraying brassica crops unexpectedly resulted in increased oviposition by *E. annulipes*. Azadirachtin can adversely affect insect reproduction, for example causing a reduction in fecundity and fertility of *Chrysoperla carnea* (Stephens) (Medina *et al.* 2004) and *Ceraeocrhysa cubana* (Hagen) (Rugno *et al.* 2019). In general, reduced fecundity following insecticide exposure may be related to the need for increased metabolism of the toxic compound, resulting in a trade-off between fecundity and insecticide detoxification (Rodrigues *et al.* 2020). Nevertheless, *E. annulipes* exposure to azadirachtin resulted in increased oviposition. The earwig *Forficula auricularia* exhibited high activity of detoxifying enzymes (carboxylesterases) in its abdominal tissues (Malagnoux *et al.* 2014), which may enhance this insect's tolerance to indoxacarb (Jana *et al.* 2021).

Exposure to indoxacarb did not affect survival or oviposition of *E. annulipes* in the current study. The reason for high survival of *E. annulipes* to insecticides tested here deserves further investigation. In a previous study this same species had only 20% survival when confined to indoxacarb dry residues on glass following an application rate used for cotton pests (120 g a.i./ha) (Potin *et al.* 2022), in contrast to the 100% survival at 24 g a.i./ha used in this study, which is a 5-fold lower concentration. Therefore, these findings suggest a dose-dependent toxicity of indoxacarb

to *E. annulipes*. The studied earwig population also showed 100% survival to pymetrozine, cyantraniliprole, chlorantraniliprole, and pyriproxyfen at field application rates registered against cotton pests (Potin *et al.* 2022). Other insecticides tested at label rates for cotton pest control ranged from intermediate toxicity (thiamethoxam) to highly toxic (chlorpyrifos, lambda-cyhalothrin, malathion, and dimethoate) to nymph and adult *E. annulipes*. However, when lambda-cyhalothrin and thiamethoxam were applied to plant foliage and the predatory earwig was allowed to undergo its natural behavior of nesting on the ground and to forage for treated prey on plant foliage at night, there was a significant reduction of earwig mortality (Potin *et al.* 2022). Similarly, methomyl applied to a glass surface at 320 g a.i./ha gave 100% mortality of *Labidura riparia* (Pallas) (Dermaptera: Labiduridae), but when exposed to treated peanut foliage or fall armyworm larvae fed treated peanut leaves, only a maximum of 35% mortality occurred (Rivero & Poe, 1981). These differences might be related to the kind of surface where the insecticide was applied, because when predators are allowed to forage on treated foliage and hide on untreated areas mortality can be drastically reduced. Our laboratory experiments suggest label application rates for insecticides registered for use in Brazil on brassica crops are compatible with *E. annulipes*, and in the field where earwigs have reduced contact with insecticide residues on plant foliage due to their dominant ground-dwelling behavior, effects are likely to be even less pronounced.

Insecticide residues we tested did not compromise earwig predation upon DBM larvae. Survival of natural enemies following insecticide exposure is very important in integrated pest management, but surviving natural enemies also need to produce offspring while suppressing populations of the target pest, especially pest individuals surviving or escaping from the insecticide control. All tested insecticides caused low mortality of DBM larvae during the 24 h assessment period used in our experiments. Despite that, there is some limitation of the result that needs to be considered. The short evaluation window could partially be affecting the result of low mortality

observed, especially for insecticides such as teflubenzuron and azadirachtin. In addition, the DBM larvae used in our experiment is originated from Agreste, Pernambuco, which have shown high levels of tolerance to insecticidal active ingredients included in this study such as indoxacarb and methomyl (Santos *et al.* 2011), deltamethrin (Oliveira *et al.* 2011), chlorantraniliprole (Ribeiro *et al.* 2017), spinosad and chlorantraniliprole (Lima Neto *et al.* 2016), and to inhibitors of chitin biosynthesis lufenuron like teflubenzuron (Arruda *et al.* 2020), among others. For example, when spinosad initially became registered and available for use offering effective control of DBM larvae due to the high susceptibility (Oliveira *et al.* 2011), brassica growers addressed multiple successive applications pointing to a rapid insecticide resistance development. This scenario happened with spinosad and has been repeated with resistance to chlorantraniliprole, indoxacarb and chlorfenapyr (Santos *et al.* 2011, Lima Neto *et al.* 2016, Ribeiro *et al.* 2017, Lima Neto *et al.* 2021). The addition of the predator with the insecticide significantly increased mortality of DBM, which is encouraging for IPM and IRM. In other studies, insecticides have impaired predatory behavior of earwigs. For example, *E. annulipes* that consumed food contaminated with pymetrozine, a chordotonal organ channel modulator insecticide, had significantly reduced predatory behavior (Potin *et al.* 2022). Pyrethroid insecticides such as deltamethrin can irritate and repel natural enemies (Cordeiro *et al.* 2010, Spíndola *et al.* 2013), altering maternal care behavior of *F. auricularia* (Meunier *et al.* 2020).

Simultaneous exposure to indoxacarb and *E. annulipes* caused 90% DBM mortality. The sodium channel blocking activity of indoxacarb occurs after its ingestion (Wing *et al.* 2000, 2005), and exposure to dry residues is relatively less harmful. We predicted consumption of indoxacarb exposed DBM would impede *E. annulipes*, but this was not observed in our experiment, probably due to lower application rate used in brassica crops as previously discussed. Dry residues of indoxacarb were also harmless for *Podisus maculiventris* Say (Hemiptera: Pentatomidae) (Tillman

& Mullinix, 2004), *Geocoris punctipes* (Say) (Hemiptera: Lygaeidae) (Tillman *et al.* 2002), and *Harmonia axiridis* (Pallas) (Coleoptera: Coccinellidae) (Galvan *et al.* 2006).

The DBM larvae exposed to methomyl residues did not affect the predation by *E. annulipes*, with 92% DBM mortality resulting from methomyl + predator exposure in our experiments. Similarly, methomyl was 517-fold more toxic to *S. frugiperda* than to the predatory earwig *D. luteipes* (Campos *et al.* 2011). Methomyl acts by inhibiting the enzyme acetylcholinesterase. Intoxication by methomyl causes hyperexcitation of the nervous system (Padilla, 2005), and as a broad-spectrum insecticide is considered highly toxic to natural enemies (Theiling & Croft, 1988). Using a toxicity rate from 1 (0% mortality) to 5 (90-100% mortality), Theiling & Croft, (1988) reported that methomyl averaged 4.28 for 122 records with predators and 4.33 for 53 records with parasitoids. Exposure of *C. carnae* larvae to methomyl resulted in 100% mortality (Korrat *et al.* 2019), and dry residues of methomyl on pod bean leaves caused significant mortality of *C. externa* and *Eriopis connexa* (Germar) up to 31 days after application treatment (Pasini *et al.* 2021).

Both tested diamides were compatible with *E. annulipes*. Chlorantraniliprole also did not cause acute toxicity for the hover beetle, *Paederus fuscipes* (Curtis) (Khan *et al.* 2021) and other predatory insects (Martinou *et al.* 2014, Barros *et al.* 2018, He *et al.* 2019, Potin *et al.* 2022), but did reduce fecundity and predation rates of *P. fuscipes* (Khan *et al.* 2021). Exposure of larval and adult *Coccinella septempunctata* L. to chlorantraniliprole resulted in reduced fecundity, fertility, and predation upon soybean aphid, *Aphis glycines* (Matsumura) (He *et al.* 2019). Therefore, chlorantraniliprole can affect some predators, but the hazard to *E. annulipes* appears low.

Diamide insecticides, like chlorantraniliprole and cyantraniliprole, are expected to increase in use up to 4.8% until 2025, and chlorantraniliprole and cyantraniliprole are responsible for 80% of the diamide market (Frost & Sullivan, 2022). Diamides act as selective ryanodine receptor modulators in insects. Activity is primarily against lepidopteran larvae (Sparks & Nauen, 2015),

but cyantraniliprole is also active against aphids and whiteflies (Barry *et al.* 2014). Cyantraniliprole tested at label application rates against cotton pests caused mortality of *E. annulipes* nymphs and reduced consumption of contaminated food (Potin *et al.* 2022), although the label rate recommended against cotton pests (75 g a.i./ha) is more than 3-fold greater than for brassicas (20 g a.i./ha). Cyantraniliprole and chlorantraniliprole differ due to the substitution of the chlorine by the cyano group (Hughes *et al.* 2004), which appears to give cyantraniliprole broader activity against several additional pest species (Selby *et al.* 2013).

Teflubenzuron, the only insect growth regulator used in this study, did not cause mortality of both DBM or *E. annulipes* in our experiments. A longer post-exposure evaluation would be required to evaluate DBM susceptibility more completely to teflubenzuron, but this growth regulator would not be expected to harm adult earwigs. Our major focus was on the predation of DBM larvae when the predator was confined on teflubenzuron dry residues, and the results indicate no negative impact.

In summary, *E. annulipes* adults present high survivorship when exposed to different insecticides recommended for the cultivation of brassicas. Exposure to spinosad, indoxacarb, chlorantraniliprole and azadirachtin cause a delay in the first egg batch production, but with an increase in egg laying and no effects on percent egg hatch. Predation by *E. annulipes* on DBM larvae did not compromise by the exposure to insecticides, suggesting good potential of integrating application of biological control through *E. annulipes* with insecticides in Brassica production.

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Table 1. Insecticides, chemical groups, mode of action (MoA), maximum label rates against *Plutella xylostella* tested, and other target pests in brassica crops (AGROFIT 2022)

Insecticides/Market	Chemical group (MoA) ¹	Maximum label dose	Company	Target pest species ²
Chlorantraniliprole 200 SC (Premio)	Diamide (28)	7.5 mL/100L	FMC	<i>Px, Tni</i>
Cyantraniliprole 100 SC (Benevia)	Diamide (28)	100 mL/ha	FMC	<i>Px, Tni, Mp, Bt, Lh</i>
Deltamethrin 25 EC (Decis)	Pyrethroid (3A)	30 mL/100L	Bayer	<i>Px, Bb, Tni, Ds, Am, Ai</i>
Spinosad 480 SC (Tracer)	Spinosyns (5)	100 mL/ha	Dow AgroScience	<i>Px, Bb, Tni, Am, Ai, Hp</i>
Indoxacarb 300 WG (Rumo)	Oxadiazines (22A)	10 g/100L	FMC	<i>Px, Tni</i>
Methomyl 215 EC (Lanate)	Carbamate (1A)	100 mL/100L	FMC	<i>Px, Bb, Am</i>
Azadiracthin 12 SC (Neem)	Tetranortriterpenoid (UKN)	300 mL/100L	UPL	<i>Px, Bb</i>
Teflubenzuron 150 EC (Nomolt)	Benzoylureas (15)	25 mL/100 L	BASF	<i>Px, Tni, Hp, Am</i>

¹Insecticides mode of action after the Insecticide Resistance Action Committee.

²Target species in brassica crops: Ai, *Agrotis ipsilon*; Am, *Ascia monuste orseis*; Bb, *Brevicoryne brassicae*; Bt, *Bemisia tabaci*; Dp, *Diabrotica speciosa*; Hp, *Hellula phidilealis*; Lh, *Liriomyza huidobrensis*; Mz, *Myzus persicae*; Px, *Plutella xylostella*; Tni, *Trichoplusia ni*.

Table 2. Effects of residual exposure to insecticide-treated leaf discs on adult ring-legged earwig *Euborellia annulipes* survival, time to first oviposition, egg production, and egg hatching rate

Insecticide	Survival (%) ^{ns}	Time to first oviposition (days) ¹	First egg batch (no. of eggs) ¹	Hatched nymphs (%) ^{ns}
Control	100.0	4.6 ± 0.7 b	43.5 ± 6.1 b	84.3 ± 4.2
Azadiractin	98.0 ± 2.0	8.6 ± 1.0 a	56.1 ± 8.0 a	85.2 ± 7.9
Cyantraniliprole	98.0 ± 2.0	6.0 ± 1.5 b	29.2 ± 7.0 b	80.4 ± 13.4
Chlorantraniliprole	100.0	8.0 ± 0.8 a	59.6 ± 9.0 a	80.4 ± 11.2
Deltamethrin	100.0	6.2 ± 1.0 b	35.5 ± 7.9 b	87.7 ± 3.8
Indoxacarb	100.0	8.7 ± 0.4 a	55.4 ± 7.5 a	90.3 ± 3.6
Methomyl	100.0	5.9 ± 1.3 b	45.1 ± 7.8 b	89.3 ± 4.2
Spinosad	98.0 ± 2.0	9.1 ± 0.6 a	62.9 ± 4.9 a	89.9 ± 3.4
Teflubenzuron	100.0	5.1 ± 0.9 b	35.8 ± 6.7 b	84.9 ± 7.1

¹Means ± SEM followed by the same letter within a column are not significantly different

(Scott-Knott test, $\alpha = 0.05$);

^{ns} = Non-significant

Table 3. Mortality of *Plutella xylostella* larvae exposed to different insecticides in the presence or absence of the predatory earwig, *Euborellia annulipes*, and number of larvae consumed by the earwigs (x/10) over 24 h

Treatments	Predator absent	Predator present	
	% mortality	% mortality	No. Larvae consumed
Control	0.0 ± 0.0 c	71.0 ± 9.7 a*	6.0 ± 0.5 a
Azadirachtin	7.0 ± 3.1 c	78.0 ± 9.2 a*	7.1 ± 1.0 a
Cyantraniliprole	6.0 ± 3.1 c	75.0 ± 6.2 a*	6.8 ± 0.6 a
Chlorantraniliprole	23.0 ± 5.0 b	82.0 ± 7.1 a*	7.0 ± 0.7 a
Deltamethrin	6.0 ± 5.0 c	73.0 ± 7.9 a*	6.3 ± 0.7 a
Indoxacarb	61.0 ± 7.4 a	90.0 ± 6.3 a*	8.2 ± 0.7 a
Methomyl	9.0 ± 6.2 c	92.0 ± 4.9 a*	7.8 ± 0.7 a
Spinosad	49.0 ± 3.5 a	66.0 ± 7.9 a*	6.3 ± 0.8 a
Teflubenzuron	0.0 ± 0.0 c	77.0 ± 9.3 a*	7.1 ± 0.8 a
Within groups	F _{8,81} = 25.5, P < 0.001	F _{8,81} = 1.3, P = 0.10	F _{8,81} = 0.9, P = 0.56
Between groups	Mortality: F _{17,162} = 33.1, P < 0.001		

¹Mean ± SEM followed by letters compare treatments within each group consisting of insecticides without and insecticides with the predator earwig, whereas asterisks (*) compare mortality for the same treatment between groups (Scott-Knott's test, $\alpha = 0.05$).

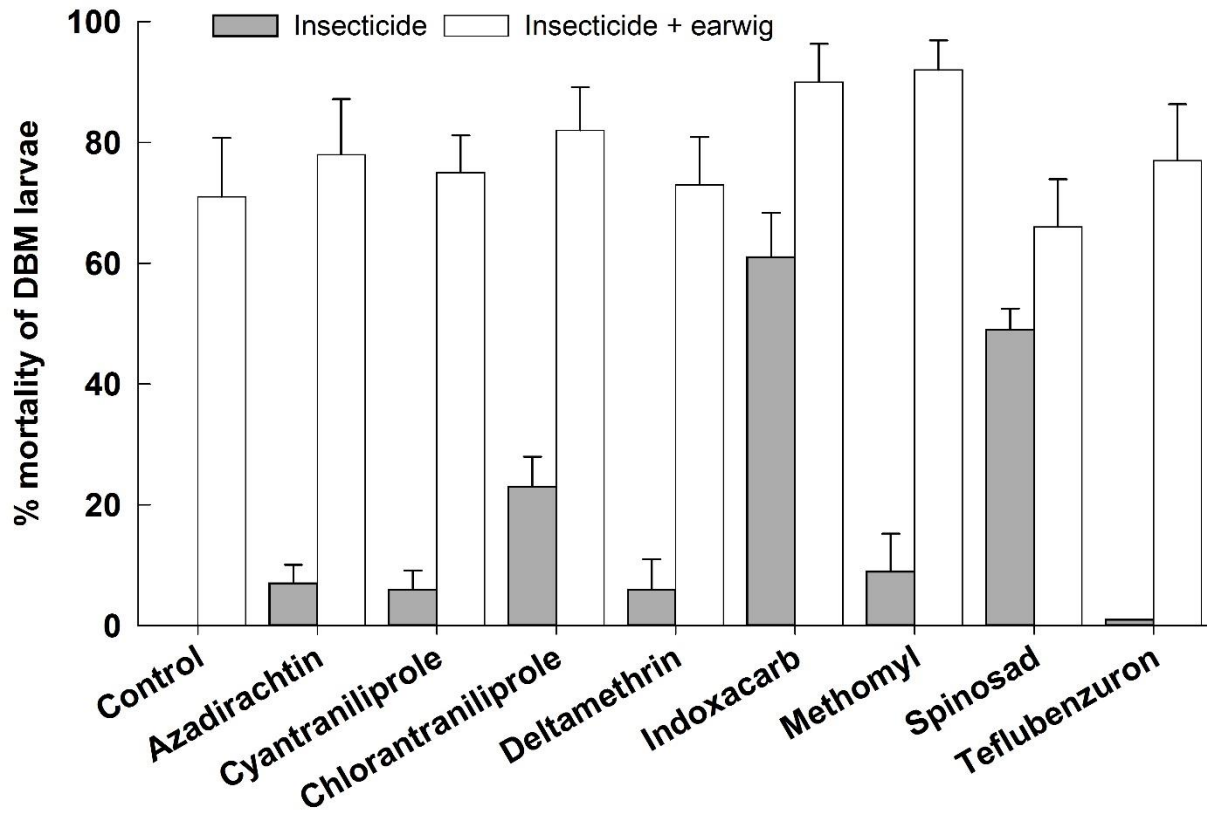


Figure 1. Additive effect of the predator *Euborellia annulipes* upon the mortality of *Plutella xylostella* larvae confined on dry residues of different insecticides over 24 h evaluation period.

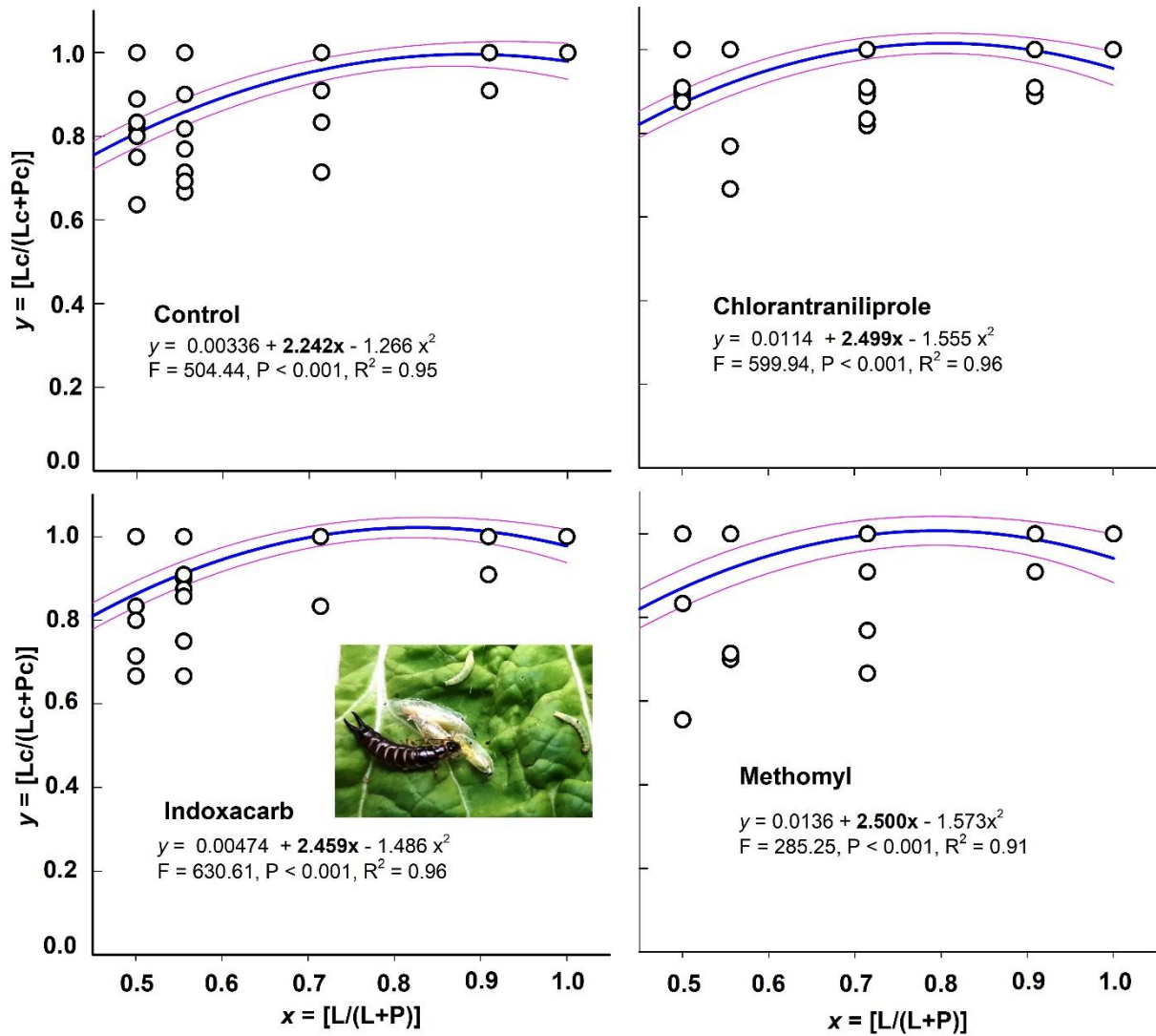


Figure 2. Preference of *Euborellia annulipes* for third instar larvae (L) vs pupae (P) of *Plutella xylostella* calculated by the ratio of prey consumed (c) $[Lc/(Lc + Pc)]$, as a function of both prey available simultaneously $[L/(L + P)]$ on insecticide-dried residues obtained on Chinese cabbage leaves. The significant slopes (in bold) indicate positive preference for larvae over pupae.

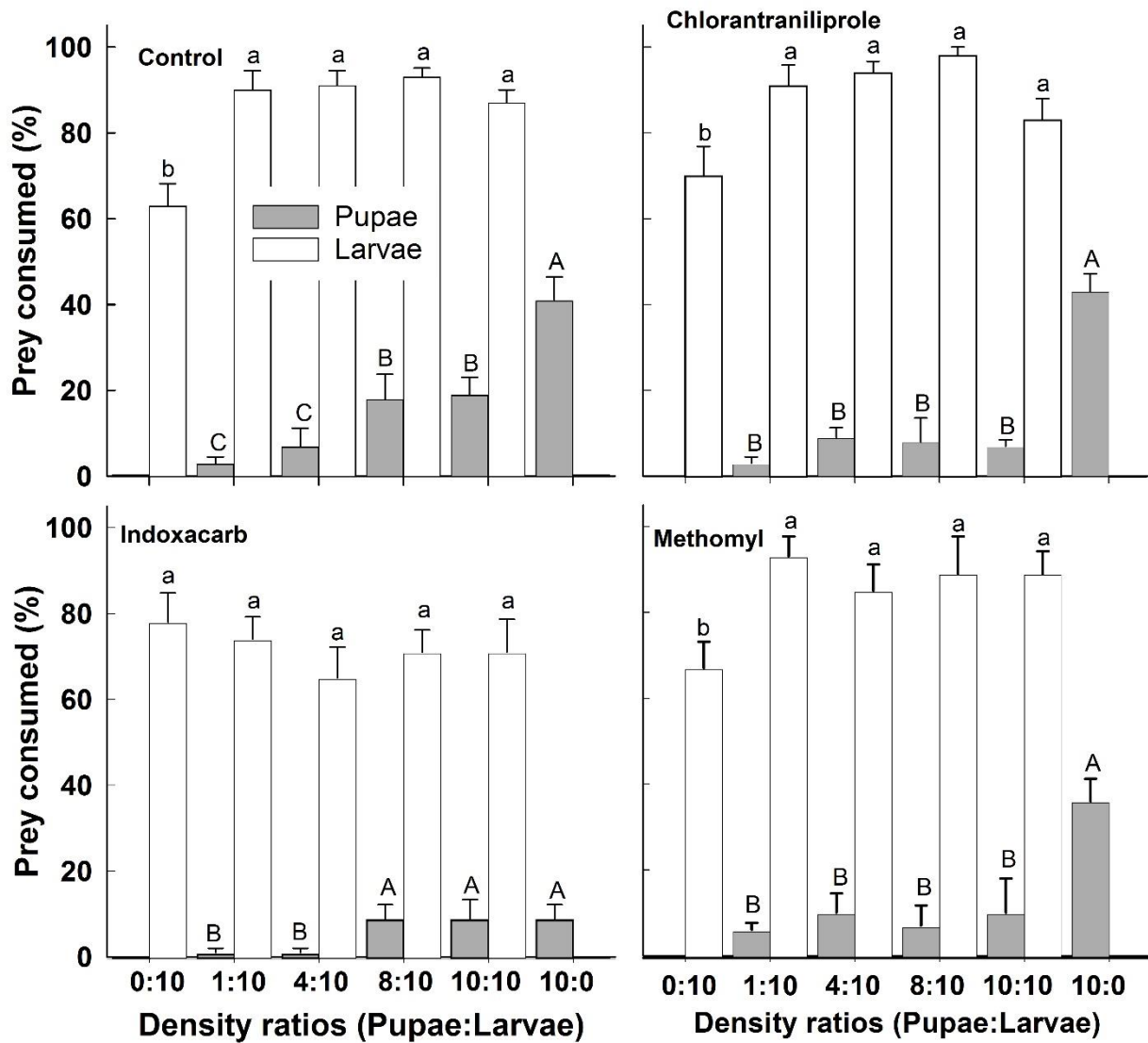


Figure 3. Mean number of pupae and larvae of *Plutella xylostella* consumed by adults of *Euborellia annulipes* within 24 h when the availability was made at different ratios. Note: Bars containing capital letters compare predation of pupae, whereas small letters compare the predation of larvae, both by the Scott-Knott test ($\alpha = 0.05$).

CAPÍTULO 3

INDOXACARB, CYANTRANILIPROLE AND THE EARWIG, *Euborellia annulipes* (LUCAS)
(DERMAPTERA: ANISOLABIDIDAE), AS OPTIONS FOR INTEGRATED DIAMONDBACK
MOTH CONTROL¹

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¹Morato, R.P. Indoxacarb, cyantraniliprole and the earwig *Euborellia annulipes* (Lucas)
(Dermaptera: Anisolabididae) as options for integrated diamondback moth control. To be
submitted.

ABSTRACT – Larvae of the diamondback moth (DBM), *Plutella xylostella* (L.), are responsible for extensive losses in brassica production worldwide. Control of DBM depends on insecticide applications, which may compromise biological control, such as that performed by the ring-legged earwig, *Euborellia annulipes* (Lucas). Brassicas' plants provide suitable habitat for ring-legged earwig residence and contact with the target pests. In this study, we investigated how the insecticides indoxacarb and cyantraniliprole, which are applied against chewing and sap-sucking pests of brassicas, affect adults and 5th instar nymphs of *E. annulipes*. Adult earwig survival was more than 90% exposed to both insecticides and control groups, but nymph survival ranged from 74% to 100%. Compared to the indoxacarb and control groups (3.4 and 3.2 days), the development of the 5th instar nymph *E. annulipes* exposed to cyantraniliprole was delayed by 16.3 days. Additionally, the females exposed to cyantraniliprole produced fewer eggs per batch and delay the first egg batch production compared to the indoxacarb and control groups. Despite that, the egg-hatching rate was greater than 80% in all treatments. Female earwigs confined on indoxacarb- or cyantraniliprole-treated or untreated plants plus DBM larvae maintained similar survival and consumption of DBM larvae. The results sublethal effects of cyantraniliprole on nymphs and adult earwigs, while indoxacarb was harmless to the earwig. Therefore, while aiming for either conservation or applied biological control of DBM larvae using *E. annulipes*, these insecticides should be taken into consideration.

KEY WORDS: *Plutella xylostella*, integrated pest management, oxadiazine, diamide

INDOXACARBE, CIANATRANILIPROLE E *Euborellia annulipes* (LUCAS)

(DERMAPTERA: ANISOLABIDIDAE) COMO OPÇÕES PARA O CONTROLE INTEGRADO DA TRAÇA-DAS-CRUCÍFERAS

RESUMO – Larvas da traça-das-crucíferas, *Plutella xylostella* (L.), são responsáveis por perdas significativas na produção de brássicas no mundo. O controle da traça-das-crucíferas depende da aplicação de inseticidas, podendo comprometer o controle biológico, como aquele realizado pela tesourinha *Euborellia annulipes* (Lucas). Plantas de brássicas fornecem habitat adequado para a *E. annulipes*, abrigo e contato com pragas alvo. Neste estudo, foi testado como adultos e ninfas de 5º instar respondem a indoxacarbe e ciantraniliprole, inseticidas comumente utilizados para controle de pragas sugadoras e mastigadoras encontradas nos cultivos de brássicas. Independentemente do tratamento, a sobrevivência de tesourinhas adultas foi superior a 90% entre os inseticidas e o controle, enquanto que a sobrevivência de ninfas variou de 74% e 100%. Tesourinhas de 5º instar expostas a ciantraniliprole atrasaram o tempo de desenvolvimento (16.3 dias) comparado com indoxacarbe e tratamento controle (3.4 e 3.2 dias). Além disso, fêmeas expostas a ciantraniliprole demandaram mais tempo para realizar a primeira oviposição e produziram menos ovos por postura, comparado a indoxacarbe e controle. Apesar disto, a taxa de eclosão dos ovos foi superior a 80% em todos os tratamentos. Fêmeas de *E. annulipes* confinadas em plantas tratadas e não-tratadas com adição de larvas de *P. xylostella* mantiveram sobrevivência e consumo da praga similares. Os resultados indicam efeitos não-letais de ciantraniliprole em ninfa e adultos de *E. annulipes* e indoxacarbe inofensivo a tesourinha. Portanto, estes inseticidas podem ser considerados quando a objetivar-se a controle biológico aplicado ou conservativo de lagartas *P. xylostella* com *E. annulipes*.

PALAVRAS CHAVE: *Plutella xylostella*, manejo integrado de pragas, oxadiazina, diamida

Introduction

The diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae), is considered the main pest of brassicas' crops and is remarkably recognized for its ability to cause yield loss to these crops (Saeed *et al.* 2019, Jaleel *et al.* 2019). The presence of this pest in brassica crops is reported in several countries. Characteristics inherent to this species, such as a high dispersal rate, a high number of annual generations, and oligophagous feeding habit upon different species of cultivated and non-cultivated brassicas, make the control of the DBM very difficult (Gu *et al.* 2010, Furlong *et al.* 2013). In addition, the control of this pest occurs, for the most part, through the use of synthetic insecticide applications, commonly resulting in continuous exposure of the pest to insecticides that, associated with its characteristics, promote the development of resistance to insecticides. Resistance to more than 100 active ingredients has been reported for DBM (Mota-Sanchez & Wise 2023). As a result, the annual cost of controlling DBM is around 5 billion US dollars (Saeed *et al.* 2010). Therefore, it is essential to pinpoint integrated methods that help control DBM. Among the practices the integration of biological control plays a fundamental role in pest control strategies based on sustainable practices (Naranjo *et al.* 2015, Overton *et al.* 2023).

Thus, control of DBM requires attention from integrated pest management (IPM) practitioners and producers. Pest control in brassica cultivation is highly dependent on applications of synthetic insecticides (Grzywacz *et al.* 2010, Liu *et al.* 2014, Zalucki *et al.* 2105) targeting DBM and other pest species. The broad adoption of synthetic insecticides to control pests of brassicas has been questioned, mainly regarding the impacts on non-target species and pest resistance (Stark *et al.* 2007), besides the potential effects on the environment and contamination of fresh vegetables for human consumption. The adoption of IPM to manage pest problems aims to reduce the abusive use of synthetic insecticides by applying a set of practices. The preservation of the natural enemies in Brassica's ecosystems is among these practices to enhance their ecological service through

biological control of the pest reducing the insecticide applications. Despite the common incompatibility of synthetic insecticides and natural enemies, there is a way of combining both when using selective insecticides that are more toxic to pests than to natural enemies (Torres & Bueno 2018).

The ecosystem composed of brassica species harbors a diversity of insects, among which the presence of natural enemies has promising characteristics for pest control. Several entomophagous species play a role in brassica's pest suppression, including earwigs (Eigenbrode *et al.* 1996, Silva-Torres *et al.* 2009, Vacari *et al.* 2013, Rana *et al.* 2019). The predatory ring-legged earwig, *Euborellia annulipes* (Lucas) (Dermaptera: Anisolabididae), is considered a natural enemy of the DBM, with predation upon larvae and pupae of this pest (Ribeiro & Gontijo 2017, Nunes *et al.* 2018, Morato *et al.* 2023). The predatory voracity of the ring-legged earwig promotes this species as a potential biological control agent for the DBM. In addition, ring-legged earwigs can prey on other key brassica pests, such as aphids, which are associated with different stages of the development of brassica crops (Nunes *et al.* 2020, Morato *et al.* 2023).

The presence of the ring-legged earwig throughout the brassica's developmental stages increases the likelihood of its exposure to the different insecticides used to control not only DBM but also other pests. Therefore, it is essential to identify the effects that insecticides may have on the earwigs, including possible sub-lethal effects and the predatory behavior of the earwig. Insecticides that cause minimal impact on the natural enemy are welcome (Anjum & Wright 2016, Lira *et al.* 2019). Thus, we hypothesized that the insecticides indoxacarb and cyantraniliprole, which offer control of brassica pests of different feeding habits (i.e., chewing and sucking sap, respectively), do not harm the ring-legged earwig using the Chinese cabbage plants. Previous studies have reported that the ring-legged earwig survives several active ingredients (Potin *et al.* 2022, Morato *et al.* 2023). Thus, the present study assessed the effects of these two insecticides on

the survival, development, and reproductive characteristics of *E. annulipes*, and determined the integration of chemical and biological control.

Material and Methods

The Ring-Legged Earwig, *Euborellia annulipes*. The earwig colony has been maintained in the Laboratory of Biological Control of the Universidade Federal Rural de Pernambuco (UFRPE), Recife, Pernambuco State, Brazil, since 2019. This colony was established from field-collected individuals and maintained following the procedures detailed by Potin *et al.* (2022). All earwig stages were reared using transparent plastic containers (13 × 20 × 7 cm in H × L × W), which were kept on a shelf covered by a black curtain to avoid direct light over the 12h light period. In addition, 8–10 layers of double sheet toilet paper (Personal Vip™, Bragança Paulista, São Paulo, Brazil) were added at the bottom of the containers as hiding and oviposition substrate. The paper sheets were moistened every two days with tap water and were replaced once a week. Each rearing container held 50-60 nymphs or 40 adults. Adults were reared at a ratio of 4 females: 1 male. The insects were fed *ad libitum* with a dry diet after Silva *et al.* (2009), which contained chicken feed (35%), wheat bran (26%), yeast (22%), powdered milk (15%), and the antimicrobial Nipagin® (2%) (Ueno Fine Chemicals Ind, Ueno, Japan). To avoid contact of the diet with the moistened paper sheets, it was placed in plastic caps (10 × 3 mm in diam × H) and replaced when necessary. Adult cages were observed daily for the presence of females and egg batches. Due to the parental care, the egg batches were transferred with the female to Petri dishes (8 × 1.5 cm in diam × H). The eggs were placed on 2 × 2 cm moistened paper sheets inside the dishes. Females were maintained with the egg batch and hatched nymphs for up to 3 days after eclosion. Next, females were returned to their original containers, while nymphs were transferred to new rearing containers with the same diet and reared to the adult stage.

The Diamondback Moth, *Plutella xylostella*. To establish the DBM colony, pupae were donated by the Laboratory of Insect-Toxic Interactions of UFRPE. This colony is a mix of individuals from collections made at Agreste region of Pernambuco State. The colony was maintained as described by Lira *et al.* (2019). Briefly, DBM larvae were reared in plastic containers (20 × 12 × 10 cm L × W × H) fed on collard leaves [*Brassica oleracea* L. var. *acephala* (Brassicaceae)] harvested from plants cultivated without insecticide application. Collard leaves were provided and replaced when needed as larvae grew. Pupae were transferred daily to 80-mL plastic pots until adult emergence. DBM adults were released inside rearing cages made of 3-L transparent plastic pots with a 10 cm diameter screw cap lid. The cage contained one 6 cm diameter opening on the side covered with anti-aphid mesh screen to allow ventilation and a 2 cm diameter opening on the bottom. An 8 cm diameter leaf disc cut from a fresh collard leaf used for oviposition was placed over a moistened filter paper of the same diameter laid inside the cap lid. Then, the cage was inverted over the cap lid holding the leaf disc and then, closed. Adults were released through the opening made on the cage bottom. The opening was closed with a cotton ball with the side of the ball left inside the cage moistened with a solution of 20% honey:water (v/v) as food for the adult moths. The collard discs were replaced every day and placed over a moistened towel paper sheet in the bottom of the rearing container for larvae. After hatch, larvae were fed by moving the old collard leaf holding the larvae to the top of a new collard leaf and cleaning the cage.

Chinese Cabbage Plants, *Brassica pekinenses*. Seeds of Chinese cabbage plants [*Brassica rapa* L. var. *pekinenses* (Brassicaceae)] produced by Isla[®] (Porto Alegre, Rio Grande do Sul, Brazil) were ordered from local market. Plant seedlings were grown in Styrofoam[™] trays filled with a 2:1 mixture of humus and soil. Seedlings at the 3-leaf stage were transplanted to 2 L plastic pots filled with sandy soil + humus (8:2) and 10 g of NPK fertilizer (10-10-10) per pot and watered daily.

Potted plants were enclosed in cages in a greenhouse and when reached 5-6 fully expanded leaves (≈ 15 cm height), they were used in the bioassays.

Toxicity of Indoxacarb and Cyantraniliprole to *Euborellia annulipes*. Dry residues of indoxacarb and cyantraniliprole were obtained by applying 2 mL of the insecticide solution split on bottom and lid of the Petri dishes (9×1.5 cm in diam \times H). Among the registered insecticides assayed by Morato *et al.* (2023), indoxacarb caused the greatest mortality of *P. xylostella* larvae, whereas cyantraniliprole is recommended against sap-sucking pests, such as aphids and whiteflies. The insecticides were applied at maximum rates recommended against *P. xylostella* in Brazil [Indoxacarb 300 WG (10g per 100L of water); cyantraniliprole 100 SC (100 mL per ha at rate of 800 L of water)], plus addition of 0.05% v/v the surfactant WilFix[®] (30 g/L, CharmonDestyl, Valinhos, São Paulo, Brasil). Control group consisted only of treatment with surfactant diluted into water. After treatment of the plates, they were left to air dry in an exhaustion chamber Nalgon mod. 3700 (Nalgon Equipamentos Científicos, Itupeva, SP, Brasil) for ≈ 2 hours. The bioassay with 5th instar earwig nymphs was carried out with 10 replications, i.e., plate dishes, with five nymphs per plate ($n = 50$ nymphs per treatment). The mortality was tallied 48 hours after confinement. The surviving nymphs were transferred to clean rearings containers and monitored assessed until the molt to adult stage.

The bioassay with earwig adults used males and females with one individual per plate to avoid cannibalism of moribund individuals. Each treatment evaluated 30 replications (15 males and 15 females). Mortality was tallied 48 h after confinement. From the surviving adults, 10 pairs were formed individually in clean rearing containers supplied with diet and paper for hiding and oviposition. The couples were monitored for egg laying with time for first egg batch produced and number of eggs per batch and eclosion of nymphs recorded.

Indoxacarb Toxicity to *Euborellia annulipes* Conveyed by Treated Plant and DBM Larvae.

Toxicity of indoxacarb is enhanced with its ingestion and enzymatic action in the midgut (Wing *et al.* 2000). Thus, this bioassay was carried out in order to assess the effect of indoxacarb mediated by ingestion of contaminated host plant and prey. Indoxacarb solution was prepared using the maximum recommended field rate to spray brassica's crop against *P. xylostella* (Indoxacarb 300 WG, 10 g per 100L of water), plus 0.05% v/v surfactant WilFix[®]. Chinese cabbage leaves were treated by dipping 9 cm diameter leaf discs into the insecticide solution for approximately 20 seconds, let to dry for \approx 2 hours, and placed into glass Petri dishes (9 \times 1.5 cm in Diam \times H). Ten larvae of 3rd-instar of *P. xylostella* were released on the leaf discs and let to feed for 24 h before offering to the earwig.

To expose the indoxacarb-treated host plant plus prey, 4 cm diameter leaf discs were cut from Chinese cabbage leaves and dipped into insecticide solution as described before. After air dried, the leaf discs were placed into Petri dishes and infested with 10 DBM larvae previously fed for 24 hours indoxacarb-treated Chinese-cabbage leaves. One female *E. annulipes* was released per plate with 15 replications per treatment that consisted of indoxacarb-treated prey and host plant, and a control group. Earwig mortality, DBM larvae and signal of plant consumption were assessed 24 hours after confinement.

Mortality of DBM by *Euborellia annulipes* on Chinese Cabbage Plants. Chinese cabbage plants with 5 to 6 full expanded leaves (ca. \approx 15 cm tall) grown in the greenhouse were sprayed with indoxacarb using the maximum recommended rate to spray brassica's crops against *P. xylostella*, as previously described. Insecticide solution was applied to the plants using a 1.25 L hand-sprayer (Guarany[®], São Paulo, SP) regulated for a flow pressure of 2.8 Kgf per cm², with liquid spray being applied to run off. The plants were transferred to laboratory \approx 2 hours after spraying (Fig. 1S). After

spraying, plants were confined into cages composed of 2.7-L plastic pots (20.5 × 15.5 in diam × H) containing two side openings with ~16 cm² diameter each sealed with anti-aphid screen.

The experimental design consisted of six treatments combining earwig and indoxacarb against DBM larvae and pupae, and one control group: T1: DBM larvae and pupae + earwig; T2: DBM larvae and pupa (control group); T3: DBM larvae and earwig; T4: DBM larvae and pupae, earwig and indoxacarb; T5: DBM larvae, earwig and indoxacarb; and T6: earwig without prey. DBM infestation consisted of 10 larvae at 3rd-instar per plant in the treatments T1, T2, T3, T4 and T5 and 5 pupae in the treatments T1, T2 and T4. The treatments combining release of the earwig T1, T3, T4, T5 and T6 received one adult female earwig between 3 and 10 days old. Each treatment was represented by 10 replications consisting of one caged plant infested DBM or earwig according to the assigned treatment. The earwig survival, mortality of DBM larvae and pupae, number of DBM pupae formed and adults of DBM emerged were tallied 120 hours after setting up the experiment.



Figure 1S. Experimental cages to test mortality of *Plutella xylostella* when interacting the predator ring-legged earwig and indoxacarb. A: Chinese cabbage plant; B: ring-legged earwig *Euborellia annulipes*; C: view of cages under evaluation.

Statistical Analysis. Data analyses were performed using the Software SAS[®] (SAS Institute 2002) or SigmaStat[®] 14.0 (SigmaPlot 2013), as follow. Nymphs survival (%) and developmental time required transformation by $\log(x+1)$ and \log , respectively, to meet normality (Shapiro-Wilk's test) and homogeneity of variance (Bartlett's test) prior ANOVA analysis. Mortality of nymphs was censored daily until they molt to adult stage and the data were used to build survival curves from insecticide exposure to molting to adult stage using the method of Kaplan-Meier and survival curves pair-wise compared by Log-Rank's test ($\alpha = 0.05$) through the SigmaStat[®] 14.0 (SigmaPlot 2017)..

Time required by female to produce the first egg batch, number of eggs and survival were submitted to ANOVA and treatment means separated by Tukey HSD's test ($\alpha = 0.05$).

The data of DBM larvae preyed by earwig and survival of the earwig after preying upon DBM larvae fed on Chinese cabbage leaves treated with indoxacarb cyantraniliprole were compared between treated and control groups by t-test (Proc ttest of SAS). Further, the survival of the earwig was compared by the 95% confidence interval overlapping rule (Proc means of SAS).

Data from combination of indoxacarb and the earwig effects upon mortality of larvae, pupae of DBM, and larvae of DBM successfully molting or reaching adult stage were submitted to ANOVA, followed by Tukey HSD's test for mean comparisons ($\alpha = 0.05$) and 95% confidence interval overlapping rule (Proc means of SAS).

Results

Toxicity of Indoxacarb and Cyantraniliprole to *Euborellia annulipes*. Significantly fewer earwig nymphs survived to cyantraniliprole dry residues (74%) compared to those from indoxacarb and control treatments (100%) ($F_{2,27} = 15.70$, $P < 0.001$; Fig. 1). Nymphs that had contact with dry residue of cyantraniliprole required more time to reach the adult stage (16.3 days) compared to nymphs confined on dry residues of indoxacarb and control groups (3.4 and 3.2 days; $F_{2,27} = 147.65$, $P < 0.001$; Fig. 1). Furthermore, the survival rate at reaching adult stage (% of adults emergence) differed among treatments ($\chi^2 = 7.65$, $df = 2$, $P = 0.021$), with 61.4%, 88.1%, and 90.1% of adults obtained in the treatments cyantraniliprole, indoxacarb, and control group, respectively (Fig. 2).

Adults of earwig survival when caged with dry residues of indoxacarb and cyantraniliprole for 48h resulted in significant effect among treatments ($F_{2,87} = 7.20$, $P = 0.0013$), but not between males and females ($F_{1,80} = 1.88$, $P = 0.3736$). Thus, the re-analysis disregarding gender effect, ratified the effect among treatments ($F_{2,87} = 7.25$, $P = 0.0012$), with an average survival of 96%

when confined on cyantraniliprole residues compared to 100% survival compared to indoxacarb and control groups.

Adult earwigs reared after confinement on dry insecticide residues had the time to produce the first egg batch ($F_{2,27} = 3.51$, $P = 0.0452$) and the number of eggs per batch ($F_{2,27} = 9.90$, $P < 0.001$) affected by the treatments (Fig. 3). Earwig females exposed to cyantraniliprole required more time (about one week) to produce the first egg batch and produced egg batches with $\approx 50\%$ fewer eggs compared to females exposed to indoxacarb and control groups. Despite that, the eggs laid showed similar rate of hatching ($F_{2,25} = 0.02$, $P = 0.979$), and above 80% among insecticides and control groups.

Indoxacarb Conveyed by Treated DBM Larvae to *Euborellia annulipes*. Earwig females showed survival greater than 93.3% and similar among treatments ($F_{3,53} = 0$, $P = 1$). Additionally, the number of DBM larvae consumed by earwig females during 24h of confinement was similar between larvae fed either indoxacarb-treated or untreated leaves (t-test = -1.62, $P = 0.12$). One female earwig consumed, on average (95% confidence intervals), 9.1 (8.2 – 9.8) and 9.8 (9.4 – 10.2) DBM larvae fed on indoxacarb-treated and -untreated Chinese cabbage leaves, respectively.

Mortality of DBM by *Euborellia annulipes* on Chinese Cabbage Plants. Mortality of DBM larvae ($F_{3,36} = 9.22$, $P < 0.001$) varied in the presence of earwig and indoxacarb (Fig. 4A-B), whereas pupae mortality was similar between treatments ($F_{1,18} = 4.20$, $P = 0.053$). The release of the earwig resulted in mortality of DBM larvae greater than 75% and near 100% when combined with indoxacarb (Fig. 4A). Additionally, earwig caused similar mortality of DBM pupae 98% (95% CI; 93.48% – 102.52%) and 84% (95% CI; 69.22% – 98.78%) with indoxacarb or without indoxacarb application, respectively (Fig. 4B).

The release of earwig and its combination with indoxacarb caused significant reduction in formation of DBM pupae ($F_{4, 45} = 36.58, P < 0.001$). In the absence of both earwig and indoxacarb, 65% of the DBM larvae reached the pupal stage (Fig. 4C). Nevertheless, the release of earwig limited formation of DBM pupa to 23%, independent of the stage of the DBM infesting the plants; and the combination of earwig with indoxacarb resulted in 0% of formation of DBM pupae. Furthermore, the combination of earwig and indoxacarb against DBM significantly resulted in the lack of adults ($F_{4, 44} = 115.44, P < 0.001$). About 46% of DBM larvae and pupae reached adult stage in the absence of earwig and indoxacarb (Fig. 4D). About 8% of DBM larvae confined with *E. annulipes* reached the adult stage. In the other treatments consisting of DBM larvae or pupae with earwig release and its combination with indoxacarb, near 0% or 0% of DBM pupae reached adult stage.

Discussion

An effective IPM program requires multiple pest control tactics, among them the conservation of natural enemies occurring either naturally or from releases. Conservation of natural enemies in crop ecosystems will require the application of compatible insecticides with the natural enemies (Zalucki *et al.* 2015, Torres & Bueno 2018, Bordini *et al.* 2021). One of the basic steps to this desired IPM is to have available effective insecticides to recommend against the target pest at the same time with low or null impact against key natural enemies, not only against the target pest but also against secondary pest, reducing the risk of pest outbreaks.

Nymph and adult earwigs exhibited relatively high survival rates and reproductive performance when exposed to dry residues of cyantraniliprole and indoxacarb, despite the observed sublethal effects of cyantraniliprole. The combination of these insecticides and earwigs was additive, aiming at the control of DBM larvae and pupae. Considering that pest management of

brassicas crops is heavily dependent on insecticide applications, these results offer options for integration with either conservation or applied biological control with ring-legged earwig, with these insecticides targeting different pest species of brassicas with different feeding habits. Cyantraniliprole is recommended for spraying brassicas crops against whiteflies, aphids, leafminers, and lepidopterans such as DBM and looper's larvae, while indoxacarb is applied against lepidopteran larvae. Furthermore, these insecticides offer different modes of action (i.e., diamide group 28 and oxadiazine group 22), which is highly relevant regarding DBM control and DBM insecticide resistance mitigation by rotation of insecticides using different modes of action. DBM is the pest species with the highest reported cases of resistance to insecticides (ca. 1022 cases), including all available insecticide modes of action (Mota-Sanchez & Wise 2023).

Insecticide applications against DBM commonly include diamides, spinosyns, carbamates, pyrethroids, and others (Khaliq *et al.* 2007, Biondi *et al.* 2012, Li *et al.* 2014, Zhang *et al.* 2016). Results from studies with recommended insecticides belonging to these groups vary the selectivity outcome from incompatible to compatible with naturally occurring parasitoids and predators in the brassicas fields (Williams *et al.* 2003, Bostanian *et al.* 2005, Gentz *et al.* 2010, Quesada & Sadof 2019, Lira *et al.* 2019, 2023), including compatible ones with the ring-legged earwig (Morato *et al.* 2023). The conservation of natural enemies in brassicas' crops is an important strategy of IPM (Furlong *et al.* 2008, Grzywacz *et al.* 2010, Lira *et al.* 2019). Numerous species of natural enemies occur in the brassica agroecosystems (Sarfraz *et al.* 2005), but are often negatively impacted by applications of non-selective insecticides (Bommarco *et al.* 2011, Li *et al.* 2015). This incompatibility results in the outbreak of secondary pest species and the survival of DBM individuals carrying resistant traits for next generations, inflating the cases of insecticide resistance and consequently, yield losses.

Nymphs and adults responded differently to the studied insecticides. Cyantraniliprole caused sublethal effects on nymphs, while indoxacarb did not. Indoxacarb belongs to the oxadiazine group and is a voltage-dependent sodium channel blocker acting on the nerve system after metabolization, suggesting a broad spectrum of action (Wing *et al.* 2000). Nevertheless, insect contamination by indoxacarb occurs predominantly by ingestion, with lesser contamination across the cuticle, thus becoming selectively more toxic to lepidopteran larvae. It is also considered a pro-insecticide, becoming active when metabolized to the active form via specific hydrolase enzymes in the midgut tissues and fat bodies (Wing *et al.* 1998, 2000, 2005). Reduced survival of *E. annulipes* 3rd-instar nymphs was found by Potin *et al.* (2022) when the nymphs were fed indoxacarb-treated prey, corroborating the indoxacarb mode of action. Indoxacarb is a pro-insecticide with reduced risks for other natural enemies such as *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), *Hippodamia convergens* (Guérin-Ménéville) (Coleoptera: Coccinellidae), and *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae) (Tillman & Mullinix 2004, Roubos *et al.* 2014). Furthermore, larval stages of the lady beetles *Harmonia axyridis* (Pallas) and *Coccinella undecimpunctata* L. (both Coleoptera: Coccinellidae), *Chrysoperla carnae* (Stephens) (Neuroptera: Chrysopidae), and *Paedorus alfieri* Koch (Coleoptera: Staphylinidae) delayed development and lethal effect resulting in a reduction on survival (Galvan *et al.* 2006, Gesraha & Ebeid 2021).

Different from indoxacarb, nymphs exposed to cyantraniliprole showed a reduction in survival and prolonged development. Furthermore, adults delayed the first egg batch production and produced fewer eggs, indicating some lethal effects for nymphs and sublethal effects for adults. A lower rate of *E. annulipes* 3rd-instar reaching adult stage after exposure to cyantraniliprole used at field rate to spray cotton crops was also found by Potin *et al.* (2022). Thus, despite the overall selectivity presented by diamides (Selby *et al.* 2013), cyantraniliprole has structural modifications

in the cyano group (Hughes *et al.* 2004), with expanded insecticidal activity against several pest species of different feeding habits (Selby *et al.* 2013). These results corroborate the variation in compatibility of cyantraniliprole with different natural enemy species compared to other diamides (Amarasekare & Shearer 2013, Mills *et al.* 2016, Jiang *et al.* 2019, Machado *et al.* 2019, Morato *et al.* 2023). Results also indicate a lack of negative impact for some natural enemies (Liu *et al.* 2014, Funderburk *et al.* 2013, Abbas *et al.* 2015). Cyantraniliprole's effect on *E. annulipes* varies with life stage, route of exposure, and to a lesser extent, with adults (He *et al.* 2019, Moreira *et al.* 2023, Potin *et al.* 2022). Therefore, the impact of cyantraniliprole on natural enemies seems to vary among species and developmental stages.

The natural enemy surviving the exposure of insecticide is the first step in integrating biological and chemical controls, but the surviving natural enemy also needs to provide mortality for the pests in the crop ecosystem (Machado *et al.* 2019, Lira *et al.* 2019, 2023). The combination of indoxacarb application and release of the ring-legged earwig in caged plants resulted in additive mortality of DBM larvae and pupae, significantly reducing pest adult emergence and reaching the expected control integration outcome.

In summary, nymphs and adults of the predatory earwig *E. annulipes* survived exposure to indoxacarb residues. In addition, the use of indoxacarb in association with the earwig applied on caged Chinese cabbage plants resulted in 100% mortality of DBM larvae or pupae, and over 90% when both larvae and pupae were available. On the other hand, the use of cyantraniliprole in association with the predatory earwig should be used with more care. The exposure of *E. annulipes* 5th-instar nymphs to cyantraniliprole resulted in a 26% reduction in adult emergence. In addition, sublethal effects of this exposure were observed as delayed development to complete nymphal stage, delayed production of the first egg batch, and a lower number of eggs produced. Thus, in conclusion, the insecticide indoxacarb shows compatibility of use in association with *E. annulipes*

to control brassica pests such as *P. xylostella*. On the other hand, even though *E. annulipes* had experienced sublethal effects when exposed to cyantraniliprole, nymph survival was over 60%, and adults produced eggs with equal viability to the control group, suggesting that the population will be sustained in the field.

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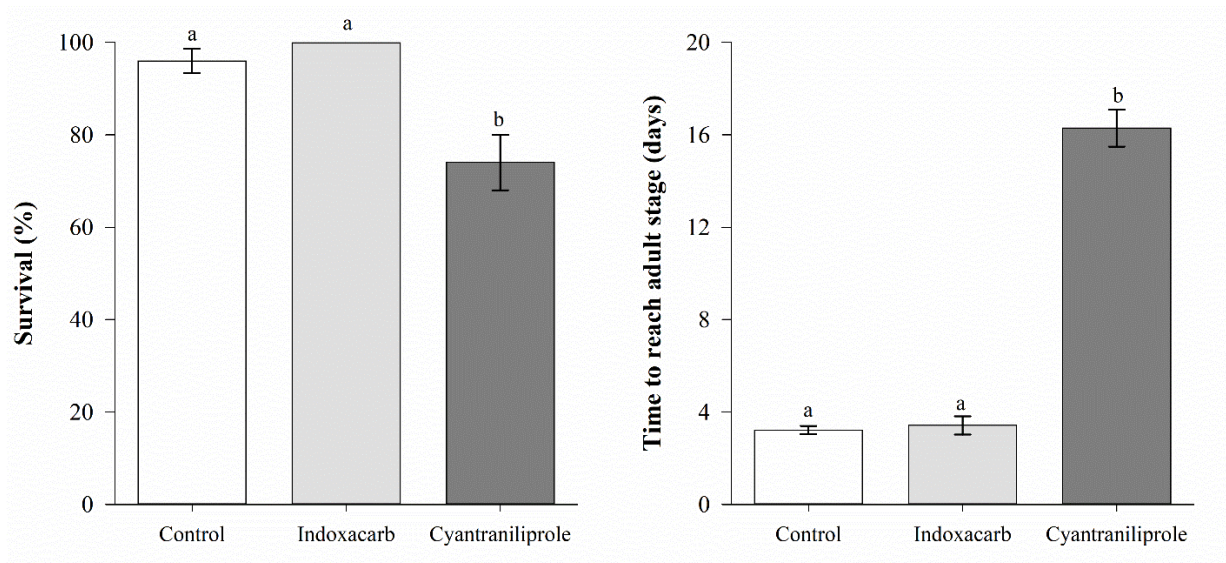


Figure 1. Survival (mean \pm SE) of 5th-instar nymphs of *Euborellia annulipes* after exposed to insecticide dried residues and control groups for 48h, and time required by the nymphs after contacting insecticide to molting to adult stage. Bars bearing different letters indicate statistical difference among treatments (Tukey HSD' test, $\alpha = 0.05$).

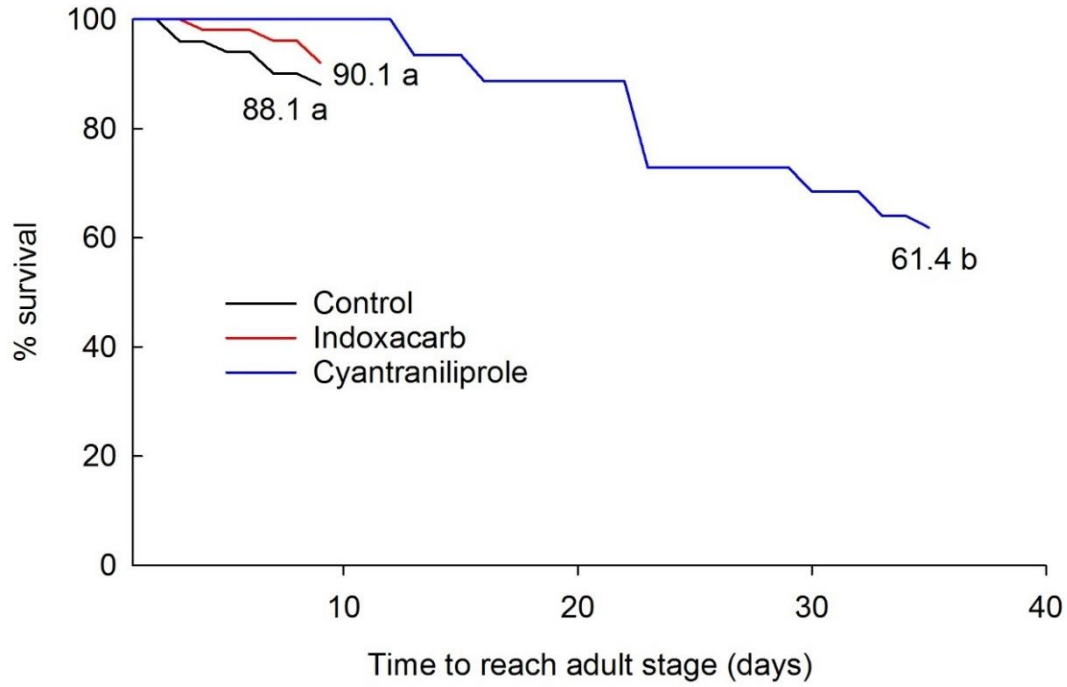


Figure 2. Survival curves for 5th-instar nymphs of *Euborellia annulipes* period between confinement on dry insecticide residues for 48h and adult emergence. Values stand for survival rate (%) and different letters indicate differences among survival curves by Log-Rank's test ($\alpha = 0.05$).

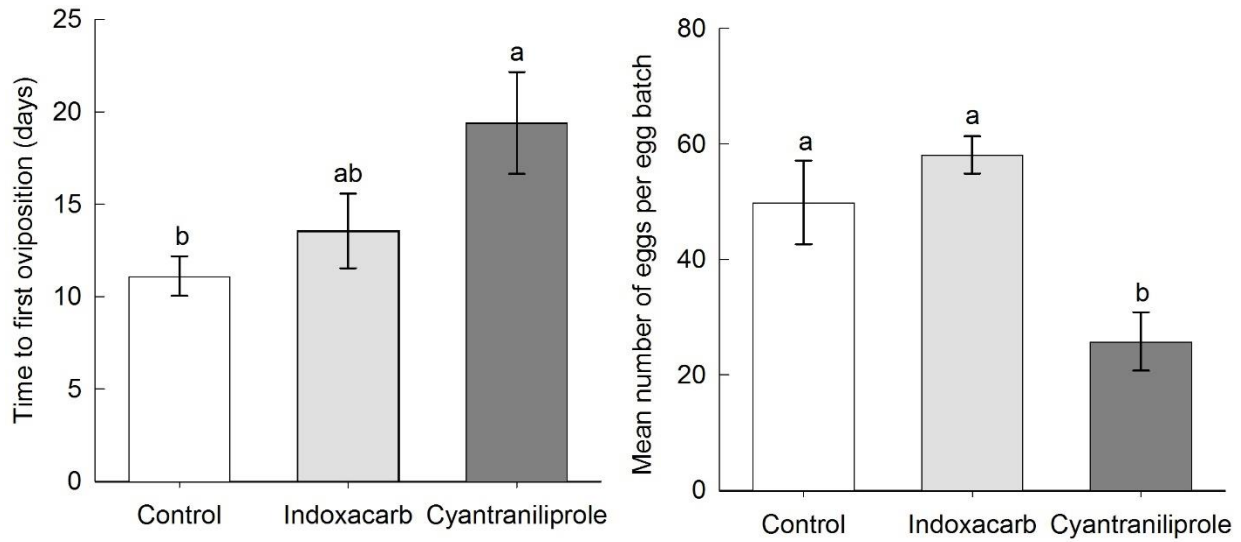


Figure 3. Time spent by *Euborellia annulipes* females to produce the first egg batch and the mean number of eggs per batch after confinement for 48h with dry insecticide residues. Bars bearing different letters indicate significance among treatments (Tukey HSD's test, $\alpha = 0.05$).

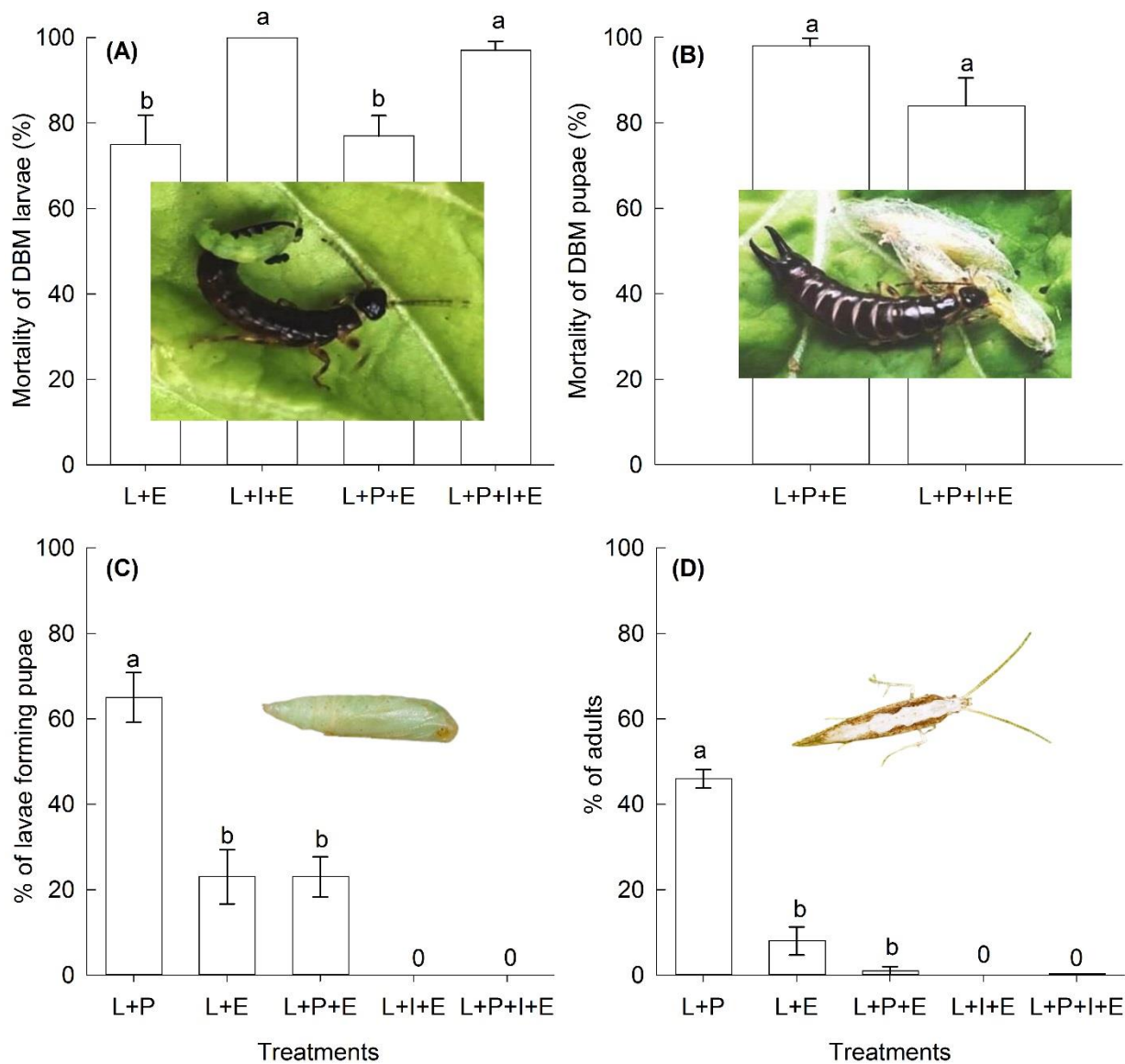


Figure 4. Predation of *Plutella xylostella* larvae (L) and pupae (P) by *Euborellia annulipes* (E) when released on Chinese cabbage plants in combination with indoxacarb (I) application. Bars bearing different letters indicate significant difference among treatments (Tukey HSD's test, $\alpha = 0.05$).

CAPÍTULO 4

CONSIDERAÇÕES FINAIS

O estudo sobre métodos complementares de controle de pragas é contínuo devido à grande dependência do uso do controle químico para o manejo de pragas. Não diferente, o cultivo de brássicas, que são hospedeiras de um vasto número de espécies-praga, mostra-se em grande parte dependente do uso de inseticidas para o manejo de pragas. Associado a isto, a frequente exposição a inseticidas promove, por sua vez, a seleção de resistência aos inseticidas utilizados, dificultando ainda mais o manejo de pragas além de perda de produção devido às falhas de controle, um exemplo comum envolvendo a traça-das-crucíferas. O desenvolvimento e a utilização de diferentes métodos, que tenham por objetivo reduzir a densidade populacional da praga, mostram-se essenciais com o intuito de promover a integração de diferentes métodos de controle, base para a implementação do Manejo Integrado de Pragas (MIP). Porém, a integração do controle químico e biológico apresenta dificuldades diante da difícil compatibilidade desses métodos de controle, uma vez que são comuns os efeitos negativos dos inseticidas sobre os agentes de controle biológico. A caracterização de inseticidas que apresentem compatibilidade com inimigos naturais relevantes para o manejo de pragas-chave das culturas abre a possibilidade de implementação de táticas de controle baseadas no MIP, com o uso de diferentes métodos de controle.

O trabalho desenvolvido demonstrou que os inseticidas utilizados no controle da traça-das-crucíferas, *Plutella xylostella*, principal praga das brássicas são compatíveis com a tesourinha predadora *Euborellia annulipes*. Os inseticidas azadiractina, clorantraniliprole, deltametrina, espinosade, indoxacarbe, metomil e teflubenzurom não ocasionaram efeitos letais sobre adultos da tesourinha predadora, não havendo redução na sobrevivência do inimigo natural e mantendo os

parâmetros reprodutivos (tempo para primeira oviposição, número de ovos e viabilidade) sem alterações significativas. O inseticida ciantraniliprole reduziu a sobrevivência de ninfas e ocasionou efeitos subletais para a reprodução dos adultos contaminados. A utilização da tesourinha predadora em associação com os inseticidas mostrou um incremento na mortalidade de *P. xylostella*, bem como a tesourinha não apresentou uma alta preferência por um estágio de desenvolvimento da praga. Estes resultados abrem a possibilidade de utilização de diferentes inseticidas oferecendo diferentes modos de ação para o controle da traça-das-crucíferas, em integração com a tesourinha predadora, permitindo a rotação de modos de ação e, com isso reduzindo a exposição prolongada da praga ao mesmo modo de ação. Desta forma, o uso contínuo de um inseticida pode ser evitado quando há várias opções para a recomendação. Os efeitos da exposição de diferentes estágios de desenvolvimento a resíduos secos dos inseticidas ciantraniliprole e indoxacarbe foram verificados, mostrando que o inseticida ciantraniliprole ocasionou efeitos letais e subletais sobre ninfas e adultos da tesourinha *E. annulipes*, ocasionando efeitos negativos no desenvolvimento das ninfas e em parâmetros reprodutivos de fêmeas. O consumo de presas contaminadas com resíduos do inseticida indoxacarbe não ocasionou efeito letal em fêmeas da tesourinha predadora, mostrando a compatibilidade deste inseticida com o inimigo. A identificação da possibilidade de integração do uso da tesourinha *E. annulipes* com inseticidas recomendados para o controle de *P. xylostella* e, de outras pragas das brássicas, abre oportunidade de implementação de táticas promissoras para o MIP das brássicas, principalmente da traça-das-crucíferas, cujo controle é dificultado pelos frequentes casos de resistência da praga aos inseticidas registrados para o seu controle.