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Junior César Somavilla

**SUSCETIBILIDADE DE POPULAÇÕES DO PERCEVEJO-MARROM
Euschistus heros (Fabricius, 1798) E DO PERCEVEJO BARRIGA-VERDE
Dichelops furcatus (Fabricius, 1775) A INSETICIDAS**

Santa Maria, RS
2020

Junior César Somavilla

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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-graduação em Agronomia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Agronomia**.

Orientador: Prof. Dr. Oderlei Bernardi

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Aprovado em 07 de fevereiro de 2020:

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Daniel Bernardi, Dr. (UFPel)

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2020

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A Deus, pelo dom da vida e por estar sempre ao meu lado em todos os momentos.

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“Ou você aumenta suas habilidades ou diminui seus sonhos, a escolha é sua.”
Jim Rohn

RESUMO

SUSCETIBILIDADE DE POPULAÇÕES DO PERCEVEJO-MARRON *Euschistus heros* (Fabricius, 1798) E DO PERCEVEJO BARRIGA-VERDE *Dichelops furcatus* (Fabricius, 1775) A INSETICIDAS

AUTOR: Junior César Somavilla
ORIENTADOR: Oderlei Bernardi

O percevejo-marrom, *Euschistus heros* (Fabricius, 1798), e o percevejo barriga-verde, *Dichelops furcatus* (Fabricius, 1775), são importantes sugadores que atacam plantas cultivadas no Brasil. O controle destas espécies é realizado principalmente com o uso de inseticidas químicos, que pertencem a poucos grupos químicos (piretroides, neonicotinoides e organofosforados). Diante disto, foram realizados bioensaios para avaliar a suscetibilidade de populações de *E. heros* e *D. furcatus* a inseticidas. No primeiro estudo, populações *E. heros* e *D. furcatus* coletadas em lavouras de produção de soja e trigo, entre 2017 e 2019 foram expostas a diferentes concentrações dos inseticidas acefato, tiametoxam, bifentrina e lambda-cialotrina em bioensaios de imersão de vagens de feijão (*Phaseolus vulgaris*). As populações de *E. heros* apresentaram baixa variação na suscetibilidade ao acefato ($CL_{50} = 172,2$ a $1.008 \mu\text{g i.a./ml}$) e tiametoxam ($CL_{50} = 28,8$ a $433,9 \mu\text{g i.a./ml}$), com razão de resistência inferior a 5,9 e 15,1 vezes, respectivamente. Em contraste, essas populações apresentaram maior variação na suscetibilidade à bifentrina ($CL_{50} = 26,7$ a $636,1 \mu\text{g i.a./ml}$) e lambda-cialotrina ($CL_{50} = 10,0$ a $636,1 \mu\text{g i.a./ml}$); razão de resistência de até 23,8 e 63,6 vezes, respectivamente. Os dados de monitoramento da suscetibilidade indicaram uma maior suscetibilidade de *E. heros* às doses registradas de acefato, lambda-cialotrina + tiametoxam e bifentrina + acetamiprido do que lambda-cialotrina. As populações de *D. furcatus* apresentaram baixa variação na suscetibilidade ao acefato ($CL_{50} = 219,2$ a $614,1 \mu\text{g i.a./ml}$), bifentrina ($CL_{50} = 62,8$ a $197,4 \mu\text{g i.a./ml}$) e lambda-cialotrina ($CL_{50} = 189,5$ a $2.538 \mu\text{g i.a./ml}$). A razão de resistência para os inseticidas foi inferior a 13,4 vezes. No segundo estudo, os inseticidas técnicos (ingrediente ativo) acefato, tiametoxam, bifentrina e lambda-cialotrina foram diluídos em acetona PA, e em bioensaios de aplicação tópica, aplicados no dorso dos insetos adultos ($2 \mu\text{l/percevejo}$). Constatou-se que as populações de *E. heros* apresentaram baixa variação na suscetibilidade ao acefato ($DL_{50} = 0,22$ a $0,69 \mu\text{g i.a./percevejo}$), bifentrina ($DL_{50} = 0,021$ a $0,10 \mu\text{g i.a./percevejo}$) e tiametoxam ($DL_{50} = 0,0046$ a $0,032 \mu\text{g i.a./percevejo}$); razão de resistência inferior a 8,2 vezes. Em contraste, houve maior variação na suscetibilidade à lambda-cialotrina ($DL_{50} = 0,073$ a $0,35 \mu\text{g i.a./percevejo}$), indicando uma razão de resistência de até 15,7 vezes. Também foram estimadas doses diagnósticas de 1,30; 0,34; 0,36 e $1,73 \mu\text{g i.a./percevejo}$ de acefato, tiametoxam, bifentrina e lambda-cialotrina, respectivamente, para o monitoramento da suscetibilidade de *E. heros*. As populações de *D. furcatus* apresentaram baixa variação na suscetibilidade ao acefato ($DL_{50} = 0,30$ a $0,53 \mu\text{g i.a./percevejo}$), tiametoxam ($DL_{50} = 0,066$ a $0,14 \mu\text{g i.a./percevejo}$) e lambda-cialotrina ($DL_{50} = 0,27$ a $0,56 \mu\text{g i.a./percevejo}$), com razão de resistência inferior a 2,1 vezes. Os resultados indicam uma menor suscetibilidade de populações de *E. heros* a bifentrina e lambda-cialotrina. As populações de *D. furcatus* tem similar suscetibilidade ao acefato, tiametoxam, bifentrina e lambda-cialotrina.

Palavras-chave: Pentatomidae. controle químico. manejo da resistência.

ABSTRACT

SUSCEPTIBILITY OF POPULATIONS OF NEOTROPICAL BROWN STINK BUG *Euschistus heros* (Fabricius, 1798) AND GREEN BELLY STINK BUG *Dichelops furcatus* (Fabricius, 1775) TO INSECTICIDES

AUTHOR: Junior César Somavilla
SUPERVISOR: Oderlei Bernardi

The neotropical brown stink bug, *Euschistus heros* (Fabricius, 1798), and the green belly stink bug, *Dichelops furcatus* (Fabricius, 1775), are important sucking pests that attack cultivated plants in Brazil. The control of these species is usually carried out with the use of chemical insecticides, which belong to a few chemical groups (pyrethroids, neonicotinoids and organophosphates). Based on this, we conducted bioassays to evaluate the susceptibility of *E. heros* and *D. furcatus* to insecticides. In the first study, populations of *E. heros* and *D. furcatus* were collected from 2017 to 2019 in soybean and wheat fields, and exposed to distinct concentrations of the acephate, thiamethoxam, bifenthrin and lambda-cyhalothrin in dip-test bioassays using fresh green bean pods (*Phaseolus vulgaris*). Field populations of *E. heros* exhibited low variation in the susceptibility to acephate ($LC_{50} = 172.2$ to $1,008 \mu\text{g a.i./ml}$) and thiamethoxam ($LC_{50} = 28.8$ to $433.9 \mu\text{g a.i./ml}$), resistance ratios less than 5.9 and 15.1-fold, respectively. In contrast, these populations had higher variation in the susceptibility to bifenthrin ($LC_{50} = 26.7$ to $636.1 \mu\text{g a.i./ml}$) and lambda-cyhalothrin ($LC_{50} = 10.0$ to $636.1 \mu\text{g a.i./ml}$); resistance ratios reaching 23.8 and 63.6-fold, respectively. Susceptibility monitoring data indicated a higher susceptibility of *E. heros* to the manufacturers field-recommended rates of acephate, lambda-cyhalothrin + thiamethoxam and bifenthrin + acetamiprid than lambda-cyhalothrin. Populations of *D. furcatus* exhibited low variation in the susceptibility to acephate ($LC_{50} = 219.2$ to $614.1 \mu\text{g a.i./ml}$), bifenthrin ($LC_{50} = 62.8$ to $197.4 \mu\text{g a.i./ml}$), and lambda-cyhalothrin ($LC_{50} = 189.5$ to $2,538 \mu\text{g a.i./ml}$). Resistance ratios for these insecticides were less than 13.4-fold. In the second study, the technical grade insecticides: acefate, thiamethoxam, bifenthrin and lambda-cyhalothrin were diluted in acetone PA, and applied topically at the dorsum of each insect (2 $\mu\text{l}/\text{stink bug}$). In this study, populations of *E. heros* presented low variation in the susceptibility to acephate ($LD_{50} = 0.22$ to $0.69 \mu\text{g a.i./stink bug}$), bifenthrin ($LD_{50} = 0.021$ to $0.10 \mu\text{g a.i./stink bug}$) and thiamethoxam ($LD_{50} = 0.0046$ to $0.032 \mu\text{g a.i./stink bug}$); resistance ratios less than 8.2-fold. In contrast, a higher variation in susceptibility to lambda-cyhalothrin was found ($LD_{50} = 0.073$ to $0.35 \mu\text{g a.i./stink bug}$); resistance ratios less than 15.7-fold. The diagnostic doses of 1.30; 0.34; 0.36 and 1.73 $\mu\text{g a.i./stink bug}$ were defined for monitoring the susceptibility of *E. heros* to acephate, thiamethoxam, bifenthrin and lambda-cyhalothrin, respectively. Field populations of *D. furcatus* had low variation in the susceptibility to acephate ($LD_{50} = 0.30$ to $0.53 \mu\text{g a.i./stink bug}$), thiamethoxam ($LD_{50} = 0.066$ to $0.14 \mu\text{g a.i./stink bug}$), and lambda-cyhalothrin ($LD_{50} = 0.27$ to $0.56 \mu\text{g a.i./stink bug}$), with resistance ratios less than 2.1-fold. The results indicate a lower susceptibility of *E. heros* populations to bifenthrin and lambda-cyhalothrin. Populations of *D. furcatus* had similar susceptibility to acephate, thiamethoxam, bifenthrin and lambda-cyhalothrin.

Keywords: Pentatomidae. chemical control. resistance management.

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1 INTRODUÇÃO

O percevejo-marrom, *Euschistus heros* (Fabricius, 1798) (Hemiptera: Pentatomidae), tem elevada ocorrência e distribuição em áreas de produção de grãos e fibras no Brasil (AQUINO et al., 2018; PANIZZI et al., 2016; SOSA-GÓMEZ et al., 2019). Na cultura da soja, os danos ocorrem principalmente durante o período reprodutivo da cultura (PANIZZI et al., 2012), com ataques aos grãos em formação, comprometendo a produtividade e a qualidade dos grãos ou sementes (BUENO et al., 2013; COSTA et al., 1998). As perdas geradas por *E. heros* em soja podem atingir até 30% (VIVAN; DEGRANDE, 2011). Estima-se que durante um ciclo de cultivo da soja esta espécie pode completar até duas gerações (CHEVARRIA et al., 2013). *Euschistus heros* também ocasiona danos em milho, algodão e girassol (MALAGUIDO; PANIZZI, 1998; PANIZZI, 2015; ROZA-GOMES et al., 2011; SORIA et al., 2017).

As espécies do gênero *Dichelops* (percevejos barriga-verde) também têm importância agrícola em milho, soja e trigo (CHOCOROSQUI; PANIZZI 2004; PANIZZI et al., 2016). Dentre as espécies, destacam-se *Dichelops furcatus* (Fabricius, 1775) (Hemiptera: Pentatomidae) e *Dichelops melacanthus* (Dallas, 1851) (Hemiptera: Pentatomidae). *Dichelops furcatus* tem maior ocorrência na região Sul do Brasil, enquanto *D. melacanthus* é encontrado a partir do norte do Paraná (PANIZZI, 2015; PANIZZI et al., 2016). Os danos de *Dichelops* spp. são mais expressivos em milho nos estágios iniciais de desenvolvimento, o que pode reduzir o tamanho das plantas e causar perfilhamento (ROZA-GOMES et al., 2011). Na cultura do trigo, os danos nos estágios vegetativos podem causar necrose do tecido foliar e, durante a fase de enchimento de grãos, a alimentação em estruturas reprodutivas ocasiona descoloração das espigas, prejudicando o desenvolvimento dos grãos (PANIZZI et al., 2016).

Além das plantas cultivadas, *E. heros* e *Dichelops* spp. também podem se desenvolver em plantas daninhas, principalmente no período da entresafra (DALAZEN et al., 2017; SMANIOTTO; PANIZZI, 2015). No inverno, especialmente no Sul do Brasil, estas espécies passam por um período de diapausa, a qual é induzida pelo fotoperíodo curto e provoca alterações no comportamento reprodutivo das espécies (CHOCOROSQUI; PANIZZI, 2003; MOURÃO; PANIZZI. 2002). Após o inverno, os insetos tornam-se ativos novamente e migram para áreas de cultivo (CORRÊA-FERREIRA, 2005; PANIZZI et al., 2016).

A principal estratégia de manejo de percevejos em soja, milho e algodão é o uso de inseticidas químicos (BUENO et al., 2013; PANIZZI, 2013). O nível de controle de percevejos na cultura da soja foi definido em 1 e 2 percevejos/m para produção de sementes e grãos, respectivamente (BUENO et al., 2013). Na cultura do milho, o nível de dano econômico de

Dichelops spp. é de atingir 0,5 percevejos/m (RODRIGUES, 2011). Em algodão, o nível de controle para *E. heros* é de 0,1 percevejo/planta (SORIA et al., 2017). Nas culturas da soja, milho e algodão, os inseticidas são pulverizados em aplicações aéreas e, no milho também são usados em tratamento de semente para evitar danos em estágios iniciais da cultura (CHIESA et al., 2016; CHOCOROSQUI; PANIZZI, 2004; CORRÊA-FERREIRA; SOSA-GÓMEZ, 2017). No entanto, os inseticidas recomendados para o controle destas espécies estão restritos aos grupos químicos dos piretroides, neonicotinoides e organofosforados (AGROFIT, 2019).

No atual sistema de produção agrícola do Brasil estima-se de duas a quatropulverizações inseticidas para o manejo de percevejos em soja e milho (BUENO et al., 2015; CONTE et al., 2018). O uso frequente de inseticidas com o mesmo modo de ação pode favorecer a seleção de indivíduos resistentes, podendo ocasionar falhas de controle (GUEDES, 2017; SOSA-GÓMEZ; OMOTO, 2012). No Brasil foram reportados casos de resistência de *E. heros* a endosulfan, monocrotofós e metamidofós (organofosforados) (SOSA-GÓMEZ et al., 2001; 2010), e falhas de controle para beta-ciflutrina e imidacloprido (GUEDES, 2017; TUELHER et al., 2018). Por outro lado, para as espécies de *Dichelops* spp. ainda não foram reportados casos de resistência a inseticidas.

De acordo com o *Insecticide Resistance Action Committee* (IRAC), a resistência é uma mudança hereditária na suscetibilidade de uma população da praga que se reflete na falha repetida de um produto em atingir o nível de controle esperado, quando utilizado de acordo com a recomendação do rótulo ou bula para determinada espécie praga. A evolução da resistência causa implicações para o manejo de pragas, tais como: aumento no número de aplicações de inseticidas, uso de doses acima do recomendado, utilização de misturas inadequadas de inseticidas e substituição por outros, normalmente mais tóxicos (GEORGHIOU, 1983). Nesse contexto, a avaliação da suscetibilidade dos insetos-praga aos inseticidas é essencial para dar suporte aos programas de Manejo Integrado de Pragas (MIP) e Manejo de Resistência de Insetos (MRI).

No que diz respeito ao MRI a inseticidas, algumas práticas podem contribuir para evitar ou retardar a evolução da resistência. Dentre as estratégias de MRI, a rotação de inseticidas com modo de ação distinto, a aplicação de inseticidas nos níveis de controle e o uso de doses recomendadas são práticas agrícolas que reduzem a probabilidade de seleção de resistentes (BUENO et al., 2015; PANIZZI, 2013; SOSA-GÓMEZ et al., 2019). Além disso, o uso de outras táticas de controle como tratamento de sementes (CORRÊA-FERREIRA; SOSA-GÓMEZ, 2017), parasitoides de ovos (LAGÔA et al., 2019; PACHECO; CORRÊA-

FERREIRA, 2000; QUEIROZ et al., 2018) e fungos entomopatogênicos (LOPES et al., 2015; ZAMBIAZZI et al., 2012), também podem auxiliar no MRI a inseticidas.

Nesse sentido, para subsidiar programas de MIP e MRI para percevejos nas culturas da soja, milho e algodão, os objetivos deste estudo foram:

- 1) Avaliar a suscetibilidade de populações de *E. heros* e *D. furcatus* a acefato, tiameoxam, bifentrina e lambda-cialotrina em bioensaios de imersão de vagens de feijão e monitorar a suscetibilidade de *E. heros* a inseticidas.
- 2) Avaliar a suscetibilidade de populações de *E. heros* e *D. furcatus* a acefato, tiameoxam, bifentrina e lambda-cialotrina em bioensaios de aplicação tópica e estimar doses diagnósticas para monitoramento da suscetibilidade de *E. heros* à inseticidas.

2 ARTIGO 1

Susceptibility of *Euschistus heros* and *Dichelops furcatus* (Hemiptera: Pentatomidae) to Selected Insecticides in Brazil

Junior C. Somavilla,¹ Alexandre C. Reis,¹ Patricia da S. Gubiani,¹ Daniela N. Godoy,¹ Glauber R. Stürmer² and Oderlei Bernardi^{1,3}

¹Department of Plant Protection, Universidade Federal de Santa Maria, Roraima avenue 1000, Santa Maria, Rio Grande do Sul 97105-900, Brazil (somavillajr@gmail.com; alexandrecr98@gmail.com; patriciagubiani70@hotmail.com; godoyufsm@yahoo.com).

²Nufarm Chemical and Pharmaceutical Company S/A (glauber.sturmer@nufarm.com).

³Corresponding author, e-mail address: oderlei.bernardi@ufsm.br

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ABSTRACT

Abstract — *Euschistus heros* (Fabricius, 1798) and *Dichelops furcatus* (Fabricius, 1775) are key pests of agricultural crops in Brazil. Chemical insecticides are the main control tactic used against these species. Here, we investigated the susceptibility of *E. heros* and *D. furcatus* from distinct regions to the mains insecticides used to stink bugs control in Brazil. Field populations of these species were collected throughout the 2017–2019 crop seasons and insects were exposed to insecticides in dip-test bioassays using fresh green bean pods. Populations of *E. heros* exhibited low variation in the susceptibility to acephate ($LC_{50} = 172.2$ to 1,008 µg a.i. per ml), and thiamethoxam ($LC_{50} = 28.8$ to 433.9 µg a.i. per ml), resistance

ratios were less than 5.9- and 15.1-fold, respectively. In contrast, these populations had higher variation in the susceptibility to bifenthrin ($LC_{50} = 26.7$ to $636.1 \mu\text{g a.i. per ml}$) and lambda-cyhalothrin ($LC_{50} = 10.0$ to $636.1 \mu\text{g a.i. per ml}$); resistance ratios reaching 23.8- and 63.6-fold, respectively. Susceptibility monitoring data indicated a higher susceptibility of *E. heros* to the manufacturers field-recommended rates of acephate, lambda-cyhalothrin + thiamethoxam and bifenthrin + acetamiprid than lambda-cyhalothrin. Populations of *D. furcatus* exhibited low variation in the susceptibility to acephate ($LC_{50} = 219.2$ to $614.1 \mu\text{g a.i. per ml}$), bifenthrin ($LC_{50} = 62.8$ to $197.4 \mu\text{g a.i. per ml}$), and lambda-cyhalothrin ($LC_{50} = 189.5$ to $2,538 \mu\text{g a.i. per ml}$); resistance ratios were less than 13.4-fold. In summary, Brazilian populations of *E. heros* are less susceptible to pyrethroids, while populations of *D. furcatus* have similar susceptibility to insecticides evaluated.

Key words: neotropical brown stink bug, green belly stink bug, chemical control, resistance management

Introduction

The neotropical brown stink bug, *Euschistus heros* (Fabricius, 1798), is an important pentatomid pest of several agricultural crops in Brazil (Silva et al. 2012, Smaniotto and Panizzi 2015, Panizzi et al. 2016). This species is widely distributed throughout areas used in crop production, commonly attacking soybean (*Glycine max L. (Merr.)*), maize (*Zea mays L.*), cotton (*Gossypium hirsutum L.*), and sunflower (*Helianthus annuus L.*) plants (Malaguido and Panizzi 1998, Roza-Gomes et al. 2011, Panizzi 2015, Soria et al. 2017, Aquino et al. 2018). The species may also be associated with non-cultivated host plants, such as weeds (Smaniotto

and Panizzi 2015, Dalazen et al. 2017). It is estimated that in soybean it can complete until two generations per season in Brazil (Chevarria et al. 2013).

On the other hand, the green belly stink bug *Dichelops furcatus* (Fabricius, 1775) (Hemiptera: Pentatomidae) occurs mainly in the southern states of Brazil, while *Dichelops melacanthus* (Dallas) (Hemiptera: Pentatomidae) can be found in the northern portion of Paraná state and northward (Panizzi et al. 2016). Species of the genus *Dichelops* are considered the major sucking pest of maize, but they also attack soybean and wheat (*Triticum* sp.) (Chocorosqui and Panizzi 2004, Panizzi et al. 2016). This species undergoes reproductive oligopause, which is induced by the short-photoperiod during the winter season, causing changes in reproductive behavior (Chocorosqui and Panizzi 2003).

Euschistus heros is a problem in the reproductive stages of soybean, while in the vegetative stages this pest has low frequency and damage. Their damage is caused by the insertion of piercing-sucking mouthparts into reproductive structures of soybean (Corrêa-Ferreira 2005, Depieri and Panizzi 2011, Silva et al. 2012). This feeding process injects salivary secretions that promote leaf retention in vegetative stages and affect grain filling, compromising yields up to 30% and quality, when feed on pods throughout reproductive stages (Corrêa-Ferreira and Azevedo 2002, Lourenção et al. 2002, Vivian and Degrande 2011). In cotton crop, injuries of *E. heros* in bolls results in reductions in fiber quality (Soria et al. 2017). In contrast, the greatest damage from *D. frucatus* occurs in maize plants on early stages of development, which cause plant death or tillering, and consequently reduce yields (Roza-Gomes et al. 2011). In wheat, they cause leaf tissue necrosis when feeding throughout vegetative stages, and in the grain filling phase feeding on ears produces discolored seed heads and undeveloped grains (Panizzi et al. 2016).

The management of *E. heros* and *D. furcatus* is mainly accomplished with insecticides in Brazil (Bueno et al. 2013, Panizzi 2013). Either insecticides are sprayed or seeds are treated to

prevent damage in the early stages of plant development (Chocorosqui and Panizzi 2004, Chiesa et al. 2016). It is estimated that in the current Brazilian crop production system, two to four insecticide sprays against these stink bugs in soybean and maize are required (Bueno et al. 2015, Conte et al. 2018). In maize, seed treatments have been widely adopted to protect against *Dichelops* species (Corrêa-Ferreira and Sosa-Gómez, 2017).

The insecticides registered to manage *E. heros* and *D. furcatus* on distinct cultivated crops belong to mainly three chemical groups (pyrethroids, neonicotinoids and organophosphates), which can be used alone or in formulated mixture. This limited availability of insecticides and the frequent use of the same mode-of-action favors the selection of resistant individuals, if Insect Resistance Management (IRM) strategies are neglected (Sosa-Gómez and Omoto 2012, Ribeiro et al. 2016). The resistance of *E. heros* to insecticides have been reported for endosulfan (cyclodien), monocrotophos and metamidophos (organophosphates) in Brazil (Sosa-Gómez et al. 2001, 2010). Control failures were also verified with respect to beta-cyfluthrin (pyrethroid) and imidacloprid (neonicotinoid) (Guedes 2017, Tuelher et al. 2018). In contrast, no resistance to insecticides have been reported for *Dichelops* species. Based on these observations, in the current study we investigated the susceptibility of *E. heros* and *D. furcatus* from distinct regions to selected insecticides used to stink bugs control in Brazil.

Material and Methods

Stink Bug Populations. To evaluate the susceptibility of stink bugs to selected insecticides, populations of *E. heros* and *D. furcatus* were collected in distinct regions in Brazil. Samples were obtained throughout the 2017/2018 and 2018/2019 crop seasons (Table 1). Stink bug populations were collected from soybean fields, with the exception of a *D. furcatus* population from Cruz Alta, which was collected on wheat. After collection, *E. heros* and *D.*

furcatus populations were transported to the laboratory being kept in aerated plastic containers (41 cm long × 29 cm wide × 13 cm high). *Dichelops furcatus* were identified based on the morphological characters (Grazia, 1978). Stink bugs were fed with fresh green bean pods (*Phaseolus vulgaris* L.), dried soybean seeds and shelled peanuts (*Arachis hypogaea* L.), and maintained in an acclimated room at $25 \pm 2^\circ\text{C}$, $60\% \pm 10\%$ RH and a photophase of 14:10 h. Insects remained in these environmental conditions for at least 24 h for acclimatization and stress reduction that resulted from collection and transport. After this time, the bioassays were started.

Insecticides. The commercial insecticides used to evaluate the susceptibility of *E. heros* and *D. furcatus* populations have been listed in Table 2.

Evaluation of Stink Bug Susceptibility to Insecticides in Dip-Test Bioassays. To perform bioassays, initially a sheet of filter paper was placed in a Petri dish (100 × 15 mm) and the paper was moistened using 1 ml distilled water. Then, five adult stink bugs (*E. heros* or *D. furcatus*) from field-collected populations were transferred to each Petri dish (each plate was considered one plot). A total of five replicates of each plot was used for each concentration and insecticide (25 stink bugs/concentration/insecticide). The untreated control was also evaluated using five replicates. Prior to bioassays, fresh green bean pods (*P. vulgaris*) were washed in 1% chlorine bleach solution and allowed to dry. After drying, pods were cut into pieces (~5 cm long). To prepare concentrations of insecticides to be tested (Table 2), each insecticide was diluted in distilled water. All solutions were agitated for 10 min and bean pod pieces were completely submerged in each concentration of insecticide for 5 seconds (IRAC Method 028). To assess controls, bean pod pieces were dipped in distilled water. Afterward, bean pods were placed onto paper towels and allowed to dry. Then, two treated bean pod pieces

were placed in each Petri dish plot containing stink bugs. Petri dishes were stored in a climatic room at $25 \pm 2^\circ\text{C}$, $60\% \pm 10\%$ RH and a photophase of 14:10 h. Mortality was assessed at 96 h (stink bugs without movement after a slight touch with a brush were considered dead).

Susceptibility Monitoring of *E. heros* Using the Field-Recommended Rates of

Insecticides. To monitoring the susceptibility of *E. heros* populations to insecticides we used the manufacturers field-recommended rates (Table 2). This study was performed with *E. heros* exclusively, due to limited number of *D. furcatus* available. In the bioassays, insecticides were diluted in accordance with a spray volume of 150 l per ha. Assays were performed and mortality we evaluated according to the methodology described above.

Statistical Analyses. To assess the susceptibility of stink bugs to selected insecticides, the lethal concentration (LC_{50}) and 95% confidence interval (CIs) were estimated using Probit analysis (PROC PROBIT, SAS Institute 2000). A likelihood ratio test was used to test the hypothesis that LC_P values (lethal concentration at which a percent mortality P is attained) were equal. If the hypothesis was rejected, pairwise comparisons were performed, and significance was stated if CIs did not overlap (Savin et al. 1977). Resistance ratios were estimated by dividing the LC_{50} values of field populations by the corresponding parameter of the most susceptible field population for each insecticide as described by Robertson et al. (2007). Susceptibility monitoring data (number of insects tested and dead) on each insecticide were used to estimate the 95% CIs for the probability of mortality, according to a binomial distribution (Dorai-Raj, 2009). For this analysis, the function *binom.probit* from the package *binom* in R 3.6.1 (R Development Core Team 2018) was used. Percent mortality values were considered significantly different when the 95% CIs among the populations did not overlap.

Percent mortality in replicates with insecticides was corrected based on the mortality of the respective untreated population using Abbott's formula (Abbott 1925).

Results

Susceptibility of *E. heros* to Insecticides in Dip-Test Bioassays. Field populations of *E. heros* had low variation with respect to susceptibility to acephate, an acetylcholinesterase inhibitor (IRAC MoA group 1B), and thiamethoxam, a competitive nicotinic acetylcholine receptor modulator (IRAC MoA group 4A). In contrast, these populations varied with respect to their susceptibility to bifenthrin and lambda-cyhalothrin, which are both sodium channel modulators (IRAC MoA group 3A) (Table 3). Slopes of the mortality-response curves also presented high variation among tested populations.

The LC₅₀ values of acephate against field populations of *E. heros* ranged from 172.2 (population from Santa Maria 2) to 1,008 (population from Restinga Seca) µg a.i. per ml. This variation represents a maximum resistance ratio of 5.9-fold. A significant variation in LC₅₀ values of thiamethoxam against *E. heros* was detected, ranging from 28.8 (population from Londrina) to 433.9 (population from Restinga Seca) µg a.i. per ml, indicating a resistance ratio up to 15.1-fold. Unlike to previous results, field populations of *E. heros* were highly variable with respect to susceptibility to bifenthrin and lambda-cyhalothrin (Table 3). Regarding bifenthrin, LC₅₀ values varied from 26.7 (population from Santa Bárbara do Oeste) to 636.1 (population from Quevedos) µg a.i. per ml indicating resistance ratios up to 23.8-fold. For lambda-cyhalothrin, LC₅₀ values ranged from 10.0 (population from Piracicaba) to 636.1 (population from Maçambará) µg a.i. per ml, representing resistance ratio up to 63.6-fold.

Susceptibility of *D. furcatus* to Insecticides in Dip-Test Bioassays. Field populations of *D. furcatus* showed a significant variation in the susceptibility to acephate, thiamethoxam, bifenthrin and lambda-cyhalothrin (Table 4). A large variation in slopes of the concentration-mortality lines of acephate and thiamethoxam were also observed. When *D. furcatus* were exposed to acephate, LC₅₀ values ranged from 219.2 (population from Cruz Alta) to 614.1 (population from São Sepé) µg a.i. per ml, indicating a resistance ratio lower than 3.0-fold. Similar variation in the susceptibility to bifenthrin (LC₅₀ from 62.8 to 197.4 µg a.i. per ml) was also detected: resistance ratio up to 3.1-fold. A higher variation in the susceptibility was verified to thiamethoxam (LC₅₀ from 171.2 to 1,431 µg a.i. per ml) and lambda-cyhalothrin (LC₅₀ from 189.5 to 2,538 µg a.i. per ml). These results indicate resistance ratios of 8.4 and 13.4-fold, respectively.

Susceptibility Monitoring of *E. heros* to Insecticides. Acephate alone and mixtures of bifenthrin + acetamiprid and lambda-cyhalothrin + thiamethoxam at the field-recommended rates showed high toxicity against *E. heros* (Fig. 1). Populations of *E. heros* from distinct Brazilian regions collected throughout the 2018/2019 soybean season exposed to the field-recommended dose of acephate showed similar mortality rates, ranging from 95.7 to 100%. However, these populations and also those collected in 2017/2018 soybean season showed significant variation in mortality rates (0 to 68%) when exposed to the registered dose of lambda-cyhalothrin. *Euschistus heros* from Santa Maria 1, Santa Bárbara do Sul, Salto do Jacuí (2017/2018 soybean season), Cruz Alta, Santiago, Restinga Seca (RS) and Londrina (PR) (2018/2019 soybean season) exposed to this insecticide had lower mortality rates than other field populations.

Populations of *E. heros* exposed to the field-recommended rates of bifenthrin + acetamiprid had mortality rates higher than 73.9% (Fig. 1). With the exception of the

population from Restinga Seca, the other field populations presented similar levels of mortality, ranging from 95.7 to 100%. In contrast, populations of *E. heros* exposed to lambda-cyhalothrin + thiamethoxam varied significantly with respect to mortality rates (58.3 to 96%). Insects from Quevedos, Santiago, Restinga Seca (RS), Londrina (PR) and Rio Verde (GO) collected throughout the 2018/2019 soybean season had the lowest mortality levels.

Discussion

Stink bugs from distinct Brazilian populations of *E. heros* had low variation in the susceptibility to acephate (acetylcholinesterase inhibitor) and thiamethoxam (competitive nicotinic acetylcholine receptor modulator), with resistance ratios lower than 5.9- and 15.1-fold, respectively. Populations of *D. furcatus* also showed low levels of variability in their susceptibility to acephate, thiamethoxam and bifenthrin (resistance ratios were less than 8.4-fold). For both species, slopes of the concentration-mortality lines were higher for some field populations, suggesting a more homogeneous response to insecticides, in which small variations in the concentrations cause greater variations in mortality. In contrast, lower slopes were verified in some field populations that resulted in different changes in the mortality rate, indicating more heterogeneity (Robertson et al. 2007; Sosa-Gómez et al. 2009). Previous reports have shown that *E. heros* from Paraná and São Paulo states were similarly variable with regard to susceptibility to endosulfan, monocrotophos and metamidophos (acetylcholinesterase inhibitors) and acephate; resistance ratios up to 8.7-fold (Sosa-Gómez et al. 2001, 2009).

The susceptibility to insecticides were also reported in other pentatomid species, *Euschistus servus* (Say, 1832) (Hemiptera: Pentatomidae) from Louisiana, United States, showed low variation in the susceptibility to bifenthrin, lambda-cyhalothrin and cypermethrin

(3.0-fold) (Willrich et al. 2003). In this country, low variation regarding susceptibility to bifenthrin, cypermethrin, and methamidophos in *Nezara viridula* (Linnaeus) and *Piezodorus guildinii* (Westwood) (Hemiptera: Pentatomidae) was also detected (Temple et al. 2013). In Brazil, *P. guildinii* from Paraná and São Paulo states also had resistance ratios to acephate, endosulfan and metamidophos (Baur et al. 2010).

An interpopulation variation in the susceptibility to insecticides is a commonly observed phenomenon in distinct insect pests (Robertson et al. 1995). From an IRM perspective, even a small level of variation in susceptibility is an indication of the potential for the selection of resistance (Carrière et al. 2010). The limited number of registered insecticides that may be used against stink bugs means that insects are more highly exposed to the same active ingredients. This may cause future control failures, which can be attributed to resistance (Guedes, 2017). Therefore, even stink bugs populations with low resistance ratio can evolve resistance, especially in central Brazil where soybean, maize and cotton are cultivated simultaneously or in succession due to favorable weather conditions, which allows their continuous exposure to insecticides. This can be intensified by the low dispersal capacity of this group of insects, as a result of limited flight activity and diapause behavior (Mourão and Panizzi 2002). The reduced dispersion of *E. heros* is in accordance with high levels of genetic similarity in insects from the same regions, what can favor the local or regional adaptation to insecticides (Sosa-Gómez et al. 2004, Soares et al. 2018). On the other hand, in southern Brazil during the winter stink bugs undergoes reproductive oligopause (Chocorosqui and Panizzi 2003), that reduce their infestation on cultivated plants and, consequently the exposure to insecticides, contributing to slow the evolution of resistance.

The relatively small level of variation in the susceptibility of the stink bug populations to acephate and thiamethoxam observed in our study suggests that, currently, the resistance level to these insecticides remains low in the field. This hypothesis is supported by the high levels

of mortalities caused by field-recommended rates of these insecticides used alone or in mixtures. Previous studies also demonstrate that the field label rate of acephate caused high mortality of *E. heros* populations from Goiás state throughout the 2014 to 2016 soybean seasons (Tuelher et al. 2018). Mixtures of lambda-cyhalothrin + thiamethoxam also caused high mortality (from 80 to 100%) in *E. heros* in Rio Grande do Sul (Marques et al. 2019). Lambda-cyhalothrin + thiamethoxam applied topically to *E. heros* from Paraná state during 2012/2013 soybean season also produced mortality rates up to 70% (Husch and Sosa-Gómez 2013).

Unlike to previous results, we detected a higher level of variation regarding the susceptibility of *E. heros* to bifenthrin and lambda-cyhalothrin (resistance ratios up to 23.8- and 63.6-fold, respectively) and *D. furcatus* to bifenthrin (up to 13.4-fold). When these insecticides were tested alone at the field-recommended dose, the susceptibility of *E. heros* varied greatly with respect to mortality rates. Previous studies also demonstrated less susceptibility of *E. heros* to beta-cyfluthrin (another pyrethroid) in soybean in Goiás (Tuelher et al. 2018).

The low susceptibility of *E. heros* populations to pyrethroids can be explained by widespread adoption of these products to manage stink bugs in soybean fields in Brazil. Formulated insecticides using pyrethroids alone or in mixture with neonicotinoids or organophosphates represent almost 50% of the insecticides registered against *E. heros* in Brazil (Agrofit 2019). These insecticides are also used against other important hemipteran pests in soybean (*P. guildinii* and *Bemisia tabaci* [Gennadius] [Hemiptera: Aleyrodidae]), cotton (*Aphis gossypii* Glover [Hemiptera: Aphididae] and *B. tabaci*) and maize (*Dalbulus maidis* [Delong & Wolcott] [Hemiptera: Cicadellidae]) (Badji et al. 2004, Baur et al. 2010, Koo et al. 2014, Dângelo et al. 2018). Therefore, it is necessary to reduce pyrethroid use against *E. heros*, and others sucking pests in soybean, maize and cotton, as well as give

preference to insecticides with low resistance ratios, and making a rotation of distinct modes of action to reduce selection pressure for resistance. Furthermore, the use of other IPM tactics, such as egg parasitoids (Silva et al. 2008, Queiroz et al. 2018) may help to retard the development of further resistance in stink bugs in Brazil.

Here, we have reported the susceptibility of populations of *E. heros* and *D. furcatus* to selected insecticides in Brazil. Future efforts should be concentrated on collecting representative stink bug populations, especially from regions used for growing substantial quantities of soybean, maize and cotton, to identify changes in the susceptibility of these insects as a result of a result of repeated exposure to these insecticides. Therefore, the continuous monitoring of insecticide susceptibility in field populations of stink bugs is essential to support IPM and IRM programs in Brazil.

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Table 1. Identification, number of insects collected, location, and date of collection of *E. heros* and *D. furcatus* populations from Brazilian soybean fields used to evaluate their susceptibility to insecticides.

Population	n	Latitude	Longitude	Date
<i>Euschistus heros</i>				
Piracicaba, SP	-	22°41'48" S	47°38'42" W	-
Salto do Jacuí, RS	211	28°59'23" S	53°13'49" W	February 2018
Santa Maria 1, RS	200	29°43'14" S	53°33'33" W	February 2018
Júlio de Castilhos, RS	150	29°22'20" S	53°59'00" W	March 2018
Santa Bárbara do Sul, RS	153	28°20'36" S	53°15'43" W	March 2018
Pantano Grande, RS	1,250	30°17'31" S	52°13'44" W	January 2019
Santa Maria 2, RS	1,551	29°42'57" S	53°44'03" W	February 2019
Quevedos, RS	1,709	29°16'18" S	53°59'29" W	February 2019
Santiago, RS	3,502	29°01'36" S	54°41'48" W	February 2019
Santa Bárbara do Oeste, SP	2,980	22°45'55" S	47°28'20" W	February 2019
Catuípe, RS	301	28°13'22" S	53°58'47" W	March 2019
Cruz Alta, RS	1,409	28°34'36" S	53°37'09" W	March 2019
Maçambará, RS	1,600	29°03'27" S	55°45'51" W	March 2019
Restinga Seca, RS	2,010	29°44'00" S	53°26'50" W	March 2019
São Sepé, RS	450	30°22'30" S	53°42'08" W	March 2019
Londrina, PR	3,248	23°09'26" S	51°15'01" W	March 2019
Rio Verde, GO	1,519	17°54'03" S	50°47'58" W	April 2019
<i>Dichelops furcatus</i>				
Cruz Alta, RS ^a	805	28°34'36" S	53°37'09" W	November 2018
Santiago, RS	253	29°01'36" S	54°41'48" W	February 2019
Catuípe, RS	302	28°13'22" S	53°58'47" W	March 2019
Restinga Seca, RS	1,012	29°44'00" S	53°26'50" W	March 2019
São Sepé, RS	857	30°22'30" S	53°42'08" W	March 2019

^aThis population were collected in wheat crop.

Table 2. Commercial insecticides used to evaluate the susceptibility of Brazilian populations of *E. heros* and *D. furcatus*.

Active Ingredient (AI)	Insecticide Class (IRAC MoA)	Trade Name	AI (%)	Company/Manufacturer	Dose (µg a.i./ml)
Concentration-mortality bioassays					
Acephate	Organophosphate (1B)	Orthene	75	Arysta Lifescience do Brasil Indústria Química e Agropecuária Ltda, Pilar do Sul, SP, Brazil	10-3,200
Bifenthrin	Pyrethroid (3A)	Talstar	10	FMC Química do Brasil Ltda, Campinas, SP, Brazil	3.2-1,800
Lambda-cyhalothrin	Pyrethroid (3A)	Kaiso	24	Nufarm A/S, Maracanaú, CE, Brazil	1.8-10,000
Thiamethoxam	Neonicotinoid (4A)	Actara	25	Syngenta Proteção de Cultivos Ltda, Paulínia, SP, Brazil	18-10,000
Susceptibility monitoring bioassays					
Acephate	Organophosphate (1B)	Orthene	75	Arysta Lifescience do Brasil Indústria Química e Agropecuária Ltda, Pilar do Sul, SP, Brazil	750
Lambda-cyhalothrin	Pyrethroid (3A)	Kaiso	24	Nufarm A/S, Maracanaú, CE, Brazil	12
Bifenthrin + Acetamiprid	Pyrethroid (3A) + Neonicotinoid (4A)	Sperto	25 + 25	UPL do Brasil Indústria e Comércio de Insumos Agropecuários S.A. Ituverava, SP, Brazil	27.5 + 27.5
Lambda-cyhalothrin + Thiamethoxam	Pyrethroid (3A) + Neonicotinoid (4A)	Engeo Pleno	10.6 + 14.1	Syngenta Proteção de Cultivos Ltda, Paulínia, SP, Brazil	21.2 + 28.2

Table 3. Concentration-mortality response (LC; µg a.i. per ml) of adults of *E. heros* populations exposed to selected insecticides in bean dip bioassays.

Population	n	Slope ± SE	LC ₅₀ (95% CI) ^{a,b}	χ ^c	df ^d	RR ₅₀ ^e	(continua)
Acephate							
Santa Maria 2, RS	150	3.03 ± 0.89	172.2 (117.6–228.1) a	3.94	3	-	
Pântano Grande, RS	175	1.80 ± 0.45	174.2 (83.4–261.2) a	0.34	4	1.0	
São Sepé, RS	150	8.24 ± 3.07	208.5 (124.3–251.7) a	4.79	3	1.2	
Santiago, RS	210	2.13 ± 0.47	294.6 (210.0–414.9) ab	6.28	4	1.7	
Cruz Alta, RS	175	3.25 ± 1.03	356.2 (169.6–469.4) ab	2.02	4	2.1	
Rio Verde, GO	175	3.05 ± 0.54	387.6 (280.21–491.4) b	1.90	4	2.3	
Piracicaba, SP	150	3.73 ± 0.92	475.1 (345.6–591.7) bc	3.32	3	2.8	
Quevedos, RS	150	3.28 ± 0.64	589.3 (385.7–764.5) bc	2.41	4	3.4	
Santa Bárbara do Oeste, SP	175	2.04 ± 0.52	656.5 (350.9–940.9) bc	1.85	4	3.8	
Maçambará, RS	150	3.82 ± 0.93	823.5 (552.8–1,028) c	2.83	3	4.8	
Restinga Seca, RS	150	2.83 ± 0.67	1,008 (675.9–1,364) c	3.19	3	5.9	
Thiamethoxam							
Londrina, PR	150	1.03 ± 0.20	28.8 (11.6–53.5) a	2.42	3	-	
Quevedos, RS	200	1.79 ± 0.33	58.1 (22.7–102.9) a	4.19	5	2.0	
Santa Bárbara do Oeste, SP	125	0.94 ± 0.26	59.3 (7.7–131.0) a	3.27	2	2.1	
Piracicaba, SP	150	2.87 ± 0.48	74.9 (53.2–98.1) a	2.53	3	2.6	
Rio Verde, GO	175	2.56 ± 0.48	122.1 (80.0–163.8) a	3.24	4	4.2	
Santiago, RS	125	1.53 ± 0.37	131.1 (53.1–228.8) a	3.31	2	4.5	
São Sepé, RS	175	2.51 ± 0.41	132.5 (94.4–176.3) a	5.24	4	4.6	
Maçambará, RS	150	2.33 ± 0.46	151.6 (86.3–221.9) a	2.51	3	5.3	
Restinga Seca, RS	175	1.67 ± 0.34	433.9 (291.0–691.9) b	3.21	4	15.1	
Bifenthrin							
Santa Bárbara do Oeste, SP	225	1.45 ± 0.20	26.7 (16.0–39.5) a	5.04	6	-	
Rio Verde, GO	175	1.67 ± 0.29	45.14 (27.9–65.0) a	0.94	4	1.7	
Londrina, PR	150	3.94 ± 1.30	51.2 (30.7–65.1) a	4.72	3	1.9	
Santa Maria 2, RS	150	1.41 ± 0.39	61.3 (12.3–120.5) ab	3.00	3	2.3	
Pântano Grande, RS	175	1.48 ± 0.31	68.3 (35.1–108.4) ab	0.82	4	2.6	
Santiago, RS	210	1.62 ± 0.25	102.4 (69.1–146.9) b	1.06	4	3.8	
Piracicaba, SP	175	3.17 ± 0.64	105.7 (76.7–133.7) b	1.73	4	4.0	
Cruz Alta, RS	200	1.54 ± 0.35	229.8 (104.4–361.4) bc	1.65	5	8.6	
Restinga Seca, RS	150	2.33 ± 0.38	362.4 (255.3–481.9) c	2.29	4	13.6	
Quevedos, RS	125	8.75 ± 2.16	636.1 (540.9–729.2) d	3.00	3	23.8	
Lambda-cyhalothrin							
Piracicaba, SP	225	1.96 ± 0.29	10.0 (6.5–13.8) a	3.81	6	-	
Santa Maria 2, RS	175	2.11 ± 0.46	82.5 (37.8–122.7) b	2.80	4	8.2	
Pantano Grande, RS	200	1.44 ± 0.23	106.8 (61.96–163.0) b	5.88	5	10.7	

Table 3. Concentration-mortality response (LC; µg a.i. per ml) of adults of *E. heros* populations exposed to selected insecticides in bean dip bioassays.

Population	n	Slope ± SE	LC ₅₀ (95% CI) ^{a,b}	χ ^c	df ^d	RR ₅₀ ^e	(continuação)
Santa Bárbara do Oeste, SP	175	4.86 ± 0.88	109.6 (89.4–130.2) b	1.83	4	11.0	
Londrina, PR	150	2.29 ± 0.46	163.2 (87.9–231.3) bc	5.05	3	16.3	
Rio Verde, GO	175	2.47 ± 0.43	172.5 (115.5–231.3) bc	2.77	4	17.3	
Cruz Alta, RS	175	2.02 ± 0.56	418.7 (212.7–621.7) c	0.34	4	41.9	
Restinga Seca, RS	150	1.71 ± 0.34	425.0 (263.2–617.9) c	5.85	3	42.5	
Santiago, RS	180	3.09 ± 0.61	451.1 (349.1–567.2) c	4.38	3	45.1	
Quevedos, RS	200	3.65 ± 0.68	529.2 (409.6–648.1) c	7.17	5	52.9	
Catuípe, RS	200	1.67 ± 0.31	534.7 (282.8–791.4) c	1.35	5	53.5	
Maçambará, RS	175	2.31 ± 0.48	636.1 (340.7–889.7) c	6.31	4	63.6	

^aLC₅₀: concentration of insecticide required to kill 50% of stinks bugs in the observation period of 4 days.

^bLC₅₀ values designated by different letters within a column are significantly different from each other through non-overlap of 95% CIs.

^cP > 0.05 in goodness of fit.

^dDegrees of freedom.

^eResistance Ratio (RR₅₀) = (LC₅₀ of field populations)/(LC₅₀ of the most susceptible population).

Table 4. Concentration-mortality response (LC; µg a.i./ml) of adults of *D. furcatus*

populations exposed to selected insecticides in bean dip bioassays.

Population	n	Slope ± SE	LC₅₀ (95% CI)^{a,b}	χ^{2c}	df^d	RR₅₀^e
<i>Acephate</i>						
Cruz Alta, RS	150	4.37 ± 0.73	219.2 (158.5–279.1) a	4.28	3	-
Restinga Seca, RS	175	1.75 ± 0.39	592.2 (290.1–896.7) b	4.15	4	2.7
São Sepé, RS	175	3.23 ± 0.60	614.1 (459.4–778.3) b	3.95	4	2.8
<i>Thiamethoxam</i>						
São Sepé, RS	125	3.06 ± 0.73	171.2 (113.1–228.3) a	3.78	2	-
Restinga Seca, RS	250	1.50 ± 0.43	1,431 (404.9–3,611) b	12.5	7	8.4
<i>Bifenthrin</i>						
Catuípe, RS	125	2.01 ± 0.43	62.8 (36.4–91.9) a	2.68	2	-
Cruz Alta, RS	125	2.07 ± 0.45	67.4 (37.6–100.6) a	2.74	2	1.1
São Sepé, RS	175	2.51 ± 0.57	92.7 (57.6–126.9) ab	1.13	4	1.5
Restinga Seca, RS	175	1.69 ± 0.36	197.4 (124.6–303.9) b	2.55	4	3.1
<i>Lambda-cyhalothrin</i>						
Catuípe, RS	125	1.75 ± 0.56	189.5 (101.0–362.3) a	3.93	2	-
Santiago, RS	150	1.09 ± 0.37	251.2 (38.5–535.6) a	1.93	3	1.3
Restinga Seca, RS	150	2.07 ± 0.46	1,107 (629.7–1,676) b	0.20	3	5.8
São Sepé, RS	200	1.54 ± 0.29	1,622 (889.6–2,534) b	4.58	5	8.6
Cruz Alta, RS	150	2.71 ± 0.60	2,538 (1,254–3,529) b	3.41	3	13.4

^aLC₅₀: concentration of insecticide required to kill 50% of stinks bugs in the observation period of 4 days.

^bLC₅₀ values designated by different letters within a column are significantly different from each other through non-overlap of 95% CIs.

^cP > 0.05 in goodness of fit.

^dDegrees of freedom.

^eResistance Ratio (RR₅₀) = (LC₅₀ of field populations)/(LC₅₀ of the most susceptible population).

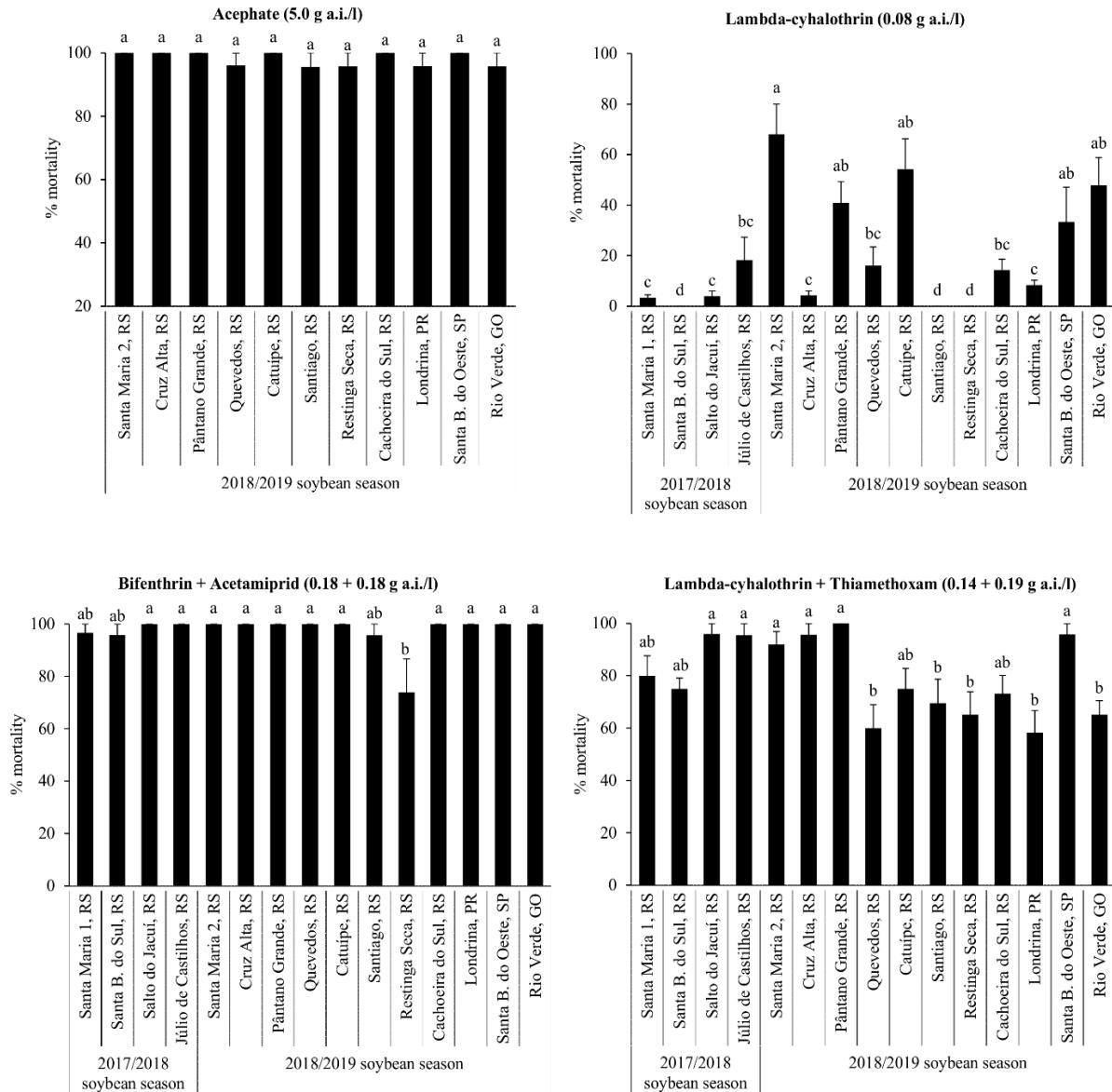


Figure 1. Susceptibility monitoring of adults of *E. heros* exposed to the manufacturers field-recommended rates of insecticides in bean dip bioassays. Bars (\pm SE) with different letters differ significantly.

3 ARTIGO 2

Susceptibility of *Euschistus heros* and *Dichelops furcatus* (Hemiptera: Pentatomidae) to insecticides determined in topical bioassays and diagnostic doses for susceptibility monitoring of *E. heros* in Brazil

Junior C. Somavilla^a, Alexandre C. Reis^a, Patricia da S. Gubiani^a, Fábio M. Führ^a, Eduardo P. Machado^a, and Oderlei Bernardi^{aa}

^aDepartment of Plant Protection, Federal University of Santa Maria (UFSM), Roraima avenue 1000 97105-900, Santa Maria/RS.

Section: Crop Protection

ABSTRACT

The neotropical brown stink bug, *Euschistus heros* (F.) and the green belly stink bug, *Dichelops furcatus* (F.) (Hemiptera: Pentatomidae), are key sucking pests that attack soybean, maize, cotton and wheat in Brazil. The management of these species is usually accomplished with chemical control. Insecticides registered for use against these species are restricted to pyrethroids, neonicotinoids and organophosphates. In this study, we evaluated the susceptibility of Brazilian populations of *E. heros* and *D. furcatus* to insecticides and define diagnostic doses for susceptibility monitoring of *E. heros*. Populations of stink bugs were collected during the 2018/2019 crop season and were exposed to technical grade insecticides in topical bioassays. Populations of *E. heros* presented low variation in the susceptibility to

^a Corresponding author. E-mail address: oderleibernardi@yahoo.com.br (O. Bernardi).

acephate (LD_{50} = 0.22 to 0.69 µg a.i./stink bug), bifenthrin (LD_{50} = 0.021 to 0.10 µg a.i./stink bug) and thiamethoxam (LD_{50} = 0.0046 to 0.032 µg a.i./stink bug), with resistance ratios less than 8.2-fold in relation to a susceptible reference population. In contrast, the same populations had higher variation in the susceptibility to lambda-cyhalothrin (LD_{50} = 0.073 to 0.35 µg a.i./stink bug), with resistance ratios ranging from 3.1 to 15.7-fold. The diagnostic doses of 1.30; 0.34; 0.36 and 1.73 µg i.a./stink bug were defined for monitoring the susceptibility of *E. heros* to acephate, thiamethoxam, bifenthrin and lambda-cyhalothrin, respectively. Field populations of *D. furcatus* showed low variation in the susceptibility to acephate (LD_{50} = 0.30 to 0.53 µg a.i./stink bug), thiamethoxam (LD_{50} = 0.066 to 0.14 µg a.i./stink bug) and lambda-cyhalothrin (LD_{50} = 0.27 to 0.56 µg a.i./stink bug), presenting resistance ratios less than 2.1-fold. Our results indicate that populations of *E. heros* had low susceptibility to lambda-cyhalothrin, while populations of *D. furcatus* had similar susceptibility to the insecticides tested.

Keywords: neotropical brown stink bug, green belly stink bug, chemical control, resistance evolution

1. Introduction

In Brazil, various sucking species have the capacity to damage important agricultural crops cultivated for grain or fiber production, causing significant yield reductions (Bueno et al., 2013; Panizzi, 2015; Soria et al., 2017; Sosa-Gómez et al., 2019). Among these species, the neotropical brown stink bug, *Euschistus heros* (F. 1798) and the green belly stink bug, *Dichelops furcatus* (F. 1775) are considered the main pentatomid pests that attack cultivated crops such as soybean (*Glycine max* L. (Merr.)), maize (*Zea mays* L.), cotton (*Gossypium*

hirsutum L.), canola (*Brassica* spp.) and wheat (*Triticum aestivum* L.), with high occurrence in the Brazilian crop production system (Smaniotto and Panizzi, 2015; Panizzi, 2015; Panizzi et al., 2016; Bianchi et al., 2019; Soares et al., 2018). These sucking species are also widely distributed in agricultural fields in Argentina, Bolivia, Paraguay and Uruguay (Grazia, 1978; Saluso et al., 2011; Panizzi, 2015).

Euschistus heros is the most abundant pentatomid species found in Brazilian soybean fields (Borges et al., 2011; Bueno et al., 2015; Aquino et al., 2018). The damage caused by this pest result from the insertion of piercing–sucking mouthparts into the soybean plants or pods, with injection of salivary secretions that facilitate the feeding process (Lucini and Panizzi, 2018). Laboratory studies have demonstrated that soybean is a food source preferred by *E. heros*, providing major adult body weight (Possebom et al., 2019). Feeding activity causes leaf retention in soybean when the attack occurs during the vegetative stages, and grain filling when they feed on pods (Lourençao et al., 2002; Depieri and Panizzi, 2011; Silva et al., 2012). This species also attacks cotton bolls, causing a reduction in fiber quality (Soria et al., 2017).

Unlike the above species, *D. furcatus* is generally more common in maize and wheat in Brazil, but in recent years' infestations in soybean fields have increased (Chocorosqui and Panizzi, 2004; Manfredi-Coimbra et al., 2005; Silva et al., 2013; Panizzi et al., 2016).

Dichelops furcatus predominates in crop production areas in southern states of Brazil, while *D. melachanthus* occurs mainly in the northern state of Paraná and northward (Bortolotto et al., 2016; Panizzi et al., 2016). Attacks by these species in maize during early stages of development cause plant death or tillering (Roza-Gomes et al., 2011; Gomes et al., 2019). On wheat they cause tissue necrosis on leaves, discolored seed heads and undeveloped grains if the ears are attacked (Panizzi et al., 2016).

The main control tactic against these stink bug species involves the use of chemical insecticides in foliar sprays or seed treatments (Bueno et al., 2013; Panizzi, 2013). The insecticides registered for the management of *E. heros* and *D. furcatus* in Brazil belong to mainly three chemical groups (pyrethroids, neonicotinoids and organophosphates), which can be used alone or in formulated mixtures (Agrofit, 2019). This limited availability of distinct modes of action favors the evolution of resistance, as a result of frequent exposure of stink bug populations to the same every season.

In Brazil, populations of *E. heros* collected from soybean fields showed low susceptibility to endosulfan, monocrotophos and metamidophos (Sosa-Gómez et al., 2001, 2010). In recent years, low susceptibility of *E. heros* to beta-cyfluthrin, bifenthrin, lambda-cyhalothrin and imidacloprid has also been detected (Guedes, 2017; Tuelher et al., 2018; Somavilla et al., 2019). The continuous evaluation of the susceptibility of stink bugs to insecticides is therefore necessary to identify potential changes in their susceptibility as a response to the selection pressure exerted by insecticides. For this reason, we evaluated the susceptibility of field-collected populations of *E. heros* and *D. furcatus* to insecticides in topical bioassays and determined diagnostic doses for resistance monitoring of *E. heros* in Brazil.

2. Material and methods

2.1 Collection sites of stink bug populations

To assess the susceptibility of *E. heros* and *D. furcatus* to insecticides, stink bugs populations were collected in distinct regions in Brazil during the 2018/2019 crop season (Table 1). After collection, the stink bugs were transported to the laboratory in aerated plastic containers (41 cm long × 29 cm wide × 13 cm high). *Dichelops furcatus* were identified based

on the morphological characters (Grazia, 1978). Insects were fed with fresh green bean pods (*Phaseolus vulgaris* L.), dried soybean seeds and shelled peanuts (*Arachis hypogaea* L.), and maintained in a room at $25 \pm 2^\circ\text{C}$, $60\% \pm 10\%$ RH and with a photoperiod of 14:10 hours. Stink bugs remained in these environmental conditions for at least 24 hours for acclimatization and stress reduction following collection and transport. After this, bioassays were started. In addition to the field populations, a susceptible reference population of *E. heros* was also used. This population has been kept in the laboratory for more than 4 years, with no exposure to insecticides. Unfortunately, a susceptible population of *Dichelops* species was not available and, because of this, the most susceptible field population was used to estimate resistance ratios.

2.2. Technical grade insecticides

The technical grade insecticides used to assess the susceptibility of *E. heros* and *D. furcatus* populations were: acephate (99.5% active ingredient (a.i.); Sigma-Aldrich, São Paulo, SP, Brazil) – an acetylcholinesterase inhibitor (IRAC MoA 1B); bifenthrin (99.1% a.i.; Sigma-Aldrich, São Paulo, SP, Brazil) and lambda-cyhalothrin (96.5% a.i.; Nufarm A/S, Maracanaú, CE, Brazil) – both sodium channel modulators (IRAC MoA 3A); and thiamethoxam (99.3% a.i.; Sigma-Aldrich, São Paulo, SP, Brazil) – a nicotinic acetylcholine receptor competitive modulator (IRAC MoA 4A).

2.3. Susceptibility of stink bugs to insecticides in topical bioassay

To perform bioassays, we used the bioassay method indicated by the *Insecticide Resistance Action Committee* (IRAC Method 029). Initially, fresh green bean pods (*P. vulgaris*) were

washed in 1% chlorine bleach solution and allowed to dry. After drying, the bean pods were cut into pieces of 4–5 cm long. Test arenas were prepared by placing a sheet of filter paper in Petri dishes (100 × 15 mm) and the paper was moistened with 1 ml distilled water, and then 2 pieces of bean pod were placed into each test arena as a food source. Five adult stink bugs were transferred to each Petri dish (each plate was considered one plot). Five replicates of each plot were used for each concentration and technical grade insecticide (25 stink bugs/concentration/insecticide). Technical grade insecticides were diluted in acetone (99.5% purity; Sigma-Aldrich, São Paulo, SP, Brazil) to prepare the concentrations to be tested (4–8 concentrations/insecticide). Each concentration was agitated for 10 minutes and then 2 µl of each solution were applied to the back (dorsum) of each stink bug using an automatic micropipette. The control treatment was composed by 5 replicates of 5 stink bugs, which were treated only with 2 µl of acetone. Mortality was assessed at 72 hours (stink bugs without movement after a slight touch with a brush were considered dead).

2.4. Statistical analyses

To estimate the lethal doses (LD_{50} and LD_{90}) and respective confidence intervals (95% CIs), dose-mortality data for each population were submitted to Probit analysis using the PROC PROBIT procedure in SAS® software (SAS Institute 2000). A likelihood ratio test was used to test the hypothesis that the LD_p values (lethal concentration at which a percent mortality P is attained) were equal. If the hypothesis was rejected, pairwise comparisons were performed and significance was assumed if 95% CIs did not overlap (Savin et al., 1977). Resistance ratios were calculated by dividing the LD_{50} values of the field populations by the corresponding parameter of the susceptible reference population as described by Robertson et al. (2007). To estimate the diagnostic doses, mortality data from all populations were

analyzed jointly, according to the method proposed by Sims et al. (1996). In this analysis, mortality data were fitted with a binomial model using the log-log complement connection function (gompit) (PROC PROBIT, SAS Institute 2000). Through this analysis, LD₉₉ and respective 95% CIs were estimated. The LD₉₉ values of each insecticide was designated as candidate diagnostic doses for the susceptibility monitoring of *E. heros* to insecticides.

3. Results

*3.1. Susceptibility of *E. heros* to insecticides in topical bioassays*

In topical bioassays, field populations of *E. heros* presented lower variation in the susceptibility to acephate, thiamethoxam and bifenthrin than lambda-cyhalothrin (Table 2). The LD₅₀ values of acephate varied significantly among populations of *E. heros*, ranging from 0.22 (population from Santiago) to 0.69 (population from Restinga Seca) µg a.i./stink bug. However, no significant variation in LD₉₀ values (0.54 to 1.49 µg a.i./stink bug) were observed. The susceptible reference population had similar LD₅₀ and LD₉₀ values to most of the field populations to acephate; 0.29 and 0.60 µg a.i./stink bug, respectively. According to LD₅₀ the values of acephate, a maximum resistance ratio of 2.4-fold was detected in Brazilian populations of *E. heros* (Table 2).

The lethal doses of thiamethoxam also varied significantly among field populations of *E. heros* (Table 2). Estimated LD₅₀ values of thiamethoxam ranged from 0.0046 (population from Londrina) to 0.032 (population from Maçambará) µg a.i./stink bug, while for the susceptible reference population the LD₅₀ was 0.0039 µg a.i./stink bug, suggesting a resistance ratio up to 8.2-fold. The LD₉₀ values of thiamethoxam also showed significant

variation, ranging from 0.011 (population from Rio Verde) to 0.072 (population from Maçambará) µg a.i./stink bug.

Variation in the susceptibility to bifenthrin was also observed in field populations of *E. heros* (Table 2). The LD₅₀ values ranged from 0.030 (population from Rio Verde) to 0.10 (population from Cruz Alta) µg a.i./stink bug. For the susceptible reference population, the estimated LD₅₀ value was 0.021 µg a.i./stink bug, indicating a resistance ratio less than 5.0-fold. In contrast, LD₉₀ values of bifenthrin did not differ significantly between field populations and the susceptible reference population (LD₉₀ values from 0.072 to 0.32 µg a.i./stink bug).

Unlike the previous results, field populations of *E. heros* were highly variable with respect to susceptibility to lambda-cyhalothrin (Table 2). The LD₅₀ values ranged from 0.073 (population from Pântano Grande) to 0.35 (population from Cruz Alta) µg a.i./stink bug, while the LD₉₀ values varied from 0.29 to 2.08 µg a.i./stink bug. In contrast, the susceptible reference population presented the highest susceptibility to lambda-cyhalothrin, with LD₅₀ and LD₉₀ values of 0.023 to 0.14 µg a.i./stink bug, respectively. According to these LD₅₀ values, a maximum resistance ratio of 15.7-fold was estimated.

Using the mortality data from all populations of *E. heros* for each dose and insecticide, we also estimated LD₉₉ values, which can be used as candidate diagnostic doses in resistance monitoring programs. According to these analyses, the estimated LD₉₉ values for each insecticide were: acephate 1.30 [CI 95% (1.16–1.50)] µg a.i./stink bug ($n = 1,355$; Slope (\pm SE) = 3.39 (± 0.19); $\chi^2 = 5.56$; df = 5); thiamethoxam 0.34 [CI 95% (0.15–1.56)] µg a.i./stink bug ($n = 1,225$; Slope (\pm SE) = 1.17 (± 0.17); $\chi^2 = 23.57$; df = 7); bifenthrin 0.36 [CI 95% (0.27–0.58)] µg a.i./stink bug ($n = 1,660$; Slope (\pm SE) = 2.22 (± 0.20); $\chi^2 = 16.76$; df = 6) and lambda-cyhalothrin 1.73 [IC 95% (1.30–2.47)] µg a.i./stink bug ($n = 1,320$; Slope (\pm SE) = 1.57 (± 0.11); $\chi^2 = 7.34$; df = 8).

3.3. Susceptibility of *D. furcatus* to insecticides in topical bioassays

We found that field populations of *D. furcatus* presented similar susceptibility to acephate, thiamethoxam and lambda-cyhalothrin (Table 3). Equivalent LD₅₀ (0.30 to 0.53 a.i./stink bug) and LD₉₀ (0.50 to 1.02 µg a.i./stink bug) values of acephate were found in field populations of *D. furcatus*. These populations also had similar susceptibility to thiamethoxam, with LD₅₀ and LD₉₀ values ranging from 0.066 to 0.14 and 0.19 to 0.47 µg a.i./stink bug, respectively. Estimated LD₅₀ (0.27 to 0.56 µg a.i./stink bug) and LD₉₀ (1.41 to 4.02 µg a.i./stink bug) values for lambda-cyhalothrin also did not differ significantly among populations of *D. furcatus*. In summary, field populations of *D. furcatus* presented resistance ratios for acephate, thiamethoxam and lambda-cyhalothrin of less than 2.1-fold.

4. Discussion

Field populations of *E. heros* from different Brazilian soybean fields presented low levels of variability in the susceptibility to acephate, bifenthrin and thiamethoxam. In contrast, the same populations were highly variable in their susceptibility to lambda-cyhalothrin. Populations of *E. heros* exposed to commercially formulated insecticides in dip-test bioassays also presented lower interpopulational variation in susceptibility to acephate, thiamethoxam and bifenthrin than lambda-cyhalothrin (Somavilla et al., 2019). Low variation in the susceptibility to lambda-cyhalothrin and thiamethoxam (resistance ratios less than 7.0-fold) was also reported in populations of *E. heros* from Paraná and São Paulo states collected during the overwintering period of 2015 (Sosa-Gómez et al., 2019).

Previous studies also revealed a low susceptibility of Brazilian populations of *E. heros* to the field-recommended rates of some pyrethroids. Somavilla et al. (2019) used dip-test

bioassays and reported a resistance ratio to lambda-cyhalothrin up to 63.6-fold. Control failures of beta-cyfluthrin (another pyrethroid) were also observed in soybean fields in Goiás state in the 2014 to 2016 crop seasons (Tuelher et al., 2018). In addition, high variation in the susceptibility of *E. heros* from São Paulo and Paraná states to acephate was also documented (resistance ratio up to 80-fold) (Sosa-Gómez et al., 2019). Unlike previous studies, the insecticides evaluated here presented high toxicity against other pentatomids species, such as *Piezodorus guildinii* (Westwood, 1837) (Baur et al., 2010) and *D. furcatus* (Somavilla et al., 2019) in Brazil, and *Acrosternum hilare* (Say, 1832), *Euschistus servus* (Say, 1832) (Willrich et al., 2003; Kamminga et al., 2009), *Nezara viridula* (L. 1758) (Willrich et al., 2003; Temple et al., 2013) and *Halyomorpha halys* (Stål, 1855) (Leskey et al., 2012, Cira et al., 2017) in the United States.

In our study, we found that Brazilian populations of *D. furcatus* had similar susceptibility to the insecticides tested. A similar susceptibility to acephate and thiamethoxam was also reported for *D. furcatus* exposed to commercially formulated insecticides in dip-test bioassays (resistance ratios less than 8.4-fold). However, a high level of variation regarding the susceptibility of *D. furcatus* to lambda-cyhalothrin was observed (resistance ratios up to 13.4-fold) (Somavilla et al., 2019). Independently of this, no control failures of acephate, thiamethoxam and lambda-cyhalothrin against *D. furcatus* have been reported in the field in Brazil (Chocorosqui and Panizzi, 2004; Ávila and Duarte, 2012; Netto et al., 2015).

The relatively lower susceptibility of *E. heros* to lambda-cyhalothrin detected in our study can be explained by the prolonged and frequent use of this active ingredient and also due to the low dispersion capacity of this species (Soares et al., 2018). In addition, the overlapping or succession of soybean, cotton and maize production in Central Brazil may expose *E. heros* to this active ingredient for a long time, favoring the evolution of resistance. This could be intensified by the genetic diversity of this species which demonstrates a distinct hybrid zone

in central Brazil between two distinct lineages of *E. heros* (northern and southern) caused by host range expansion, mainly onto cotton, favoring the process of hybridization of this species in the Brazilian Cerrado. A consequence of this hybridization process is a high risk of the development of resistance in *E. heros* populations in central Brazil (Soria et al., 2017; Corrêa et al., 2019; Zucchi et al., 2019). On the other hand, in southern Brazil stink bugs present a reproductive oligopause due to low temperatures during the winter season, reducing their infestations on cultivated plants and their exposure to insecticides. This behavior favors local or regional adaptation to insecticides, due to the low dispersal capacity and genetic similarity among populations of *E. heros* in the same geographic regions (Mourão and Panizzi, 2002; Sosa-Gómez et al., 2004; Soares et al., 2018).

Based on our results, the resistance levels to insecticides in Brazilian populations of *E. heros* and *D. furcatus* can be considered low. Regardless, it will be necessary to develop effective IRM plans to prevent the evolution of resistance. From an IRM perspective, applying insecticide when the action thresholds are reached (Bueno et al., 2013; Soria et al., 2017; Gomes et al., 2019) and rotating the use of insecticides with distinct modes of action would maintain the susceptibility of stink bugs to insecticides (IRAC, 2020). The development of resistance could also be retarded by using other available IPM tactics, such as biological control with *Telenomus podisi* (Ashmead, 1893), *Trissolcus basalis* (Wollaston, 1858) (Hymenoptera: Platygastridae) and *Beauveria bassiana* (Bals. Criv.) Vuill., 1912 (Ascomycetes: Clavicipitaceae) (Lopes et al., 2015; Queiroz et al., 2018; Lagôa et al., 2019). Eliminating non-cultivated host plants (such as weeds) that are used as a food source for stink bugs during the off-season from crop production areas could also reduce infestations and, consequently, exposure to insecticides.

In summary, in this study we report the susceptibility of *E. heros* and *D. furcatus* to insecticides in Brazil and estimated diagnostic doses for monitoring the susceptibility of *E.*

heros to acephate, bifenthrin, thiamethoxam and lambda-cyhalothrin in Brazil. Future efforts should be concentrated on collecting representative samples of this sucking species in soybean fields and subsequent exposure to the diagnostic doses defined here, to monitor the frequency of resistance.

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Table 1

Populations of *E. heros* and *D. furcatus* used to characterize the susceptibility to technical grade insecticides in topical bioassays.

Collection site	<i>n</i>	Latitude	Longitude	Date	Host plant
<i>Euschistus heros</i>					
Susceptible of reference	-	22°41'48" S	47°38'43" W	2013	Soybean
Catuípe, RS	300	28°13'23" S	53°58'47" W	March 2019	Soybean
Cruz Alta, RS	1400	28°34'36" S	53°37'09" W	March 2019	Soybean
Maçambará, RS	1600	29°03'27" S	55°45'51" W	March 2019	Soybean
Pântano Grande, RS	1250	30°17'31" S	52°13'44" W	January 2019	Soybean
Quevedos, RS	1700	29°16'18" S	53°59'29" W	February 2019	Soybean
Restinga Seca, RS	2000	29°44'00" S	53°26'50" W	March 2019	Soybean
Santa Maria, RS	1550	29°42'58" S	53°44'03" W	February 2019	Soybean
Santiago, RS	3500	29°01'36" S	54°41'48" W	February 2019	Soybean
Londrina, PR	3248	23°09'26" S	51°15'01" W	March 2019	Soybean
Rio Verde, GO	1519	17°54'03" S	50°47'58" W	April 2019	Soybean
<i>Dichelops furcatus</i>					
São Sepé 1, RS	850	30°22'30" S	53°42'08" W	March 2019	Soybean
Catuípe, RS	1000	28°11'30" S	53°59'56" W	October 2019	Wheat
Santa Maria, RS	300	29°42'57" S	53°44'03" W	October 2019	Wheat
São Sepé 2, RS	350	30°07'30" S	53°37'43" W	November 2019	Oat

Table 2

Lethal doses of technical grade insecticides (LD; µg a.i./stink bug) against adults of *E. heros* in topical bioassay.

Population	n	Fit of probit lines			LD ₅₀ (95% CI) ^{b,c}	LD ₉₀ (95% CI) ^{b,c}	RR ₅₀ ^d
		Slope ± SE	χ ² (df ^a)	P			
Acephate							
Susceptible of reference	150	4.07 ± 0.82	0.55 (3)	0.91	0.29 (0.21–0.36) a	0.60 (0.48–0.87) a	-
Santiago, RS	180	3.27 ± 0.99	3.81 (3)	0.28	0.22 (0.11–0.28) a	0.54 (0.41–1.20) a	0.8
Cruz Alta, RS	175	2.89 ± 0.71	9.62 (4)	0.13	0.25 (0.10–0.47) a	0.68 (0.38–5.61) a	0.9
Londrina, PR	125	3.17 ± 0.54	0.61 (2)	0.74	0.35 (0.26–0.46) a	0.90 (0.67–2.89) a	1.2
Santa Maria, RS	175	2.78 ± 0.62	2.92 (4)	0.57	0.39 (0.27–0.51) a	1.13 (0.81–2.33) a	1.3
Quevedos, RS	125	4.73 ± 1.00	0.70 (2)	0.70	0.39 (0.29–0.47) a	0.72 (0.58–1.06) a	1.3
Rio Verde, GO	150	3.72 ± 0.78	5.35 (3)	0.15	0.40 (0.30–0.50) a	0.89 (0.69–1.46) a	1.4
Maçambará, RS	125	5.08 ± 1.31	2.97 (2)	0.23	0.58 (0.43–0.70) ab	1.04 (0.83–1.72) a	2.0
Restinga Seca, RS	150	3.87 ± 0.86	2.65 (3)	0.45	0.69 (0.56–0.87) b	1.49 (1.11–2.89) a	2.4
Thiamethoxam							
Susceptible of reference	175	2.91 ± 0.51	1.41 (4)	0.84	0.0039 (0.0028–0.0051) a	0.011 (0.008–0.017) a	-
Londrina, PR	175	2.94 ± 0.50	3.22 (4)	0.52	0.0046 (0.0033–0.0058) a	0.012 (0.009–0.019) a	1.2
Rio Verde, GO	150	3.28 ± 0.53	4.92 (3)	0.18	0.0047 (0.0037–0.0058) a	0.011 (0.009–0.017) a	1.2
Santiago, RS	150	1.78 ± 0.40	1.44 (3)	0.70	0.0097 (0.0048–0.015) a	0.051 (0.030–0.14) b	2.5
Santa Maria, RS	175	1.89 ± 0.44	9.35 (4)	0.08	0.011 (0.0031–0.023) a	0.052 (0.024–0.64) b	2.8
Restinga Seca, RS	150	2.77 ± 0.61	3.69 (3)	0.30	0.013 (0.0058–0.019) a	0.037 (0.026–0.061) b	3.3
Maçambará, RS	150	3.60 ± 0.87	2.81 (3)	0.42	0.032 (0.021–0.040) b	0.072 (0.056–0.12) b	8.2
Bifenthrin							
Susceptible of reference	150	2.15 ± 0.80	5.16 (3)	0.16	0.021 (0.0019–0.034) a	0.082 (0.053–0.43) a	-
Rio Verde, GO	150	3.36 ± 0.86	3.89 (3)	0.27	0.030 (0.018–0.038) a	0.072 (0.055–0.13) a	1.4
Londrina, PR	150	2.76 ± 0.53	4.00 (3)	0.26	0.033 (0.023–0.043) a	0.097 (0.070–0.17) a	1.6
Santa Maria, RS	175	2.38 ± 0.41	0.54 (4)	0.97	0.038 (0.026–0.050) ab	0.13 (0.10–0.22) a	1.8
Restinga Seca, RS	175	3.07 ± 0.65	1.00 (4)	0.91	0.045 (0.023–0.064) ab	0.12 (0.087–0.18) a	2.1
Pântano Grande, RS	175	2.07 ± 0.38	1.76 (4)	0.78	0.046 (0.029–0.064) ab	0.19 (0.13–0.37) a	2.2
Quevedos, RS	150	2.67 ± 0.72	2.07 (3)	0.56	0.061 (0.025–0.087) ab	0.18 (0.13–0.36) a	2.9
Santiago, RS	210	3.17 ± 0.68	8.48 (4)	0.07	0.066 (0.031–0.10) ab	0.17 (0.11–0.54) a	3.1
Maçambará, RS	150	3.84 ± 1.00	1.84 (3)	0.61	0.084 (0.044–0.11) b	0.18 (0.14–0.27) a	4.0
Cruz Alta, RS	175	2.56 ± 0.43	2.66 (4)	0.62	0.10 (0.076–0.13) ab	0.32 (0.23–0.57) a	4.9
Lambda-cyhalothrin							
Susceptible of reference	175	1.66 ± 0.35	1.45 (4)	0.84	0.023 (0.0092–0.040) a	0.14 (0.08–0.35) a	-
Pântano Grande, RS	150	2.12 ± 0.46	3.08 (3)	0.38	0.073 (0.042–0.10) b	0.29 (0.19–0.68) ab	3.1
Londrina, PR	150	1.84 ± 0.35	0.20 (3)	0.98	0.081 (0.051–0.12) bc	0.40 (0.25–0.96) ab	3.6
Santiago, RS	270	1.57 ± 0.29	3.72 (6)	0.71	0.12 (0.060–0.17) bc	0.76 (0.51–1.53) b	5.4
Rio Verde, GO	175	1.61 ± 0.30	1.80 (4)	0.77	0.12 (0.075–0.17) bc	0.74 (0.44–2.00) b	5.4
Quevedos, RS	200	2.13 ± 0.48	1.54 (5)	0.91	0.17 (0.072–0.25) bc	0.67 (0.47–1.22) b	7.6
Santa Maria, RS	200	1.85 ± 0.32	5.00 (5)	0.41	0.17 (0.11–0.24) c	0.84 (0.55–1.71) b	7.6
Restinga Seca, RS	175	2.59 ± 0.72	8.26 (4)	0.08	0.24 (0.054–0.43) bc	0.69 (0.38–11.42) b	10.8
Cruz Alta, RS	175	1.65 ± 0.28	2.67 (4)	0.61	0.35 (0.22–0.51) c	2.08 (1.27–4.91) b	15.7

^aDegrees of freedom.

^bLD₅₀: dose of technical grade insecticide required to kill 50% of stinks bugs in the observation period of 72 hours. Similarly, LD₉₀ is the dose of insecticide required to kill 90% of stinks bugs tested.

^cLD₅₀, LD₉₀ and RR₅₀ values designated by different letters within a column are significantly different from each other through non-overlap of 95% CIs.

^dResistance Ratio (RR₅₀) = (LD₅₀ of field population)/(LD₅₀ of susceptible of reference).

Table 3

Lethal doses of technical grade insecticides (LD; µg a.i./stink bug) against adults of *D. furcatus* in topical bioassay.

Population	n	Fit of probit lines			LD ₅₀ (95% CI) ^{b,c}	LD ₉₀ (95% CI) ^{b,c}	RR ₅₀ ^d
		Slope ± SE	χ ² (df ^a)	P			
Acephate							
São Sepé 2, RS	150	4.13 ± 0.84	0.48 (3)	0.92	0.30 (0.21–0.38) a	0.59 (0.48–1.46) a	-
Catuípe, RS	175	4.25 ± 0.94	6.31 (4)	0.18	0.53 (0.38–0.65) a	1.02 (0.83–1.46) a	1.8
Thiamethoxam							
Catuípe, RS	200	1.81 ± 0.31	3.29 (5)	0.65	0.066 (0.038–0.094) a	0.30 (0.21–0.55) a	-
Santa Maria, RS	150	2.82 ± 0.48	3.51 (3)	0.32	0.073 (0.052–0.094) a	0.19 (0.15–0.34) a	1.1
São Sepé 1, RS	175	2.23 ± 0.41	3.04 (4)	0.55	0.14 (0.08–0.19) a	0.47 (0.33–0.76) a	2.1
Lambda-cyhalothrin							
Santa Maria, RS	150	1.57 ± 0.38	2.07 (3)	0.56	0.27 (0.13–0.40) a	1.57 (0.94–5.57) a	-
São Sepé 2, RS	175	1.93 ± 0.98	3.49 (4)	0.48	0.34 (0.21–0.47) a	1.41 (0.93–3.09) a	1.3
Catuípe, RS	225	1.41 ± 0.27	1.00 (6)	0.99	0.56 (0.32–0.88) a	4.02 (2.20–12.52) a	2.1

^aDegrees of freedom.

^bLD₅₀: dose of technical grade insecticide required to kill 50% of stinks bugs in the observation period of 72 hours. Similarly, LD₉₀ is the dose of insecticide required to kill 90% of stinks bugs tested.

^cLD₅₀, LD₉₀ and RR₅₀ values designated by different letters within a column are significantly different from each other through non-overlap of 95% CIs.

^dResistance Ratio (RR₅₀) = (LD₅₀ of field population)/(LD₅₀ of susceptible of reference).

4 DISCUSSÃO

O manejo dos percevejos *E. heros* e *D. furcatus* em áreas de produção de soja, milho, trigo e algodão é um dos grandes desafios da agricultura brasileira. A principal tática de controle destas espécies é a utilização de inseticidas químicos sintéticos, o que pode favorecer a evolução da resistência. Populações geograficamente distintas de *E. heros* apresentaram pequena variação na suscetibilidade ao acefato e tiameksam (razão de resistência de até 15,1), em bioensaios de imersão de vagens. No entanto, as mesmas populações foram menos suscetíveis a bifentrina (razão de resistência de até 23,8 vezes) e lambda-cialotrina (razão de resistência de até 63,6 vezes). Além disso, quando populações de *E. heros* foram expostas a lambda-cialotrina, em bioensaios de aplicação tópica, ocorreu variação de até 15,7 vezes na suscetibilidade de populações de campo. Além disso, na dose de campo de lambda-cialotrina, as taxas de mortalidade de populações de *E. heros* variaram de 0 a 68%. Por outro lado, as doses registradas de acefato, lambda-cialotrina + tiameksam e bifentrina + acetamiprido ocasionaram mortalidade variando de 58,3 a 100%. Nesse mesmo estudo, detectou-se que populações de *D. furcatus* apresentaram pequena variação na suscetibilidade a acefato, tiameksam, bifentrina e lambda-cialotrina.

A menor suscetibilidade de populações de *E. heros* a bifentrina e lambda-cialotrina indica uma maior probabilidade para evolução da resistência. Diante destes resultados recomenda-se reduzir o uso de inseticidas do grupo químico dos piretroides, especialmente bifentrina e lambda-cialotrina para o manejo de *E. heros* no Brasil. Para evitar ou retardar a resistência é essencial realizar a rotação de inseticidas com modo de ação distintos e usar inseticidas somente quando o nível de controle for atingido, dando preferência para aqueles que apresentam maior toxicidade para *E. heros*. Esforços futuros devem se concentrar no monitoramento da suscetibilidade de *E. heros* e *D. furcatus*, especialmente em regiões ou áreas de produção com histórico de altas infestações dessas espécies, para identificar mudanças na suscetibilidade, devido a exposição constante os inseticidas. Além do controle químico, a utilização outras estratégias de MIP, como parasitoides de ovos e fungos entomopatogênicos, pode auxiliar no controle destas espécies e também retardar a evolução da resistência aos inseticidas químicos sintéticos.

5 CONCLUSÕES

Em bioensaios de imersão de vagens, populações de *E. heros* tem maior variação na suscetibilidade a lambda-cialotrina e bifentrina e menor variação na suscetibilidade ao acefato e tiametoxam. As populações de *D. furcatus* apresentam similar suscetibilidade a acefato, tiametoxam, bifentrina e lambda-cialotrina.

Em bioensaios de aplicação tópica, populações de *E. heros* demonstram maior variação na suscetibilidade a lambda-cialotrina do que a acefato, tiametoxam e bifentrina. As populações de *D. furcatus* tem similar suscetibilidade a acefato, tiametoxam e lambda-cialotrina.

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