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Regis Felipe Stacke

RESISTÊNCIA DE *Chrysodeixis includens* (LEPIDOPTERA: NOCTUIDAE) À INSETICIDAS: LEVANTAMENTO, HERANÇA E CUSTO ADAPTATIVO

Santa Maria, RS
2020

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Tese apresentada ao Programa de Pós-Graduação em Agronomia, Área de concentração em Produção Vegetal, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Doutor em Agronomia**.

Orientador: Prof. Dr. Jerson V. Carús Guedes

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Aos meus pais Ivanete Batista e Regio Stacke, aos meus irmãos Regina Stacke e Giliardi Brair Stacke por sempre estarem ao meu lado, e pelo incentivo e apoio em minha formação pessoal e profissional.

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“O certo é certo, mesmo que ninguém o faça. O errado é errado, mesmo que todos se enganem sobre ele.”

G. K. Chesterton

“Tudo o que um sonho precisa para ser realizado é alguém que acredite que ele possa ser realizado.”

Roberto Shinyashiki

RESUMO

RESISTÊNCIA DE *Chrysodeixis includens* (LEPIDOPTERA: NOCTUIDAE) À INSETICIDAS: LEVANTAMENTO, HERANÇA E CUSTO ADAPTATIVO

Autor: Regis Felipe Stacke
Orientador: Prof. Dr. Jerson Vanderlei Carús Guedes

Chrysodeixis includens (Walker, 1858) (Lepidoptera: Noctuidae) é uma importante espécie-praga da soja e do algodão no Brasil. O uso de inseticidas é uma das táticas de controle utilizada para essa praga. Visando subsidiar os programas de Manejo Integrado de Pragas (MIP) e o Manejo de Resistência a Insetos (MRI), no primeiro estudo nós caracterizamos a suscetibilidade das populações brasileiras de *C. includens* a inseticidas e, no segundo estudo, nós selecionamos uma linhagem resistente à lambda-cialotrina e avaliamos a herança da resistência, o custo adaptativo e a resistência cruzada a outros piretroides. No primeiro estudo, populações de campo de *C. includens* foram coletadas em áreas de soja durante as safras de 2016-2017 e 2017-2018. No laboratório, larvas L2/L3 foram expostas a inseticidas aplicados sobre a dieta ou em bioensaio com aplicação tópica. Populações de campo de *C. includens* apresentaram níveis baixos de resistência aos inseticidas espinetoram (CL_{50} = 0,074 a 0,25 μg i.a. cm^{-2}), indoxacarbe (CL_{50} = 0,46 a 0,94 μg i.a. cm^{-2}), tiodicarbe (CL_{50} = 9,14 a 36,61 μg i.a. cm^{-2}), clorantraniliprole (CL_{50} = 0,15 a 0,57 μg i.a. cm^{-2}), flubendiamide (CL_{50} = 0,45 a 2,01 μg i.a. cm^{-2}) e clorfenapir (CL_{50} = 0,15 a 0,25 μg i.a. cm^{-2}); com Razão de Resistência (RR) inferior a 16 vezes. Em contraste, as populações de *C. includens* apresentaram níveis elevados de resistência à lambda-cialotrina (CL_{50} = 3,71 a 9,54 μg i.a. cm^{-2}), metoxifenozide (CL_{50} = 0,67 a 4,23 μg i.a. cm^{-2}), novaluron (CL_{50} = 27,52 a 77,63 μg i.a. cm^{-2}) e teflubenzuron (CL_{50} = 13,41 a 73,02 μg i.a. cm^{-2}). Para estes inseticidas a RR foi de 62,5, 63, 1.553 e 5.215 vezes, respectivamente. No segundo estudo, os valores estimados de DL_{50} para lambda-cialotrina das linhagens suscetível e resistente foram de 0,0015 e 0,11 μg i.a. larva^{-1}, respectivamente, representando uma RR de 73,3 vezes. Os valores de DL_{50} dos heterozigotos foram 0,016 e 0,017 μg i.a. larva^{-1}, indicando uma herança autossômica da resistência. Nos ensaios com folhas de soja pulverizadas com o inseticida lambda-cialotrina, a linhagem resistente apresentou sobrevivência > 85%. Por outro lado, linhagens heterozigotas e suscetíveis tiveram sobrevivência <64 e 8%, respectivamente, indicando uma resistência incompletamente dominante na dose de campo de lambda-cialotrina. A linhagem resistente também mostrou resistência cruzada à deltametrina (RR = 6,2 vezes) e cipermetrina (RR = 22,5 vezes). Além disso, os insetos resistentes tiveram redução de 71% na sobrevivência até a fase adulta e de 48% na taxa reprodutiva, quando comparadas à linhagem suscetível, indicando a presença de custo adaptativo da resistência. A seleção de uma linhagem de *C. includens* resistente à lambda-cialotrina demonstrou que o padrão de herança da resistência é autossômico, incompletamente dominante e associado com custo adaptativo. Os resultados apresentados aqui são importantes para programas de MIP e MRI em *C. includens* no Brasil.

Palavras chaves: Lagarta-falsa-medideira. Controle químico. Herança da resistência. Modulador de canal de sódio. Manejo da Resistência de Insetos

ABSTRACT

RESISTANCE OF *Chrysodeixis includens* (LEPIDOPTERA: NOCTUIDAE) TO INSECTICIDES: SURVEY, INHERITANCE AND FITNESS COST

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ADVISOR: PROF. DR. JERSON VANDERLEI CARÚS GUEDES

Chrysodeixis includens (Walker, 1858) (Lepidoptera: Noctuidae), is an important pest of soybean and cotton in Brazil. The use of insecticides is one of the main control tactics against this pest. To support Integrated Pest Management (IPM) and Insect Resistance Management (IRM) programs, in the first study, we characterized the susceptibility of Brazilian populations of *C. includens* to insecticides and; in the second study, we selected a strain with resistance to lambda-cyhalothrin and evaluated the inheritance, fitness costs and cross-resistance to other pyrethroids. In the first study, field populations were collected from soybean fields during the 2016–2017 and 2017–2018 crop seasons. In the laboratory, late L2/early L3 larvae were exposed to insecticides in diet-overlay or topical bioassays. Field populations of *C. includens* showed low levels of resistance to spinetoram (LC_{50} = 0.074 to 0.25 μg a.i. cm^{-2}), indoxacarb (LC_{50} = 0.46 to 0.94 μg a.i. cm^{-2}), thiodicarb (LC_{50} = 9.14 to 36.61 μg a.i. cm^{-2}), chlorantraniliprole (LC_{50} = 0.15 to 0.57 μg a.i. cm^{-2}), flubendiamide (LC_{50} = 0.45 to 2.01 μg a.i. cm^{-2}), and chlorfenapyr (LC_{50} = 0.15 to 0.25 μg a.i. cm^{-2}); the resistance ratios (RR) were less than 16-fold. In contrast, *C. includens* populations showed high levels of resistance to lambda-cyhalothrin (LC_{50} = 3.71 to 9.54 μg a.i. cm^{-2}), methoxyfenozide (LC_{50} = 0.67 to 4.23 μg a.i. cm^{-2}), novaluron (LC_{50} = 27.52 to 77.63 μg a.i. cm^{-2}), and teflubenzuron (LC_{50} = 13.41 to 73.02 μg a.i. cm^{-2}). The RRs relative to a susceptible population was up to 62.5-, 63-, 1,553- and 5,215-fold, respectively. Based on the second study, estimated LD_{50} values of lambda-cyhalothrin for the susceptible and resistant strains were 0.0015 and 0.11 μg a.i. $larva^{-1}$, respectively, representing a resistance ratio (RR) of 73.3-fold. The LD_{50} values of heterozygous were 0.016 and 0.017 μg a.i. $larva^{-1}$, indicating an autosomal inheritance of resistance. On lambda-cyhalothrin sprayed-leaves, the resistant strain showed > 85% survival. By contrast, heterozygous and susceptible strains had < 64 and 8% survival, respectively, indicating an incompletely dominant resistance at the field dose of lambda-cyhalothrin. The resistant strain also showed cross-resistance to deltamethrin (RR = 6.2-fold) and cypermethrin (RR = 22.5-fold). Fitness costs studies showed that resistant insects had 71 and 48% lower survival until adult and reproductive rates, respectively, when compared to the Sus strain. Thus, the selection of a *C. includens* strain resistant to lambda-cyhalothrin showed that resistance inheritance patterns were autosomal, incompletely dominant and associated with substantial fitness costs. The results presented here support the IPM and IRM programs for *C. includens* in Brazil.

Key words: Soybean looper. Chemical control. Resistance inheritance. Sodium channel modulator. Insect Resistance Management.

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1 INTRODUÇÃO

Chrysodeixis includens (Walker, 1858) (Lepidoptera: Noctuidae), conhecida como lagarta-falsa-medideira, é uma das principais espécies-praga da soja e do algodão, que gera preocupação aos agricultores (SANTOS, 2011; PANIZZI, 2013; BALDIN et al., 2014; BORTOLOTTO et al., 2015; GUEDES et al., 2015). A ocorrência de *C. includens* já foi reportada em 175 plantas hospedeiras, distribuídas em 39 famílias (BALDIN et al., 2014; SPECHT et al., 2015). Apesar de se desenvolver em uma grande variedade de hospedeiros, essa espécie apresenta melhor adaptação nas culturas da soja (*Glycine max* (L.) Merrill) e do algodão (*Gossypium hirsutum* L.), e com menor ocorrência em feijão (*Phaseolus vulgaris* L.) e girassol (*Helianthus annuus* L.) (EICHLIN; CUNNINGHAM, 1978; BALDIN et al., 2014; ANDRADE et al., 2016).

Até meados da década de 1990, *C. includens* era considerada uma praga-secundária em soja (MORAES et al., 1991; GUEDES et al., 2015) mas, a partir de 1990 tem se tornado um sério problema fitossanitário, com vários surtos isolados ou associados à lagarta-da-soja (BERNARDI, 2012). As modificações que ocorreram no sistema de produção de soja no Brasil com o uso intensivo de fungicidas para o controle da ferrugem asiática, contribuiu para a redução de fungos que realizam o controle biológico natural de *C. includens* (SOSA-GÓMEZ, 2005), intensificando a ocorrência da lagarta-falsa-medideira. Além disso, a capacidade dessa espécie de se desenvolver em diferentes plantas cultivadas (SPECHT et al., 2015), sua capacidade reprodutiva com várias gerações por ano (REID; GREENE, 1973; BOLDT et al., 1975; WILLE et al., 2017) e dispersão entre regiões e culturas (PALMA et al., 2015), favorecem a ocorrência de infestações durante todas as safras de soja e do algodão no Brasil.

O controle da lagarta-falsa-medideira tem sido realizado pelo uso de inseticidas químicos sintéticos (MARTINS; TOMQUELSKI, 2015; RAMOS et al., 2017; PERINI et al., 2019) e plantas transgênicas que expressam proteínas inseticidas de *Bacillus thuringiensis* (Bt) (BERNARDI et al., 2012; SORGATTO et al., 2015; YANO et al., 2015; MARQUES et al., 2016). Recentemente, o baculovirus (por exemplo, *Chrysodeixis includens* nucleopolyhedrovirus - ChinNPV) também está sendo utilizado para o controle dessa espécie (MURARO et al., 2018; GODOY et al., 2019). Atualmente, são realizadas de duas a seis pulverizações de inseticidas contra lepidópteros-praga na cultura da soja (PANIZZI, 2013; BORTOLOTTO et al., 2015). Entretanto, falhas no controle de *C. includens* têm sido constatadas com frequência, pois essa espécie é mais tolerante aos inseticidas normalmente utilizados para o seu controle, por apresentar maior dificuldade de controle quando comparada a outras espécies que ocorrem em soja (BERNARDI, 2012). A maior tolerância da lagarta *C. includens* aos inseticidas, quando comparada a outras espécies, se deve a capacidade desta de destoxificar e excretar determinados inseticidas antes mesmo da ativação do composto *in vivo* (MARTIN; BROWN, 1984). Capacidade relacionada a presença de algumas enzimas, como glutationa-transferases, monooxigenases e hidrolases que são as principais responsáveis pela baixa suscetibilidade da lagarta-falsa-medideira a inseticidas moduladores dos canais de sódio, como os piretroides (DOWD; SPARKS, 1986; ROSE et al.,1990; THOMAS; BOETHEL, 1995; THOMAS et al., 1996).

No entanto, muitos dos casos de falhas de controle também estão associados as dificuldades de o inseticida em atingir o alvo, pois essa espécie tem o hábito de se abrigar no terço médio do dossel foliar, o que reduz a exposição aos inseticidas e favorece a sua sobrevivência (GUEDES et al., 2015; ZULIN et al., 2018; FUNICHELLO et al., 2019). Além destes aspectos, o uso constante de inseticidas para o controle de *C. includens* pode ter selecionado populações resistentes, estando as dificuldades de controle associadas à evolução da resistência. Nos EUA, o primeiro caso de resistência de *C. includens* a um inseticida foi relatado para o piretroide permetrina em 1987 (LEONARD et al., 1990). Segundo a base de dados da Universidade Estadual de Michigan (www.pesticideresistance.org), essa espécie possui relatos de resistência nos EUA ao acefato, BHC, DDT, cipermetrina, deltametrina, permetrina, teflutrim, fenvalerato, metomil, paratiom metílico, tiodicarbe e *Bacillus thuringiensis* (FELLAND et al., 1990; BOETHEL et al., 1992; THOMAS et al., 1996; MASCARENHAS; BOETHEL 1997; MASCARENHAS; BOETHEL 2000).

O processo determinante para a evolução da resistência de insetos a inseticidas é o uso excessivo ou inadequado do mesmo ingrediente ativo, que exerce grande pressão de seleção nas populações do insetos-praga. Do ponto de vista prático, a resistência marca a mudança na composição genética de uma população em resposta a pressão de seleção, o que pode afetar o controle a campo (SAWICKI, 1987). A evolução e estabilidade da resistência são determinadas pela maior adaptação dos indivíduos resistentes em relação aos indivíduos suscetíveis na presença do agente de seleção. Essa adaptação é favorecida pelo grande número de gerações da praga, que ocorrem durante o ciclo da cultura, porém os indivíduos resistentes, em geral, apresentam um maior custo adaptativo do que os indivíduos suscetíveis (SOSA-GÓMEZ; OMOTO, 2012). Este maior custo adaptativo dos indivíduos resistentes, como uma menor capacidade reprodutiva pode comprometer a reprodução e sobrevivência da espécie em ambientes livres da pressão de seleção (GEORGHIOU, 1972; ROUSH; MCKENZIE, 1987; GASSMANN et al., 2009). Quanto mais rápido é o ciclo de vida do inseto, não havendo custo adaptativo associado à resistência, mais rapidamente os indivíduos resistentes serão capazes de se estabelecer e aumentar de frequência no agroecossistema.

A constatação, por parte dos agricultores, de falhas de controle de um inseticida pelo simples fato de encontrarem indivíduos sobreviventes a uma determinada dose do princípio ativo aplicado, faz com que os produtores aumentem a dose e possivelmente a frequência do uso de determinados produtos, o que resulta em maior pressão de seleção e, consequentemente, maior perda de indivíduos suscetíveis, ocasionando um aumento na proporção de indivíduos resistentes. Assim, devido *C. includens* apresentar elevado fluxo gênico entre populações no Brasil (PALMA et al., 2015), os indivíduos resistentes têm o potencial de “dispersar” o alelo da resistência entre as diferentes populações da praga.

Nesse contexto, avaliar a suscetibilidade de *C. includens* aos inseticidas utilizados em soja é essencial para subsidiar estratégias de controle para o Manejo Integrado de Pragas (MIP) e para o Manejo da Resistência de Insetos (MRI). Para o estabelecimento de um programa de MRI é necessária a caracterização da suscetibilidade de populações geograficamente distintas da praga-alvo de determinado inseticida. Estudos adicionais como o entendimento da herança e o custo adaptativo da resistência, e a resistência cruzada entre moléculas inseticidas, também são fundamentais para subsidiar estratégias de MRI.

2 REVISÃO DE LITERATURA

2.1 ASPECTOS BIOECOLÓGICOS DE *Chrysodeixis includens* (WALKER, 1858)

A lagarta-falsa-medideira, *C. includens,* é a espécie de maior ocorrência e maior importância econômica da subfamília Plusiinae que ataca a cultura da soja no Brasil (MORAES et al., 1991). Sua ocorrência foi reportada desde o Norte dos EUA até o Sul da América do Sul (ALFORD; HAMMOND JUNIOR, 1982) e também na Austrália (EICHLIN; CUNNINGHAM, 1978). No Brasil, a lagarta-falsa-medideira ocorre em todas as regiões produtoras de soja, com surtos populacionais mais frequentes e abundantes (SOSA-GÓMEZ; OMOTO, 2012). Essa espécie também tem importância crescente no algodão, pois essa cultura é usualmente cultivada após a soja, de onde as mariposas dispersam e causam infestações (SANTOS, 2011).

Chrysodeixis includens foi considerada praga secundária da soja no Brasil até a década de 90, porém as mudanças fitossanitárias verificadas entre os anos 1990 e 2010 alteraram a dinâmica das espécies de lagartas da soja, acarretando um aumento da ocorrência da lagarta-falsa-medideira (BUENO et al., 2007; GUEDES et al., 2015). Dentre as principais mudanças está a ocorrência da ferrugem asiática da soja, *Phakopsora pachyrhizi*, que ocasionou um aumento das aplicações de fungicidas e como consequência a menor incidência de fungos entomopatogênicos, como a *Nomuraea rileyi*, que contribuía para o controle biológico natural de *C. includens*.

A lagarta-falsa-medideira é uma espécie polífaga com capacidade de se desenvolver em 175 plantas hospedeiras, pertencentes a 39 famílias (BALDIN et al., 2014; SPECHT et al., 2015). Embora se alimente de uma vasta gama de hospedeiros, essa espécie tem melhor adaptação à soja e ao algodão, com ocorrências menos frequentes em feijão e girassol (EICHLIN; CUNNINGHAM, 1978; BALDIN et al., 2014; ANDRADE et al., 2016). A polifagia é uma característica que pode colaborar com a dinâmica populacional e condição de praga, uma vez que as populações podem se desenvolver simultaneamente em diferentes plantas hospedeiras, o que favorece sua dispersão dentro de uma região ou entre regiões (MOSCARDI et al., 2012).

Os adultos de *C. includens* são mariposas com 35 mm de envergadura, asas anteriores de coloração escura, com duas manchas prateadas brilhantes na parte central, e asas posteriores de coloração marrom (GALLO et al., 2002; SOSA-GÓMEZ et al., 2010). As fêmeas ovipositam em média 700 ovos, que são depositados na face inferior das folhas (JOST; PITRE, 2002). As lagartas são de coloração verde-clara, com listras longitudinais brancas e pontuações pretas, atingindo até 45 mm de comprimento no último instar larval (SOSA-GÓMEZ et al., 2010). Após a eclosão, as larvas iniciam a alimentação e permanecem no terço inferior da planta de soja (ZULIN et al., 2018; FUNICHELLO et al., 2019). O comportamento de *C. includens* de habitar os terços médios e inferiores do dossel da cultura da soja dificulta o controle, fato que determina uma atenção especial à tecnologia de aplicação, pois os inseticidas precisam atingir a parte média e inferior das plantas (GUEDES et al., 2015).

2.2 RESISTÊNCIA DE *C. includens* A INSETICIDAS

Um aspecto preocupante em relação a *C. includens* é sua tolerância natural a inseticidas sintéticos (MARTIN; BROWN, 1984; MOSCARDI et al., 2012; GUEDES et al., 2015). Essa maior tolerância aos inseticidas, quando comparada a outras espécies, está relacionada com a capacidade desta de destoxificar e excretar determinados inseticidas, antes mesmo da ativação do composto *in vivo* (MARTIN; BROWN, 1984). A lagarta-falsa-medideira tem sido controlada mediante uso de inseticidas de quase todos os grupos químicos registrados no Brasil, no entanto, casos de resistência ainda não foram reportados. Todavia, nos EUA, *C. includens* evoluiu para resistência a inseticidas do grupo químico dos piretroides (LEONARD et al., 1990; MASCARENHAS; BOETHEL, 2000). Os primeiros casos de resistência a piretroides foram documentados na Louisiana, após sete anos de utilização desses produtos em campo (FELLAND et al., 1990). O aumento na presença de enzimas esterases é dada como um dos principais mecanismos de resistência a piretroides em *C. includens* (KRANTHI et al., 1997; BYRNE et al., 2000). No Brasil, relatos de falhas de controle de *C. includens* quando do uso de piretroides têm sido frequentes, sendo que atualmente os inseticidas desse grupo químico têm sido pouco utilizados, devido à baixa eficácia.

Por sua vez, o uso de plantas geneticamente modificadas que expressam proteínas inseticidas de *Bacillus thuringienis* Berliner (Bt) como soja e algodão Bt tem sido uma tática adicional de controle de *C. includens* nos últimos anos (BERNARDI et al., 2012; SORGATTO et al., 2015; YANO et al., 2015; MARQUES et al., 2016). Atualmente, estão liberadas para cultivo três eventos de soja Bt (Cry1Ac; Cry1Ac/Cry1F e Cry1A.105/Cry2Ab/Cry1Ac) e sete eventos de algodão Bt (Cry1Ac; Cry1Ac/Cry1F; Cry2Ab2/Cry1Ac; Cry1Ab/Cry2Ae; Vip3A/CrylAc/Cry2Ab2; Cry1Ac/Cry1F/Vip3A e Vip3A/Cry1Ac/Cry1F) (CTNBio, 2019). O uso de soja e algodão Bt se constituem em importantes estratégias para o controle de insetos resistentes a inseticidas, como reportado para *Helicoverpa armigera* (Lepidoptera: Noctuidae) resistente a piretroides na China e na África, que após a utilização de algodão Bt, houve redução do número de aplicações de inseticidas químicos e restabelecimento da suscetibilidade das populações aos piretroides (WU et al., 2005; DJIHINTO et al., 2009; ACHALEKE; BRÉVAULT, 2010).

No entanto, no Brasil, o controle da lagarta-falsa-medideira pelo uso de inseticidas químicos sintéticos é amplamente realizado (MARTINS; TOMQUELSKI, 2015; RAMOS et al., 2017; PERINI et al., 2019). Os inseticidas recomendados e frequentemente utilizados para o manejo de *C. includens* são tiodicarbe e metomil (carbamato), espinetoram e espinosade (espinosinas), indoxacarbe (oxadiazina), metoxifenozide (regulador de crescimento de insetos), lufenuron e teflubenzuron (benzoiluréias), flubendiamide e clorantraniliprole (diamidas) (BROWN, 2012; MARTINS; TOMQUELSKI, 2015; RAMOS et al., 2017; PERINI et al., 2019). Atualmente, são realizadas de duas a seis pulverizações de inseticidas contra lepidópteros-praga na cultura da soja (PANIZZI, 2013; BORTOLOTTO et al., 2015). Essa forte dependência do uso de inseticidas para o controle de *C. includens* favorece a seleção de indivíduos resistentes. Nesse sentido, para prolongar a vida útil dos inseticidas e estabelecer estratégias de manejo da resistência de *C. includens*, se faz necessário entender a suscetibilidade dessa espécie aos inseticidas recomendados para seu controle, bem como identificar possíveis casos de resistência no campo.

2.3 ESTRATÉGIAS DE MANEJO DA RESISTÊNCIA DE INSETOS A INSETICIDAS

O gradual aumento da resistência de insetos a inseticidas tem revelado as limitações dessa estratégia de manejo, alertando para a necessidade de maximizar o tempo de “vida útil” dos inseticidas e planejar sua utilização, de modo a evitar ou retardar a evolução da resistência (GEORGHIOU; TAYLOR, 1986). O planejamento do sistema de produção de cultivos, rotação de inseticidas e o contínuo desenvolvimento de produtos com novos mecanismos de ação são vitais para o manejo da resistência (BERNARDI; OMOTO, 2014).

O termo MRI é usado para descrever um conjunto de práticas que devem ser adotadas com o objetivo de reduzir o potencial de evolução da resistência nas populações das pragas-alvo (ANDOW, 2008) e tem se tornado um importante componente de MIP e vice-versa (CROFT, 1990; MCGAUGHEY; WHALON, 1992; GLASER; MATTEN, 2003). As estratégias de MRI podem ser caracterizadas como proativas ou reativas (ANDOW, 2008). As estratégias reativas são implementadas em decorrência de falhas de controle resultantes da resistência, enquanto as estratégias proativas visam evitar ou retardar a resistência antes da ocorrência de uma falha de controle no campo (BROWN, 1981; DENNEHY, 1987; SAWICKI; DENHOLM, 1987).

O MRI se baseia em quatro fatores: (1) diversificação das causas de mortalidade para que uma praga não seja selecionada para um único mecanismo; (2) redução da pressão de seleção para cada mecanismo de mortalidade; (3) manutenção de um refúgio ou imigração para promover a mistura de indivíduos suscetíveis e resistentes; e (4) predição da suscetibilidade usando monitoramento (MCGAUGHEY; WHALON, 1992). Assim, as estratégias de manejo da resistência são divididas em três grupos: manejo por moderação, manejo por saturação e manejo por ataque múltiplo (GEORGHIOU, 1983).

O manejo por moderação tem como princípio básico a redução da pressão de seleção para preservar os indivíduos suscetíveis numa determinada população. O uso de áreas de refúgio, a redução da frequência de aplicação de inseticidas, o uso de doses reduzidas do inseticida, o uso de produtos de baixa persistência e a recomendação de níveis de controle mais elevados, são estratégias que se colocadas em prática, reduzem a pressão de seleção e podem aumentar a frequência de insetos suscetíveis em uma população da praga e, consequentemente, retardar a evolução de resistência (BERNARDI; OMOTO, 2014; ONSTAD, 2014). Porém, sem o apoio de um programa de MIP eficaz, as tentativas de aumentar o número de indivíduos suscetíveis em uma população, podem simplesmente levar a mais danos causados pela praga. Por isso essas práticas não são amplamente utilizadas em campo.

O manejo por saturação tem por objetivo reduzir as vantagens adaptativas dos indivíduos resistentes mediante uso de altas doses do inseticida. Em teoria, com a utilização de alta dose, o indivíduo heterozigoto se comportaria “fenotipicamente” como homozigoto suscetível, o que acarretaria na sua morte. A mortalidade dos heterozigotos é um dos pontos fundamentais para o MRI, pois no início do processo de evolução da resistência são os principais carreadores dos alelos da resistência (GOULD, 1998). Mas apesar de resultar na morte dos heterozigotos e retardar a evolução da resistência, é preciso considerar os impactos ambientais, econômicos e suas implicações no MIP e no sistema de produção. Além disso, em casos extremos, com o passar do tempo pode haver a seleção dos indivíduos super-resistentes, ou seja, a seleção de resistentes dentro da população resistente (BERNARDI; OMOTO 2014).

O manejo por ataque múltiplo envolve a utilização de dois ou mais produtos com diferentes modos de ação em rotação ou mistura. O princípio da rotação visa a utilização de produtos com diferentes modos de ação. Essa estratégia de manejo da resistência está baseada no fato de que a frequência de resistência a um produto (A) diminui quando produtos alternativos (por ex.: B e C) são utilizados (GEORGHIOU, 1983; TABASHNIK, 1989; ROUSH, 1989). O princípio da mistura de dois produtos (A e B) se baseia no fato que os indivíduos resistentes ao produto A serão controlados pelo produto B e vice-versa. Porém, existe possibilidade de ocorrer indivíduos resistentes ao produto A e B através da resistência múltipla ou resistência cruzada. Sendo assim, as condições para o sucesso da mistura dependem da existência de uma baixa frequência de resistência, ausência de resistência múltipla e persistência biológica semelhante para os dois produtos usados em mistura (ROUSH, 1989; TABASHNIK, 1989).

3 OBJETIVOS

3.1 OBJETIVO GERAL

Estabelecer as bases de um programa de manejo da resistência de *C. includens* a inseticidas utilizados na cultura da soja no Brasil.

3.2 OBJETIVOS ESPECÍFICOS

Caracterizar a suscetibilidade de populações geograficamente distintas de *C. includens* a inseticidas utilizados em soja no Brasil.

Selecionar uma população de *C. includens* resistente ao inseticida lambda-cialotrina.

Caracterizar a herança e o custo adaptativo da resistência de *C. includens* ao inseticida lambda-cialotrina.

Verificar a existência de resistência cruzada com outros piretroides em população de *C. includens* resistente ao inseticida lambda-cialotrina.

4 ARTIGO 1

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Soybean Looper to Insecticides

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Insecticide Resistance and Resistance

Management

Susceptibility of Brazilian Populations of *Chrysodeixis includens* (Lepidoptera: Noctuidae) to Selected Insecticides

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Abstract — Soybean looper (SBL), *Chrysodeixis includens* (Walker, [1858]) (Lepidoptera: Noctuidae), is an important pest of soybean and cotton in Brazil. The use of insecticides is one of the main control tactics against this pest. To support Integrated Pest Management (IPM) and Insect Resistance Management (IRM) programs, we characterized the susceptibility of Brazilian populations of SBL to insecticides. Field populations were collected from soybean fields during the 2016–2017 and 2017–2018 crop seasons. In the laboratory, late L2/early L3 larvae were exposed to insecticides in diet-overlay or topical bioassays. Field populations of SBL showed high susceptibility to spinetoram (LC_{50} = 0.074 to 0.25 μg a.i. per cm^2), indoxacarb (LC_{50} = 0.46 to 0.94 μg a.i. per cm^2), thiodicarb (LC_{50} = 9.14 to 36.61 μg a.i. per cm^2), chlorantraniliprole (LC_{50} = 0.15 to 0.57 μg a.i. per cm^2), flubendiamide (LC_{50} = 0.45 to 2.01 μg a.i. per cm^2), and chlorfenapyr (LC_{50} = 0.15 to 0.25 μg a.i. per cm^2); the resistance ratios were less than 16-fold. In contrast, SBL populations have reduced susceptibility to lambda-cyhalothrin (LC_{50} = 3.71 to 9.54 μg a.i./cm^2), methoxyfenozide (LC_{50} = 0.67 to 4.23 μg a.i. per cm^2), novaluron (LC_{50} = 27.52 to 77.63 μg a.i. per cm^2), and teflubenzuron (LC_{50} = 13.41 to 73.02 μg a.i. per cm^2). The resistance ratios relative to a Lab population (susceptible of reference) was up to 38-, 63-, 1,553- and 5,215-fold, respectively. These high resistance ratios can be associated with resistance evolution. Implications of these findings to IPM and IRM programs are discussed.

Key words: soybean looper, chemical control, toxicity, resistance management

Introduction

In Brazil, soybean (*Glycine max* (L.) Merrill) and cotton (*Gossypium hirsutum* L.) are planted on upwards of 33 million and 940 thousand hectares per year, respectively (Céleres 2017). Among the pests attacking these crops, the soybean looper (SBL), *Chrysodeixis includens* (Walker, [1858]) (Lepidoptera: Noctuidae), is one of the main lepidopteran pest (Santos 2011, Panizzi 2013, Baldin et al. 2014, Bortolotto et al. 2015, Guedes et al. 2015). This species has been reported from 170 other hosts distributed in 39 families (Specht et al. 2015). Although they can develop on several host plants, this species is better adapted to soybean and cotton, but it is also commonly found on common bean (*Phaseolus vulgaris* L.) and sunflower (*Helianthus annuus* L.) (Eichlin and Cunningham 1978, Baldin et al. 2014, Andrade et al. 2016). In the current Brazilian crop production system, this species has several outbreaks per season in soybean and cotton (Bortolotto et al. 2015). Based on life history traits of SBL developing on soybean, they can complete up to seven generations per year (Reid and Greene 1973, Boldt et al. 1975, Wille et al. 2017).

The main Integrated Pest Management tactics for SBL management are the use of chemical control and transgenic plants (soybean and cotton) expressing *Bacillus thuringiensis* Berliner (Bt) proteins. Currently, Bt soybean and cotton represents more than 59 and 54% of the total area used by these crops in Brazil, respectively (Céleres 2017). However, the use of chemical insecticides remains the main tactic against SBL in soybean, cotton, common bean and sunflower crops. In Brazil, two to six insecticide sprays are needed against this pest per soybean season (Panizzi 2013, Bortolotto et al. 2015). Chemical control of this species with insecticides is difficult because it is naturally tolerant to some chemistries (Dowd and Sparks 1986) and larvae are less exposed to insecticide sprays due to their habit of remaining sheltered under the plant's canopy (Papa and Celoto 2007). Therefore, the widespread use of chemical insecticides against SBL associated with the natural tolerance and habit of this species favors the development of resistance to insecticides, if Insect Resistance Management strategies are neglected.

Field-evolved resistance to insecticides in SBL populations has been reported since the 1980s. The first case of resistance to an insecticide in SBL was reported for the pyrethroid permethrin in 1987 in the USA (Leonard et al. 1990). This pest also evolved resistance to DDT, acephate, cypermethrin, tefluthrin, fenvalerate, methomyl, parathion-methyl and thiodicarb (Felland et al. 1990, Boethel et al. 1992, Thomas et al. 1996, Mascarenhas and Boethel 1997, Mascarenhas and Boethel 2000). In Brazil, even though almost all discovered insecticides are used against this pest, no resistance cases have been documented; however, control failures were reported when pyrethroids were applied (Sosa-Gómez and Omoto 2012, Perini 2018). In this context, the objective of the current study was to characterize the susceptibility to selected insecticides in geographically distinct populations of SBL to support IPM and IRM programs.

Material and Methods

Populations. To characterize the susceptibility of SBL to insecticides, 21 field populations were collected during the 2016–2017 and 2017–2018 crop seasons, from representative soybean and cotton growing areas in Brazil (Table 1 and Fig. 1). Insects were collected from commercial fields of non-Bt soybean (500–1200 larvae per location). After collections, SBL larvae were taken to the laboratory and kept on an artificial diet proposed by Greene et al. (1976). In addition to the field populations, a susceptible reference population (Lab) was also tested. The Lab population was collected in soybean in Paulínia, São Paulo, Brazil

(23º45'03"S and 47º07'05"W) and has been kept in the laboratory for more than 6 years, free of selection pressure by insecticides, Bt proteins and microbial-based products.

Insecticides. The commercial and technical grade insecticides used to characterize the susceptibility of Brazilian populations of SBL are listed in Table 2.

Susceptibility to Insecticides in Diet-Overlay Bioassays. In these bioassays, we used the artificial diet proposed by Greene et al. (1976), which, after its preparation, was poured onto 24-well acrylic plates (Costar®, São Paulo, SP, Brazil) (1 ml per well). Afterwards, the insecticides were diluted in distilled water to prepare the range of concentrations to be tested (Table 2). Triton X-100 (Sigma-Aldrich, São Paulo, SP, Brazil) at 0.1% was added to obtain a uniform spread of the solution over the diet surface. The control treatment was composed of distilled water + surfactant. For each SBL population, six to nine concentrations of each insecticide were tested, which were applied to the diet surface with a replication pipette (30 µl per well). The diet surface area in each well was 1.88 cm^2. After a drying period, a single late L2/early L3 larva was added to each well. The plates were sealed with their covers and then placed in a climate controlled room at 27 ± 1°C, 60 ± 10% relative humidity, and a photoperiod of 14:10 [L:D] h. The bioassays were repeated twice for each population, with each concentration being repeated twice per bioassay (two replications of 48 larvae per concentration). Mortality was assessed at 48 (spinetoram, indoxacarb, lambda-cyhalothrin, thiodicarb and chlorfenapyr), 96 (chlorantraniliprole and flubendiamide) or 120 (methoxyfenozide, novaluron and teflubenzuron) h. Larvae without movement after a slight touch with a brush were considered dead.

Susceptibility to Insecticides in Topical Bioassays. In these bioassays, technical grade insecticides were diluted in acetone (99.5% purity; Sigma-Aldrich, São Paulo, SP, Brazil) to prepare the tested concentrations (Table 2). For each insecticide, six to nine concentrations were tested. The concentrations were applied topically to the dorsal thoracic region of late L2/early L3 larvae (1 μl per larva) using a micropipette. Then, larvae were placed in the same acrylic plates containing the artificial diet and environmental conditions described above. The bioassays were repeated twice for each population, with each concentration being repeated twice per bioassay (two replications of 32 larvae per concentration). Mortality was assessed at 48 h, using the same criterion described above.

Statistical Analyses. To assess the relative toxicity of selected insecticides against SBL populations, the LC_{50} and LC_{90} lethal concentrations and their 95% confidence intervals (CIs) were estimated using the Probit analysis (PROC PROBIT, SAS Institute 2000). A likelihood ratio test was used to test the hypothesis that the LCp 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 confidence intervals did not overlap (Savin et al. 1977). Resistance ratios were calculated by dividing the LC_{50} values of the field populations by the corresponding parameter of the Lab population (susceptible of reference). The 95% CIs of resistance ratios were estimated as described by Robertson et al. (2007).

Results

Susceptibility to Nerve Action Insecticides. Larvae from SBL populations were susceptible to insecticides that act on the nervous system, such as spinetoram, indoxacarb and thiodicarb

(Table 3). Field populations exposed to spinetoram, an allosteric modulator of the nicotinic acetylcholine receptor (IRAC MoA group 5), had a significant geographic variation in LC_{50} values, which ranged from 0.074 (population BA-1) to 0.25 (population RS-1) µg a.i. per cm^2. In contrast, the Lab population showed higher susceptibility to this insecticide ($LC_{50} = 0.029$ µg a.i. per cm^2) than field populations. The resistance ratios also varied significantly among the field populations of SBL, from 2.5- to 8.6-fold. Significant variation in susceptibility to spinetoram was also verified for LC_{90} values: 0.30 (population BA-1) to 1.17 (population PR-1) µg a.i. per cm^2.

The lethal concentrations of indoxacarb, a voltage dependent sodium channel blocker (IRAC MoA sub-group 22A), also varied significantly among the field populations of SBL (Table 3). The LC_{50} values ranged from 0.46 (population RS-1) to 0.94 (population BA-1) µg a.i. per cm^2 and LC_{90} from 2.14 (population RS-1) to 6.22 (population BA-1) µg a.i. per cm^2. However, high susceptibility was detected in the Lab population, with LC_{50} and LC_{90} of 0.14 and 1.31 µg a.i. per cm^2, respectively. Based on the LC_{50} values, the resistance ratio to indoxacarb was up to 6.7-fold. A significant variation in susceptibility was also observed in field populations of SBL exposed to the acetylcholinesterase inhibitor thiodicarb (IRAC MoA sub-group 1A) in diet-overlay (LC_{50} from 9.14 to 36.61 µg a.i. per cm^2) and topical bioassays (LC_{50} from 0.09 to 0.25 µg a.i. per larva) (Table 3). The Lab population showed LC_{50} of 6.57 µg a.i. per cm^2 and 0.07 µg a.i. per larva, respectively. These results indicate a significant variation in the resistance ratios, from 1.4- to 5.6-fold resistance.

In contrast to previous insecticides, field populations of SBL presented low susceptibility to the sodium channel modulator lambda-cyhalothrin (IRAC MoA sub-group 3A) in diet-overlay and topical bioassays (Table 3). The LC_{50} values of lambda-cyhalothrin differ significantly among the field populations of SBL, ranging from 3.71 (population BA-2) to 9.54 (population RS-1) µg a.i. per cm^2 in diet-overlay bioassays. On the other hand, the Lab population showed LC_{50} of 0.26 µg a.i. per cm^2 (resistance ratio up to 36.7-fold). Significant variation in LC_{90} values, from 30.59 (population BA-2) to 129.04 (population MS-1) µg a.i. per cm^2, were also detected. When field populations of SBL were exposed to lambda-cyhalothrin in topical bioassays, a lower variation in LC_{50} values was verified (0.04 to 0.10 µg a.i. per larva) (Table 3). However, the Lab population presented a significant higher susceptibility to this insecticide (LC_{50} = 0.0016 µg a.i. per larva) than field populations, indicating a significant variation in the resistance ratios: from 25- to 62.5-fold. The LC_{90} values also had a significant variation among the SBL populations, ranging from 0.20 (population MT-2) to 0.58 (population MS-2) µg a.i. per larva.

Susceptibility to Muscle Action Insecticides. Field populations of SBL also were susceptible to insecticides that act on the muscle system – ryanodine receptor modulators (IRAC MoA group 28) – such as chlorantraniliprole and flubendiamide (Table 4). The LC_{50} of chlorantraniliprole against field populations of SBL varied significantly from 0.15 (population RS-4) to 0.57 (population BA-2) µg a.i. per cm^2, while higher susceptibility was observed for the Lab population LC_{50} = 0.05 µg a.i. per cm^2, indicating a significant variation in the resistance ratios, from 3.0- to 11.4-fold. Significant geographic variation in susceptibility was also verified for LC_{90} values, which ranged from 0.62 (population RS-3) to 3.28 (population MS-2) µg a.i. per cm^2.

Geographic variation in susceptibility of field populations of SBL to flubendiamide was also detected (Table 4). The LC_{50} values ranged from 0.45 (population PR-2) to 2.01 (population RS-3) µg a.i. per cm^2. In contrast, the Lab population had significant higher susceptibility to this insecticide (LC_{50} = 0.13 µg a.i. per cm^2) than field populations, indicating a resistance ratio up to 15.4-fold. Variation in the response to flubendiamide also occurred in LC_{90} values, which ranged from 2.36 (population MT-3) to 17.39 (BA-2) µg a.i. per cm^2.

However, the Lab population (LC_{90} = 2.22 μg a.i. per cm^2) had similar susceptibility of some field populations, such as MT-3, PR-2 and PR-3.

Susceptibility to Energy Metabolism Insecticides. Field populations of SBL had high susceptibility to chlorfenapyr, an inhibitor (uncoupler) of oxidative phosphorylation disrupting the proton gradient (IRAC MoA group 13) (Table 5). No geographic differences in susceptibility to chlorfenapyr were observed in LC_{50} (0.15–0.25 μg a.i. per cm^2) and LC_{90} (0.34–0.48 μg a.i. per cm^2) values among the field populations of SBL and also when compared with the Lab population (0.15 and 0.31 μg a.i. per cm^2, respectively). The resistance ratios (less than 1.7-fold) did not differ among field populations.

Susceptibility to Insect Growth Regulator Insecticides. When exposed to insecticides that act as growth regulators (methoxyfenozide, novaluron and teflubenzuron), larvae from field populations of SBL showed reduced susceptibility (Table 6). For methoxyfenozide, an ecdysone receptor agonist (IRAC MoA group 18), the LC_{50} values ranged from 0.67 (population PR-3) to 4.23 (population BA-2) μg a.i. per cm^2, while LC_{90} varied from 5.29 (population PR-3) to 20.17 (population MT-2) μg a.i. per cm^2. In contrast, the Lab population presented LC_{50} and LC_{90} of 0.067 and 0.44 μg a.i. per cm^2, respectively. Based on LC_{50} values, the resistance ratios to methoxyfenozide varied significantly among field populations, from 11.2- to 63.1-fold.

Larvae from Brazilian populations of SBL showed even less susceptibility to insecticides that act as inhibitors of chitin biosynthesis, type 0 (IRAC MoA group 15) (Table 6). The LC_{50} values of novaluron ranged from 27.52 (population GO-1) to 77.63 (population RS-4) μg a.i. per cm^2, while the Lab population had a significant higher susceptibility (LC_{50} = 0.05 μg a.i. per cm^2) than field populations. These results indicate a significant variation in the resistance ratios, from 550.4- to 1,553-fold. The LC_{90} values of novaluron ranged from 357.85 (population GO-1) to 1,150 (population MS-2) μg a.i. per cm^2, differing from the LC_{90} of the Lab population (0.19 μg a.i. per cm^2).

When SBL larvae were exposed to teflubenzuron, a significant geographic variation in susceptibility was also observed. The LC_{50} values ranged from 13.41 (population MT-2) to 73.02 (population PR-4) μg a.i. per cm^2. The Lab population showed higher susceptibility (LC_{50} = 0.014 μg a.i. per cm^2) to teflubenzuron than field populations, indicating a resistance ratio up to 5,215-fold. Significant variation was also verified in LC_{90} values, which ranged from 298.16 (population MS-3) to 1,229 (population MS-2) μg a.i. per cm^2, while the Lab population had LC_{90} = 0.05 μg a.i. per cm^2.

Discussion

Larvae from Brazilian populations of SBL were susceptible to insecticides that act on the nerve system (i.e. spinetoram, indoxacarb and thiodicarb), muscle system (i.e. chlorantraniliprole and flubendiamide), and insect energy metabolism (i.e. chlorfenapyr). For these insecticides, the resistance ratio was inferior to 8.6-, 15.4- and 1.7-fold, respectively. Similar resistance ratios were reported in SBL populations from the USA to spinosad (2.0-fold) and thiodicarb (3.1-fold) (Mascarenhas and Boethel 1997), from Brazil to methomyl (lower than 1.8-fold) (Yano 2012), flubendiamide (up to 7.5-fold) and indoxacab (3.7-fold) (Schneider, 2015), and from Puerto Rico to thiodicarb: 15-fold (Mascarenhas and Boethel 1997). A similar resistance ratio was also verified in SBL populations from soybean fields in Mississippi and Louisiana, USA to chlorantraniliprole (up to 6.2-fold) and flubendiamide (up to 9.2-fold) (Owen et al. 2013). In other lepidopteran pests, a comparable resistance ratio was reported in *Plutella xylostella* L. (Lepidoptera: Plutellidae) from Central China to chlorfenapyr (less than 13.4-fold) (Xia et al. 2014, Zhang et al. 2016), to indoxacarb in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) from Pakistan (2.0-fold) (Ahmad et al. 2003) and Australia (3.5-fold) (Bird 2015).

Unlike these studies, a larger resistance ratio was reported in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) from Pakistan to indoxacarb: up to 52-fold resistance (Ahmad et al. 2018), *P. xylostella* in Brazil to spinetoram (up to 149-fold resistance) (Lima Neto et al. 2016), and to thiodicarb and methomyl in *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) from Mexico and Puerto Rico (up to 124- and 223-fold resistance, respectively) (Gutierrez-Moreno 2017). For diamides, a similar resistance ratio to chlorantraniliprole was detected in *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) from the USA (4.5-fold resistance) (Temple et al. 2009). Resistance ratios from 1.2- to 21.7-fold to flubendiamide was also verified in *S. exigua* from China (Zhang et al. 2014).

In contrast to previous insecticides, a reduced susceptibility to the sodium channel modulator lambda-cyhalothrin, the ecdysone receptor agonist methoxyfenozide and inhibitors of chitin biosynthesis (i.e. novaluron and teflubenzuron) were detected in Brazilian populations of SBL. For these insecticides, the resistance ratios were up to 62.5-, 63.1-, 1,553- and 5,215-fold, respectively. A large resistance ratio was also reported to permethrin, another sodium channel modulator, in SBL populations from the USA (41- to 426-fold resistance) (Felland et al. 1990, Leonard et al. 1990, Thomas and Boethel 1994, Thomas et al. 1996, Mascarenhas and Boethel 1997). In contrast, a resistance ratio inferior to 8-fold was reported to permethrin in SBL populations from Brazil (Yano 2012), and to lambda-cyhalothrin and methoxyfenozide (up to 5.4-fold) in SBL populations from Mississippi and Louisiana, USA (Leonard et al. 1990, Owen et al. 2013).

In other lepidopterans pests, a low resistance ratio to methoxyfenozide was verified in *S. frugiperda* and *H. armigera* populations from Brazil (less than 5-fold) (Amado 2017) and *S.*

exigua from Mexico (up to 13-fold) (Osorio et al. 2008). In contrast, a higher resistance ratio to methoxyfenozide was detected in *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) from Pakistan and China: 43- and 672-fold resistance, respectively (Zhang et al. 2014, Saleem et al. 2016). A large variation in the resistance ratios, from 2.0- to 59-fold, were also detected in *S. exigua* and *S. litura* to lufenuron, another chitin synthesis inhibitor, in Pakistan (Ishtiaq et al. 2012, Saleem et al. 2016).

An interpopulation variation in the susceptibility to chemical or microbial insecticides is a common phenomenon when bioassays are repeated (Robertson et al. 1995). This variation may be associated with several factors such as natural intraspecific genetic diversity, distinct geographic environments, host plants varieties, bioassay methods, generation tested, time of exposure, artificial diets, and management practices (Dulmage et al. 1978, Tabashnik et al. 1997, Luttrell et al. 1999, Blanco et al. 2009, Zheng et al. 2011). From an IRM perspective, even a small variation in susceptibility is an indication of the potential for selection of resistance as the insects adapt to the insecticide (Carrière et al. 2010). Therefore, the repeated exposure of SBL populations to insecticides with the same mode of action, even for those SBL populations with low resistance ratios, provides opportunities for resistance evolution, especially in areas where these crops are cultivated simultaneously or in succession (higher outbreaks of SBL), as in Mato Grosso do Sul, Mato Grosso, Goiás and Bahia states. In addition, the resistance evolution can be also exacerbated by the gene flow amongst populations in both crops (Palma et al. 2015, Silva et al. 2018).

The small geographic variation in the susceptibility of Brazilian populations of SBL to spinetoram, indoxacarb, thiodicarb, chlorantraniliprole, flubendiamide and chlorfenapyr suggests that, currently, the frequency of resistance to these insecticides is low in the field. This hypothesis is supported by high efficacy (more than 90%) of some insecticides, such as spinetoram, indoxacarb and chlorfenapyr against SBL during 2014–2016 (Perini 2018) and

2017–2018 soybean seasons (Castro et al. 2018), and also by low frequency of alleles for resistance to these insecticides (Unpublished data).

Unlike to these insecticides, the reduced susceptibility of SBL populations to lambda-cyhalothrin, methoxyfenozide, teflubenzuron and novaluron may be a reflection of increased occurrence of SBL in soybean in Brazil. This also increased the exposure to insecticides, and consequently the resistance frequencies, due to longtime use of these products for the management of this species. The low susceptibility of SBL to teflubenzuron was also detected in field studies during the 2006–2007 and 2007–2008 soybean seasons, which presented efficacy less than 66% (Martins and Tomquelski 2015). The insecticides lufenuron and lambda-cyhalothrin also showed low efficacy (less than 51%) against SBL in cotton during the 2008–2009 season (Andrade Junior and Vilela 2009). Low efficacy of lambda-cyhalothrin and inhibitors of chitin biosynthesis against SBL was also reported in field studies conducted over three years (2014–2016): less than 45% control (Perini 2018).

The low susceptibility of Brazilian populations of SBL to teflubenzuron, novaluron and lambda-cyhalothrin verified here and in previous field studies suggest that this species evolved resistance to these chemistries. Based on this, it is necessary to reduce the use of these active ingredients, as well as to give preference to use insecticides with lower resistance frequencies making a rotation of distinct modes of action. Furthermore, the use of other available and effective IPM tactics, such as Bt soybean and cotton (Ashfaq et al. 2001, Greenberg et al. 2010, Bernardi et al. 2012, Sorgatto et al. 2015), egg parasitoids (Bueno et al. 2009), and baculovirus-based insecticides (e.g. *Chrysodeixis includens* nucleopolyhedrovirus – ChinNPV) (Lima et al. 2018, Muraro et al. 2018), may contribute to retard the development of further resistance.

In summary, we have reported the susceptibility of Brazilian populations of SBL to several insecticides. Future efforts should be concentrated on collecting representative individuals of SBL, especially from major soybean and cotton growing areas, to identify possible changes in the susceptibility to those insecticides with high control efficacy as a result of repeated exposure to these chemistries. Therefore, the continuous monitoring of insecticide susceptibility in field populations of SBL is essential to support IPM and IRM programs in Brazil.

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Table 1. Identification, location and date of collection of SBL populations from major Brazilian soybean fields used to characterize the susceptibility of insecticides.

Pop. code	City, State	Farm	Latitude	Longitude	Date
Lab	Paulínia, SP	Estação de Pesquisa	22º45'03"S	47º07'05"W	-
Soybean season 2016–2017					
TO-1	Novo Jardim, TO	Campo Novo do Jardim	11º49'40"S	46º34'06"W	Feb. 2017
BA-1	Barreiras, BA	Biselo	11º73'20"S	45º90'68"W	Feb. 2017
BA-2	Luis Eduardo Magalhães, BA	Decisão Rio Branco	11º49'24"S	46º10'50"W	April 2017
MT-1	Lucas do Rio Verde, MT	Fundação Rio Verde	12º59'88"S	55º58'02"W	April 2017
MS-1	São Gabriel do Oeste, MS	Crop Solution	19º27'31"S	54º36'42"W	Jan. 2017
PR-1	Santa Helena, PR	-	24º53'31"S	54º18'40"W	Jan. 2017
PR-2	Cascavel, PR	-	25º05'42"S	53º28'27"W	Feb. 2017
PR-3	Cafelândia, PR	-	24º37'00"S	53º17'58"W	Feb. 2017
RS-1	Santa Maria, RS	UFSM	29º43'20"S	53º33'34"W	Feb. 2017
RS-2	Coqueiros do Sul, RS	-	28º10'54"S	52º44'46"W	Feb. 2017
Soybean season 2017–2018					
BA-3	Luis Eduardo Magalhães, BA	Circulo Verde	12º07'01"S	45º49'56"W	Jan. 2018
BA-4	Correntina, BA	Guarani	12º53'71"S	46º12'34"W	Mar. 2018
GO-1	Rio Verde, GO	Universidade de Rio Verde	17º46'35"S	50º57'58"W	Feb. 2018
MT-2	Campo Verde, MT	Boa Vista	17º10'43"S	54º17'11"W	Jan. 2018
MT-3	Sapezal, MT	Pai Nosso	12º09'45"S	58º10'48"W	Feb. 2018
MS-2	Chapadão do Sul, MS	André Machado	18º37'25"S	52º54'13"W	Jan. 2018
MS-3	Campo Grande, MS	Grupo Produza	20º44'18"S	54º30'03"W	Mar. 2018
SP-1	Leme, SP	Sítio Conceição	22º07'41"S	47º20'28"W	Mar. 2018
PR-4	Bela Vista do Paraiso, PR	Val Paraiso	23º02'45"S	51º12'58"W	Feb. 2018
RS-3	Santa Maria, RS	UFSM	29º43'20"S	53º33'34"W	Feb. 2018
RS-4	Victor Graeff, RS	Agropecuária Kuhn	28º33'36"S	52º44'52"W	Mar. 2018

Table 2. Commercial and technical grade insecticides used to characterize the susceptibility of Brazilian populations of SBL.

Active Ingredient (AI)	IRAC MoA Classification	Insecticide Class	Trade Name	AI (%)	Company/Manufacturer	Dose Range
Diet-overlay bioassay						**(μg a.i. per cm²)**
Lambda-cyhalothrin	Sub-group 3A	Pyrethroid	Kaiso CS	25	Nufarm A/S, Maracanaú, CE, Brazil	0.016–80.0
Thiodicarb	Sub-group 1A	Carbamate	Larvin WG	80	Bayer CropScience Ltda., São Paulo, SP, Brazil	0.896–160.0
Indoxacarb	Sub-group 22A	Oxadiazine	Avatar EC	15	Du Pont do Brasil S.A, Barueri, SP, Brazil	0.005–3.0
Spinetoram	Group 5	Spinosyn	Exalt SC	12	Dow AgroSciences Industrial Ltda., São Paulo, SP, Brazil	0.005–0.9
Flubendiamide	Group 28	Diamide	Belt SC	48	Bayer CropScience Ltda., São Paulo, SP, Brazil	0.051–9.0
Chlorantraniliprole	Group 28	Diamide	Premio SC	20	Du Pont do Brasil S.A, Barueri, SP, Brazil	0.016–3.0
Chlorfenapyr	Group 13	Pyrroles	Pirate SC	24	BASF S.A., São Paulo, SP, Brazil	0.016–0.5
Methoxyfenozide	Group 18	Diacylhydrazine	Intrepid SC	24	Dow AgroSciences Industrial Ltda., São Paulo, SP, Brazil	0.0009–16.0
Teflubenzuron	Group 15	Benzoylurea	Nomolt SC	15	BASF S.A., São Paulo, SP, Brazil	0.0016–320.0
Novaluron	Group 15	Benzoylurea	Rimon Supra SC	10	Adama Brasil S/A, Londrina, PR, Brazil	0.0086–320.0
Topical bioassay						**(μg a.i. per larva)**
Lambda-cyhalothrin	Sub-group 3A	Pyrethroid	Lambda-cyhalothrin	96.5	Nufarm A/S, Maracanaú, CE, Brazil	0.00032–0.32
Thiodicarb	Sub-group 1A	Carbamate	Thiodicarb Pestanal	99.8	Sigma-Aldrich International	0.032–5.0

Table 3. Concentration-mortality response of SBL populations exposed to nerve action insecticides in diet-overlay (LC; µg a.i. per cm^2) and topical (LC; µg a.i. per larva) bioassays.

Pop. code	Generation	*n*	Slope ± SE	LC_{50} (95% CI)[a,b]	LC_{90} (95% CI)[a,b]	χ^2[c]	df[d]	RR_{50} (95% CI)[b,e]
Spinetoram (overlay bioassays)								
Lab	-	767	1.65 ± 0.22	0.029 (0.016–0.047) a	0.17 (0.10–0.45) a	14.61	5	–
BA-1	F_3	669	2.11 ± 0.24	0.074 (0.053–0.093) b	0.30 (0.25–0.38) a	1.03	4	2.5 (1.2–3.2) a
BA-2	F_3	669	2.65 ± 0.43	0.18 (0.12–0.24) cd	0.54 (0.37–1.29) ab	8.92	4	6.2 (4.9–7.8) b
GO-1	F_1	576	1.80 ± 0.24	0.11 (0.08–0.14) bc	0.58 (0.43–0.92) ab	5.22	5	3.8 (2.5–5.3) ab
MT-2	F_1	742	2.08 ± 0.27	0.13 (0.10–0.15) c	0.53 (0.41–0.77) ab	5.43	5	4.5 (2.7–5.8) ab
MS-1	F_2	768	2.31 ± 0.24	0.15 (0.13–0.17) c	0.54 (0.43–0.75) ab	5.00	5	5.2 (4.2–6.7) b
PR-1	F_3	504	1.46 ± 0.74	0.19 (0.11–0.38) cd	1.17 (0.51–11.15) b	13.18	4	6.5 (5.0–8.6) b
PR-2	F_2	768	1.39 ± 0.12	0.14 (0.11–0.16) c	1.12 (0.78–1.82) b	1.96	5	4.8 (2.9–6.5) ab
PR-3	F_3	768	1.93 ± 0.36	0.14 (0.08–0.20) bc	0.66 (0.42–2.06) ab	11.71	5	4.8 (2.7–6.6) ab
RS-1	F_2	768	2.44 ± 0.19	0.25 (0.22–0.28) d	0.83 (0.68–1.06) b	6.35	5	8.6 (6.3–11.6) b
RS-2	F_2	766	2.08 ± 0.28	0.16 (0.14–0.19) c	0.68 (0.51–1.07) b	7.60	5	5.5 (4.4–7.3) b
Indoxacarb (overlay bioassays)								
Lab	-	667	1.32 ± 0.11	0.14 (0.11–0.19) a	1.31 (0.92–2.08) a	0.97	4	–
TO-1	F_3	765	1.63 ± 0.13	0.70 (0.59–0.83) bc	4.29 (3.19–6.32) cd	0.96	5	5.0 (3.9–7.1) ab
BA-1	F_3	672	1.56 ± 0.34	0.94 (0.60–1.22) c	6.22 (3.85–18.95) d	4.25	5	6.7 (4.9–8.7) b
BA-2	F_3	763	1.55 ± 0.12	0.65 (0.55–0.78) bc	4.41 (3.24–6.62) cd	3.52	5	4.6 (3.2–5.9) ab
GO-1	F_1	672	2.06 ± 0.28	0.64 (0.44–0.82) bc	2.69 (2.16–3.68) bc	5.46	4	4.5 (3.3–5.8) ab
MT-2	F_1	696	1.74 ± 0.25	0.66 (0.47–0.84) bc	3.61 (2.66–5.92) bcd	6.60	5	4.7 (3.5–6.2) ab
MS-1	F_3	768	1.51 ± 0.17	0.82 (0.60–1.04) c	5.78 (4.10–9.54) d	6.58	5	5.9 (4.3–7.9) b
RS-1	F_3	768	1.93 ± 0.21	0.46 (0.33–0.59) b	2.14 (1.73–2.82) ab	0.56	5	3.3 (2.3–4.1) a
PR-2	F_3	767	1.87 ± 0.18	0.58 (0.45–0.71) bc	2.81 (2.23–3.79) bc	3.52	5	4.1 (3.1–5.5) ab
PR-3	F_4	768	1.52 ± 0.12	0.73 (0.62–0.88) c	5.09 (3.67–7.84) cd	1.18	5	5.2 (3.9–7.0) ab
Thiodicarb (overlay bioassays)								
Lab	-	671	2.56 ± 0.53	6.57 (4.05–8.84) a	20.81 (13.62–82.22) ab	9.29	4	–
BA-2	F_3	769	2.04± 0.21	36.61 (29.24–43.51) c	155.66 (127.46–204.72) c	8.99	5	5.6 (4.7–6.9) c
MS-1	F_3	765	1.90 ± 0.23	17.62 (12.41–22.55) b	83.14 (66.30–113.27) b	6.46	5	2.7 (2.2–3.2) b
PR-2	F_3	575	1.84 ± 0.26	9.14 (4.32–15.12) ab	45.61 (27.53–97.37) ab	12.37	5	1.4 (1.1–1.6) a
PR-3	F_3	672	2.32 ± 0.27	11.48 (8.70–14.07) ab	40.99 (33.85–52.85) a	2.28	4	1.7 (1.5–2.1) ab
RS-1	F_2	768	1.43 ± 0.32	14.93 (2.07–30.50) abc	117.86 (61.74–501.20) bc	13.65	5	2.3 (1.1–3.6) ab
RS-2	F_3	503	2.49 ± 0.26	16.23 (13.72–18.96) b	52.95 (42.19–72.65) ab	3.78	4	2.5 (2.0–2.9) b
Thiodicarb (topical bioassays)								
Lab	-	504	2.15 ± 0.26	0.07 (0.05–0.10) a	0.29 (0.23–0.39) a	4.12	4	–
BA-3	F_1	576	1.42 ± 0.18	0.09 (0.04–0.16) ab	0.75 (0.50–1.16) b	2.19	6	1.2 (0.9–1.6) a
GO-1	F_1	640	1.43 ± 0.18	0.25 (0.14–0.38) b	1.99 (1.44–2.98) c	2.55	7	3.5 (2.6–4.3) b
MT-2	F_1	448	0.97 ± 0.14	0.20 (0.08–0.40) ab	4.13 (2.09–10.10) c	6.02	4	2.9 (2.0–4.1) b

MS-2	F_1	512	1.00 ± 0.13	0.13 (0.05–0.27) ab	2.52 (1.40–4.98) c	4.88	5	1.8 (1.5–2.9) ab
MS-3	F_1	576	1.26 ± 0.14	0.20 (0.11–0.30) b	2.06 (1.37–3.48) c	3.85	6	2.9 (2.2–3.8) b
SP-1	F_1	576	1.38 ± 0.16	0.17 (0.10–0.25) ab	1.44 (1.01–2.27) bc	3.36	6	2.4 (1.2–3.1) ab
PR-4	F_1	512	1.35 ± 0.15	0.22 (0.14–0.33) b	1.98 (1.32–3.35) c	5.54	5	3.1 (2.3–4.2) b
RS-3	F_1	512	1.28 ± 0.15	0.16 (0.09–0.25) ab	1.60 (1.04–2.77) bc	8.20	5	2.3 (1.9–3.0) b
Lambda-cyhalothrin (overlay bioassays)								
Lab	-	765	1.65 ± 0.12	0.26 (0.22–0.31) a	1.56 (1.14–2.33) a	7.18	5	–
BA-2	F_3	768	1.40 ± 0.10	3.71 (3.01–4.53) b	30.59 (22.60–44.81) b	5.52	5	14.3 (10.8–18.8) a
MS-1	F_3	743	1.04 ± 0.07	7.52 (5.78–9.82) c	129.04 (83.49–223.75) c	6.03	4	28.9 (20.9–40.0) b
PR-2	F_3	767	1.54 ± 0.12	8.22 (6.93–9.69) c	55.57 (41.93–80.16) b	4.77	5	31.6 (24.6–40.6) b
PR-1	F_3	576	1.88 ± 0.25	7.02 (4.45–9.51) bc	33.82 (26.36–47.22) b	6.54	5	27.0 (22.5–36.4) b
RS-1	F_2	864	1.64 ± 0.12	9.54 (8.04–11.25) c	57.80 (44.25–81.41) b	3.22	6	36.7 (28.5–47.2) b
Lambda-cyhalothrin (topical bioassays)								
Lab	-	448	1.62 ± 0.24	0.0016 (0.0008–0.0025) a	0.010 (0.007–0.016) a	0.88	4	–
BA-3	F_1	448	1.56 ± 0.23	0.06 (0.04–0.10) bc	0.43 (0.29–0.74) c	2.20	4	37.5 (29.0–48.3) a
GO-1	F_1	512	1.65 ± 0.27	0.06 (0.03–0.08) bc	0.33 (0.24–0.52) bc	3.93	5	37.5 (28.3–50.8) ab
MT-2	F_1	512	2.1 ± 0.24	0.04 (0.04–0.06) bc	0.20 (0.16–0.27) b	3.24	5	25.0 (20.1–31.7) a
MS-2	F_1	477	1.68 ± 0.27	0.10 (0.06–0.14) c	0.58 (0.41–0.99) c	1.16	4	62.5 (50.7–74.2) b
MS-3	F_1	512	1.69 ± 0.21	0.05 (0.03–0.06) bc	0.27 (0.20–0.41) bc	8.88	5	31.2 (23.7–42.0) a
PR-4	F_1	512	1.65 ± 0.15	0.04 (0.03–0.05) b	0.23 (0.17–0.34) bc	3.78	5	25.0 (19.9–30.3) a
RS-3	F_1	525	1.70 ± 0.26	0.06 (0.04–0.08) bc	0.34 (0.25–0.55) bc	7.95	5	37.5 (28.7–49.5) a

[a] LC$_{50}$: concentration of insecticide required to kill 50% of larvae in the observation period of 2 d. Similarly, LC$_{90}$ is the concentration of insecticide required to kill 90% of larvae tested.

[b] LC$_{50}$, LC$_{90}$ and RR$_{50}$ values designated by different letters within a column are significantly different from each other through nonoverlap of 95% CIs.

[c] $P > 0.05$ in the goodness-of-fit test.

[d] Degrees of freedom.

[e] Resistance Ratio (RR$_{50}$) = (LC$_{50}$ of field population)/(LC$_{50}$ of Lab population).

Table 4. Concentration-mortality response (LC; µg a.i. per cm^2) of SBL populations exposed to muscle action insecticides in diet-overlay bioassays.

Pop. code	Generation	*n*	Slope ± SE	LC_{50} (95% CI)[a,b]	LC_{90} (95% CI)[a,b]	χ^{2c}	df[d]	RR_{50} (95% CI)[b,e]
Chlorantraniliprole								
Lab	-	504	3.18 ± 0.27	0.05 (0.04–0.05) a	0.12 (0.10–0.15) a	6.04	4	–
BA-2	F_3	633	1.97 ± 0.29	0.57 (0.40–0.72) e	2.56 (2.00–3.82) ef	4.99	4	11.4 (8.7–16.2) c
BA-3	F_1	576	2.02 ± 0.23	0.28 (0.21–0.35) cd	1.20 (0.95–1.63) cd	3.82	5	5.6 (4.6–7.1) b
GO-1	F_1	576	1.52 ± 0.15	0.19 (0.13–0.23) bc	1.11 (0.80–1.67) bcd	8.16	5	3.8 (3.3–5.2) a
MT-2	F_1	576	2.14 ± 0.25	0.38 (0.30–0.46) de	1.52 (1.20–2.14) de	1.07	5	7.6 (6.6–9.6) bc
MT-3	F_1	504	2.60 ± 0.33	0.28 (0.20–0.35) bcd	0.88 (0.72–1.12) bc	4.88	4	5.6 (4.8–6.9) b
MS-1	F_3	648	1.95 ± 0.15	0.27 (0.23–0.33) d	1.24 (0.99–1.64) cd	0.57	4	5.4 (4.3–7.0) b
MS-2	F_1	504	1.57 ± 0.28	0.50 (0.30–0.69) de	3.28 (2.19–6.95) f	7.44	4	10.0 (8.6–13.8) c
MS-3	F_1	576	1.97 ± 0.27	0.28 (0.19–0.36) bcd	1.25 (0.98–1.75) cd	0.90	5	5.6 (4.6–7.1) b
SP-1	F_1	576	1.69 ± 0.18	0.19 (0.11–0.24) bcd	1.01 (0.75–1.43) bcd	2.84	5	3.8 (3.3–5.0) ab
PR-4	F_1	577	2.54 ± 0.39	0.51 (0.40–0.61) e	1.62 (1.29–2.38) def	7.31	5	10.2 (8.8–12.5) c
RS-3	F_1	504	2.51 ± 0.36	0.19 (0.13–0.24) bcd	0.62 (0.50–0.81) b	2.09	4	3.8 (3.3–4.7) a
RS-4	F_1	576	1.92 ± 0.23	0.15 (0.09–0.20) b	0.68 (0.53–0.92) b	4.73	5	3.0 (2.5–3.8) a
Flubendiamide								
Lab	-	768	1.03 ± 0.10	0.13 (0.09–0.17) a	2.22 (1.50–3.76) ab	3.45	5	–
BA-2	F_3	952	1.27 ± 0.09	1.72 (1.41–2.08) f	17.39 (12.71–25.80) e	3.52	7	13.2 (9.1–19.7) b
BA-3	F_1	648	1.76 ± 0.21	0.78 (0.48–1.08) bcd	4.17 (3.23–5.65) bc	2.11	6	6.0 (4.2–8.9) ab
MT-2	F_1	648	1.42 ± 0.18	1.35 (0.86–1.87) def	10.83 (7.71–17.47) de	2.69	6	10.3 (6.6–14.9) b
MT-3	F_1	576	2.04 ± 0.24	0.55 (0.36–0.75) bc	2.36 (1.85–3.12) a	2.95	5	4.2 (2.9–6.4) ab
MS-1	F_3	768	1.52 ± 0.12	1.10 (0.92–1.31) de	7.63 (5.65–11.25) cd	7.30	5	8.5 (5.8–12.5) b
PR-2	F_3	672	1.73 ± 0.21	0.45 (0.28–0.61) b	2.46 (1.92–3.36) ab	7.70	4	3.4 (2.4–5.2) a
PR-3	F_4	672	1.43 ± 0.27	0.58 (0.25–1.06) bcd	4.56 (2.12–34.63) abcde	14.25	4	4.5 (3.0–6.8) ab
PR-4	F_1	576	1.42 ± 0.19	1.27 (0.74–1.87) cdef	10.45 (7.20–17.72) de	6.97	5	9.7 (6.4–14.3) b
RS-3	F_1	648	1.47 ± 0.28	2.01 (1.13–2.76) ef	14.95 (10.04–32.51) de	4.13	6	15.4 (10.1–23.3) b

[a] LC_{50}: concentration of insecticide required to kill 50% of larvae in the observation period of 4 d. Similarly, LC_{90} is the concentration of insecticide required to kill 90% of larvae tested.

[b] LC_{50}, LC_{90} and RR_{50} values designated by different letters within a column are significantly different from each other through nonoverlap of 95% CIs.

[c] *P* > 0.05 in the goodness-of-fit test.

[d] Degrees of freedom.

[e] Resistance Ratio (RR_{50}) = (LC_{50} of field population)/(LC_{50} of Lab population).

Table 5. Concentration-mortality response (LC; µg a.i. per cm^2) of SBL populations exposed to the energy metabolism insecticide chlorfenapyr in diet-overlay bioassays.

Pop. code	**Generation**	***n***	**Slope ± SE**	**LC_{50} (95% CI)^{a,b}**	**LC_{90} (95% CI)^{a,b}**	**χ^{2c}**	**df^d**	**RR_{50} (95% CI)^{b,e}**
Lab	-	432	4.02 ± 1.07	0.15 (0.05–0.24) a	0.31 (0.20–11.17) a	10.69	3	–
BA-3	F_1	504	3.76 ± 0.34	0.20 (0.18–0.22) a	0.43 (0.37–0.53) a	1.47	4	1.3 (1.1–1.6) a
GO-1	F_1	504	3.91 ± 0.37	0.19 (0.17–0.21) a	0.40 (0.35–0.49) a	4.37	4	1.2 (0.9–1.5) a
MT-2	F_1	504	4.09 ± 0.41	0.19 (0.17–0.21) a	0.39 (0.34–0.48) a	2.57	4	1.2 (1.0–1.5) a
MT-3	F_1	504	3.61 ± 0.33	0.15 (0.13–0.17) a	0.34 (0.29–0.42) a	2.53	4	1.0 (0.9–1.5) a
MS-2	F_1	504	3.57 ± 0.42	0.21 (0.18–0.24) a	0.48 (0.40–0.62) a	3.19	4	1.4 (1.2–1.7) a
MS-3	F_1	504	3.63 ± 0.34	0.16 (0.15–0.19) a	0.38 (0.33–0.47) a	2.35	4	1.1 (0.9–1.4) a
SP-1	F_1	504	4.06 ± 0.46	0.19 (0.16–0.21) a	0.39 (0.33–0.47) a	1.39	4	1.2 (0.9–1.4) a
PR-4	F_1	432	4.50 ± 0.68	0.25 (0.21–0.27) a	0.47 (0.41–0.60) a	4.90	3	1.7 (1.4–1.9) a
RS-3	F_1	504	4.11 ± 0.50	0.18 (0.16–0.20) a	0.36 (0.32–0.45) a	5.56	4	1.2 (1.0–1.4) a
RS-4	F_1	504	3.23 ± 0.31	0.18 (0.16–0.20) a	0.45 (0.38–0.57) a	3.21	4	1.2 (1.0–1.5) a

[a] LC_{50}: concentration of insecticide required to kill 50% of larvae in the observation period of 2 d. Similarly, LC_{90} is the concentration of insecticide required to kill 90% of larvae tested.

[b] LC_{50}, LC_{90} and RR_{50} values designated by different letters within a column are significantly different from each other through nonoverlap of 95% CIs.

[c] $P > 0.05$ in the goodness-of-fit test.

[d] Degrees of freedom.

[e] Resistance Ratio (RR_{50}) = (LC_{50} of field population)/(LC_{50} of Lab population).

Table 6. Concentration-mortality response (LC; µg a.i. per cm^2) of SBL populations exposed to the insect growth regulator insecticides in diet-overlay bioassays.

Pop. code	Generation	*n*	Slope ± SE	LC$_{50}$ (95% CI)[a,b]	LC$_{90}$ (95% CI)[a,b]	χ^2[c]	df[d]	RR$_{50}$ (95% CI)[b,e]
Methoxyfenozide								
Lab	-	912	1.42 ± 0.11	0.067 (0.05–0.07) a	0.44 (0.33–0.65) a	4.69	4	–
BA-1	F_3	692	1.77 ± 0.22	1.97 (1.55–2.43) cd	10.43 (7.38–17.72) bcd	4.83	5	29.4 (22.1–42.7) bc
BA-2	F_3	861	1.97 ± 0.17	4.23 (3.61–4.93) e	18.91 (14.78–26.26) d	4.48	6	63.1 (47.9–79.2) d
BA-3	F_1	581	1.96 ± 0.25	2.18 (1.61–2.72) cd	9.83 (7.55–14.55) bc	2.38	5	36.3 (28.0–46.2) c
MT-2	F_1	648	1.54 ± 0.18	2.98 (2.09–3.92) de	20.17 (14.61–31.84) d	9.61	6	49.7 (37.4–65.6) bcd
MS-1	F_2	930	1.43 ± 0.24	1.61 (0.96–2.34) bcd	12.62 (7.01–46.04) bcd	10.97	6	24.0 (18.7–30.5) bc
PR-2	F_3	765	1.36 ± 0.13	1.79 (1.46–2.23) cd	15.58 (10.22–28.16) cd	5.31	5	26.7 (21.1–33.4) bc
PR-3	F_4	764	1.43 ± 0.21	0.67 (0.26–1.16) b	5.29 (3.13–12.27) bc	9.24	5	11.2 (8.5–15.8) a
PR-4	F_1	576	2.14 ± 0.18	1.78 (1.53–2.07) c	7.07 (5.64–9.47) b	4.21	5	29.7 (23.6–36.9) bc
RS-1	F_2	765	1.48 ± 0.13	1.51 (1.25–1.83) c	11.11 (7.75–18.19) bcd	5.78	5	22.5 (19.0–27.1) b
Novaluron								
Lab	-	504	2.26 ± 0.38	0.05 (0.03–0.07) a	0.19 (0.12–0.47) a	10.11	4	–
BA-4	F_1	576	1.36 ± 0.15	46.68 (34.41–59.76) bc	411.52 (286.41–696.77) b	3.34	5	933.6 (625.2–1,234) ab
GO-1	F_1	648	1.15 ± 0.11	27.52 (21.11–35.01) b	357.85 (233.90–650.43) b	6.24	6	550.4 (406.6–733.7) a
MS-2	F_1	576	0.96 ± 0.16	52.56 (21.73–93.87) bcd	1,150 (602.13–3,339) b	5.10	5	1,051 (772.3–1,460) b
MS-3	F_1	720	1.07 ± 0.10	46.77 (35.51–60.54) cd	725.35 (459.72–1,365) b	3.85	7	935.4 (679.8–1,261) ab
SP-1	F_1	720	1.26 ± 0.12	75.83 (59.94–95.59) d	789.65 (523.85–1,390) b	5.70	7	1,517 (1,080–1,991) b
PR-4	F_1	504	1.72 ± 0.37	77.12 (13.64–144.70) bcd	428.43 (235.17–1,803) b	9.69	4	1,542 (1,146–2,013) b
RS-4	F_1	720	1.41 ± 0.19	77.63 (51.38–103.96) cd	627.40 (438.85–1,089) b	7.23	7	1,553 (1,158–1,989) b
Teflubenzuron								
Lab	-	576	1.69 ± 0.19	0.014 (0.010–0.020) a	0.05 (0.03–0.10) a	5.09	5	-
BA-3	F_1	648	0.82 ± 0.08	26.73 (18.95–37.71) b	999.10 (525.84–2,441) c	8.08	6	1,909 (1,276–2,690) ab
GO-1	F_1	576	1.12 ± 0.12	24.83 (17.39–33.10) b	345.07 (226.69–630.94) bc	5.50	5	1,773 (1,213–2,439) ab
MT-2	F_1	504	0.79 ± 0.12	13.41 (4.67–27.53) b	564.54 (263.58–1,814) bc	4.60	4	957.8 (720.3–1,564) a
MT-3	F_1	504	0.88 ± 0.15	37.10 (12.85–70.08) bc	1,062 (551.61–3,229) c	7.42	4	2,650 (2,030–4,114) bc
MS-2	F_1	450	0.82 ± 0.14	34.01 (11.61–129.82) bc	1,229 (249.33–3,463) bc	6.49	3	2,429 (1,496–3,714) abc
MS-3	F_1	576	1.12 ± 0.10	21.19 (14.74–28.68) b	298.16 (206.79–478.66) b	6.90	5	1,513 (1,022–2,108) a
SP-1	F_1	576	0.86 ± 0.09	16.58 (11.25–23.29) b	507.87 (284.34–1,168) bc	8.76	5	1,184 (778.6–1,695) a
PR-4	F_1	576	1.17 ± 0.14	73.02 (55.24–97.00) c	904.69 (529.60–2,042) c	6.99	5	5,215 (3,699–6,925) c
RS-3	F_1	557	1.18 ± 0.17	32.44 (17.07–60.67) bc	392.28 (169.32–2,019) bc	13.81	5	2,317 (1,738–3,243) b
RS-4	F_1	504	0.86 ± 0.09	22.90 (15.09–33.04) b	708.97 (384.27–1,730) bc	4.28	4	1,635 (1,048–2,402) a

[a] LC$_{50}$: concentration of insecticide required to kill 50% of larvae in the observation period of 5 d. Similarly, LC$_{90}$ is the concentration of insecticide required to kill 90% of larvae tested.

[b] LC$_{50}$, LC$_{90}$ and RR$_{50}$ values designated by different letters within a column are significantly different from each other through nonoverlap of 95% CIs.

[c] $P > 0.05$ in the goodness-of-fit test.

[d] Degrees of freedom.

[e] Resistance Ratio (RR_{50}) = (LC_{50} of field population)/(LC_{50} of Lab population).

5 ARTIGO 2

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Inheritance of lambda-cyhalothrin resistance, fitness costs and cross-resistance to other pyrethroids in soybean looper, *Chrysodeixis includens* (Lepidoptera: Noctuidae)

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ABSTRACT

Soybean looper (SBL), *Chrysodeixis includens* (Lepidoptera: Noctuidae), is one of the main lepidopteran pests of soybean and cotton crops. In this study, we selected a strain of SBL with resistance to lambda-cyhalothrin and evaluated the inheritance, fitness costs and cross-resistance to deltamethrin and cypermethrin. In inheritance pattern studies, SBL larvae were exposed to technical grade insecticides in topical bioassays. To investigate fitness costs, SBL strains were reared on artificial diet without insecticide and the survival, development and reproductive rates were evaluated. Estimated LD_{50} values of lambda-cyhalothrin for the susceptible and resistant strains were 0.0015 and 0.11 μg a.i./larva, respectively, indicating a resistance ratio (RR) of 73.3-fold. The LD_{50} values of heterozygous were 0.016 and 0.017 μg a.i./larva, suggesting an autosomal inheritance of resistance. On lambda-cyhalothrin sprayed-leaves, the resistant strain showed > 85% survival. By contrast, heterozygous and susceptible strains had < 64 and 8% survival, respectively, indicating an incompletely dominant resistance against the field dose of lambda-cyhalothrin. The resistant strain also showed cross-resistance to deltamethrin (RR = 6.2-fold) and cypermethrin (RR = 22.5-fold). Fitness costs studies showed that resistant insects had 71 and 48% lower survival and reproductive rates, respectively, than the susceptible strain (62.8% survival). The inheritance patterns of lambda-cyhalothrin resistance in SBL was autosomal, incompletely dominant and associated with fitness costs. SBL had low cross-resistance to deltamethrin and cypermethrin, indicating the potential to develop resistance to other pirethroids. Therefore, to preserve the useful life of chemical control against SBL are required the development of multi-tactic resistance management programs along with a reduction in insecticides applied.

Keywords: soybean looper, heritability, sodium channel modulator, insect resistance management

Highlights

We selected a soybean looper (SBL) strain with 73.3-fold resistance to lambda-cyhalothrin.

The inheritance pattern, fitness cost and cross-resistance to other pyrethroids were evaluated.

The resistance of SBL to lambda-cyhalothrin is autosomal and incompletely dominant traits.

The resistance of SBL to lambda-cyhalothrin is associated with significant fitness costs.

The lambda-cyhalothrin-resistant SBL strain had low cross-resistance to other pyrethroids.

1. Introduction

Soybean looper (SBL), *Chrysodeixis includens* (Walker, 1858) (Lepidoptera: Noctuidae), causes economic damage to important agricultural crops from South to North America (Alford and Hammond Junior, 1982). In Brazil and other South American countries, this species is considered one of the main lepidopteran pests of soybean (*Glycine max* (L.) Merrill), cotton (*Gossypium hirsutum* L.) and common bean (*Phaseolus vulgaris* L.) (Panizzi, 2013; Bortolotto et al., 2015; Sorgatto et al., 2015). The ability of SBL to complete the development on different cultivated plants (Specht et al., 2015), their high reproductive capacity with several generations per year (Moonga and Davis, 2016; Zulin et al., 2018) and dispersion between regions and crops (Palma et al., 2015), favors the occurrence of high infestations during the crop seasons.

The management of SBL can be performed with chemical insecticides (Ramos et al., 2017; Perini et al., 2019), transgenic plants expressing *Bacillus thuringiensis* (Bt) proteins (Bernardi et al., 2012; Marques et al., 2016), host plant resistance (Wille et al., 2017; Schlick-Souza et al., 2018), and baculovirus-based insecticides (Muraro et al., 2018; Godoy et al., 2019). Most of chemical insecticides, biological-based products and Bt plants have efficacy against SBL. Alternative control strategies such as host plant resistance are available, but are rarely used. Currently, two to six insecticide sprays are performed against lepidopteran pests in Brazilian soybean fields (Panizzi, 2013; Bortolotto et al., 2015). The widespread use of chemical insecticides against SBL associated with its natural tolerance to insecticides (Dowd and Sparks, 1986) and the habit of remaining on the lower or middle third of plant's canopy (Zulin et al., 2018; Funichello et al., 2019), favors the survival and, consequently, resistance evolution.

In the United States, since the 1980s, the resistance of SBL to insecticides has been reported to cypermethrin, permethrin, tefluthrin, fenvalerate, acephate, methomyl, parathion-methyl and thiodicarb (Felland et al., 1990; Leonard et al., 1990; Boethel et al., 1992; Mascarenhas and Boethel, 1997; Mascarenhas and Boethel, 2000). Until now, resistance cases have not been documented in SBL in Brazil; however, evidence of resistance to lambda-cyhalothrin (pyrethroid), teflubenzuron and novaluron (chitin synthesis inhibitors) were reported (Stacke et al., 2019). In Brazilian soybean fields, control failures occurred when pyrethroids were applied against SBL (Sosa-Gómez and Omoto, 2012; Perini et al., 2019). The major mechanisms related with resistance to pyrethroids in SBL and other lepidopteran pests are reduced neuronal sensitivity and increased monooxygenase activity by cytochrome P450 content (Thomas et al., 1996; Carvalho et al., 2013; Silva et al., 2015; Durigan et al., 2017). These mechanisms affect the affinity of pyrethroids to the sodium channel receptors or causes faster detoxification due to increase in enzyme activity (O'Reilly et al., 2006; Pu et al., 2016).

Understanding the genetic basis of resistance to insecticides is essential to develop Insect Resistance Management (IRM) strategies to delay or prevent resistance evolution (Georghiou and Taylor, 1977; Roush and McKenzie, 1987). It is also important to develop insecticide application techniques, such as mixtures or rotation of insecticides with distinct modes of action to delay resistance development. In addition, investigating fitness costs associated with the resistance can help to understand if the susceptibility could be restored in the absence of insecticide sprays (Kliot and Ghanim, 2012). In this study, we selected a lambda-cyhalothrin-resistant SBL strain from a field population and studied the inheritance, fitness costs and cross-resistance to other pyrethroids.

2. Material and Methods

2.1. SBL strains

The susceptible reference population of SBL (hereafter Sus) was collected in soybean in Paulínia, São Paulo, Brazil (23º45'03"S and 47º07'05"W) and has been kept in the laboratory for more than six years, free of selection pressure by insecticides. During the 2017/2018 season, the resistant strain (hereafter Lambda-res) was selected from 1250 larvae collected in a soybean crop from Chapadão do Sul, MS, Brazil (18°37'25" S and 52°54'13" W). In this area, there is a history of use of lambda-cyhalothrin for the management of lepidopteran and stink bug pests. To test heterozygous genotypes, reciprocal crosses were performed between resistant × susceptible strains (Lambda-res♀ × Sus♂ and Lambda-res♂ × Sus♀). All SBL strains were reared on artificial diet (Greene et al., 1976) and maintained at 25 ± 2°C, 60 ± 10% RH and a 12:12 h (light:dark) photoperiod.

2.2 Selection of lambda-cyhalothrin-resistant SBL strain

The Lambda-res colony was isolated from a field population using massal selection method. During three generations (F_2, F_3 and F_4), late L2/early L3 larvae (~1.0 cm length) from the field population mentioned above were exposed to technical grade lambda-cyhalothrin (96.5% purity, Nufarm A/S, Maracanaú, CE, Brazil) diluted in acetone (99.5% purity; Sigma Aldrich, São Paulo, SP, Brazil). In this process, a single dose of 0.32 µg a.i./larva was applied topically to the dorsal thoracic region of each larva (1 µl/larva) using a micropipette (Biohit *e*Line, Sartorius, São Paulo, SP, Brazil). This dose represents the LD_{90} value of lambda-cyhalothrin obtained in a joint analysis of dose-mortality data from seven field populations of SBL (Stacke et al., 2019). Larvae of the resistant strain in F_5 and F_6 generations were used in the characterization of resistance

2.3 Inheritance of resistance

Late L2/early L3 larvae from Lambda-res, Sus or heterozygous strains were exposed to seven doses of technical grade lambda-cyhalothrin (0.00032–0.32 μg a.i./larva). These doses were defined in previous bioassays and caused mortality ranging from ∼5 to 95%. Insecticides were diluted in acetone and applied to the dorsal thoracic region of each larva (1 μl/larva) using a micropipette. Larvae were then placed in 24-well acrylic plates (Costar®, São Paulo, SP, Brazil) containing artificial diet (Greene et al., 1976). Acrylic plates were closed and maintained in a growth room at 25 ± 2C, 60 ± 10% RH, and a 12:12 h (light:dark) photoperiod. Each dose was repeated twice per strain in distinct bioassays (2 replications of 48 larvae/dose). Mortality was assessed at 48 h after insecticide exposure. Larvae that did not show movement were considered dead. Dose-mortality data were subjected to Probit analysis to estimate the LD_{50} and LD_{90} lethal doses and their 95% confidence intervals using the PROC PROBIT procedure in SAS® 9.1 (SAS Institute, 2002). A likelihood ratio test was performed to test the hypothesis that the LD values were equal. If the hypothesis was rejected, pairwise comparisons were performed and significance was stated when confidence intervals (95% CI) did not overlap (Savin et al., 1977; Robertson et al., 2007). Resistance ratios were calculated by dividing the LD_{50} values of Lambda-res or heterozygous by the corresponding parameter for the Sus strain. Parallelism and equality tests were also performed to compare the angular and linear coefficients obtained for the regression lines of each strain (Robertson et al., 2007).

2.4 Dominance of resistance

To estimate the dominance of resistance (*D*), reciprocal crosses were performed between Lambda-res and Sus strains (50 couples per cross). Late L2/early L3 larvae from each strain were then subjected to the dose-response bioassays as described above.

The degree of dominance (*D*) was calculated using the equation [1] proposed by Bourguet et al. (2000):

$$D = (M_{RS} - M_{SS})/(M_{RR} - M_{SS}) \qquad [1]$$

where, M_{SS}, M_{RR} and M_{RS} are the mortalities of the Sus, Lambda-res and heterozygote strains, respectively. For *D* values close to 0 ($D \cong 0$) the inheritance was characterized as incompletely recessive, and for values close to 1 ($D \cong 1$), the resistance was considered as incompletely dominant.

According to Stone (1968) method, the degree of dominance (*D*) is given by equation [2]:

$$D = (2X_F - X_R - X_S)/(X_R - X_S) \qquad [2]$$

where X_F, X_R and X_S are logarithms of the LD_{50} estimated for the heterozygous, Lambda-res and Sus strains, respectively. For *D* values close to – 1 ($D \cong -1$) the resistance inheritance was considered as incompletely recessive, and for values close to 1 ($D \cong 1$), it was incompletely dominant.

2.5 Survival on soybean leaves sprayed with lambda-cyhalothrin

The survival of Lambda-res, Sus and heterozygous larvae were also evaluated in bioassays with soybean plants (BMX Ativa RR, Brasmax, Passo Fundo, RS, Brazil) cultivated in a greenhouse at 28 ± 4°C, 60 ± 15% RH and a 14:10 h (light:dark) photoperiod. At V_6 growth stage (six unfolded trifoliolate leaves), plants were sprayed with lambda-cyhalothrin (Kaiso

CS, 250 g lambda-cyhalothrin/L, Nufarm A/S, Maracanaú, CE, Brazil) at a rate of 80 ml/ha diluted in 150 L of water (equivalent to 20 g a.i./ha), that represents the field dose against SBL in soybean. The control treatment was composed by unsprayed plants. After a drying period (~30 min), leaves of the upper third of the plants were collected and placed in 100 ml plastic pots. Then, a single late L2/early L3 larvae from Lambda-res, Sus or heterozygous were individualized in each pot. Sixty to 100 larvae/strain/treatment were evaluated (each replication was composed by 10 larvae). Before bioassays, SBL larvae of each strain were fed with soybean leaves for 24 h to reduce the possible effects of food change. Larval survival was evaluated at 48 h after insecticide exposure, and those without movement were considered dead. A generalized linear model for quasibinomial distribution was adjusted to analyze the data of larval survival. Good adjustment of the data to the model was obtained by using a half normal probability graph with a simulated envelope (Moral et al., 2017). Statistical differences were determined with the Tukey's honestly significant difference test. These analyses were performed in R software (R Development Core Team, 2017).

2.6 Cross-resistance of lambda-cyhalothrin to other pyrethroids in SBL

To evaluate cross-resistance, late L2/early L3 larvae from Lambda-res and Sus strains were exposed to the technical grade insecticides cypermethrin (93.6% purity, Sigma Aldrich, São Paulo, SP, Brazil) and deltamethrin (99.6% purity, Sigma Aldrich, São Paulo, SP, Brazil). Eight doses of cypermethrin (0.00032–0.56 μg a.i./larvae) and deltamethrin (0.00056–1.0 μg a.i./larvae) were tested. These doses were obtained in previous bioassays and cause mortality from ~7 to 98%. Each dose was repeated twice per strain in distinct bioassays (2 replications of 48 larvae/dose). The same statistical procedure described in the inheritance resistance study were used.

2.7 Fitness costs

To estimate fitness costs associated with the resistance to lambda-cyhalothrin, 150 neonates (10 replications of 15 larvae) from each strain were reared on an artificial diet in 50 ml plastic cups. The following biological parameters were recorded: survival and duration of the egg, larva, pupa and egg-to-adult periods; oviposition period; and number of eggs per female. The number of eggs was obtained from 15 to 20 couples per strain maintained in plastic cups (500 ml), internally covered with yellow paper. Eggs were counted daily until the death of the female. The embryonic period and survival were determined from 100 eggs obtained of the second or third oviposition of each pair. For this, eggs were stored into plastic cups and the number of hatched larvae were counted daily. Biological parameters data were subjected to studentized residuals analysis to confirm the assumption of normality with a Shapiro-Wilk test (PROC UNIVARIATE) and for homogeneity of variances with a Bartlett test (PROC GLM) in SAS® 9.1 (SAS Institute, 2002). Statistical differences were determined with the least-square means statement (PROC GLM) with Tukey adjustment in SAS® 9.1 (SAS Institute, 2002).

A fertility life table was also obtained by estimating the mean generation time (T), the net reproductive rate (R_0), and the intrinsic rate of increase (r_m). These parameters were determined with the '*lifetable.sas*' procedure developed by Maia et al. (2000) in SAS® 9.1 (SAS Institute, 2002). The relative fitness cost of the Lambda-res and heterozygous were calculated according to the equation [3] proposed by Cao and Han (2006):

$$Relative\ fitness\ cost = (R_0\ Lambda - res)/(R_0\ Sus) \qquad [3]$$

where R_0 is the net reproductive rate parameter from the life table.

3. Results

3.1 Selection of lambda-cyhalothrin-resistant SBL strain

The field-collected population of SBL in the F_1 generation in laboratory showed a higher LD_{50} (0.10 µg a.i./larva) to lambda-cyhalothrin than the Sus strain (0.0016 µg a.i./larva), indicating a resistance ratio of 62.5-fold (Stacke et al., 2019). After three generations of selection pressure with technical grade lambda-cyhalothrin at a dose of 0.32 µg a.i./larva (LD_{90} of field populations), there was an increase in LD_{50} value and resistance ratio of selected insects. These surviving insects were used to establish the lambda-cyhalothrin-resistant colony.

3.2 Inheritance of resistance

The LD_{50} value of lambda-cyhalothrin was higher for the Lambda-res strain (0.11 µg a.i./larva) than the Sus strain (0.0015 µg a.i./larva), resulting in a resistance ratio of 73.3-fold, differing by the equality ($\chi^2 = 221$; df = 2; $p < 0.0001$) and parallelism ($\chi^2 = 19.44$; df = 1; $p < 0.0001$) tests (Table 1 and Fig. 1). Estimated LD_{50} values of lambda-cyhalothrin against heterozygous was similar (0.016 and 0.017 µg a.i./larva), indicating a resistance ratio less than 10-fold relative to the Sus strain (Table 1). However, these LDs differed from those of the Lambda-res and Sus strains. The LD_{90} values of lambda-cyhalothrin also differed between the Lambda-res (0.95 µg a.i./larva) and Sus (0.015 µg a.i./larva) strains. In contrast, heterozygous larvae had similar LD_{90} values (0.12 and 0.14 µg a.i./larva), but were significantly different from those of Lambda-res and Sus strains (Table 1). The similar LD_{50} and LD_{90} values in heterozygous strains indicated by the overlapping of their 95% confidence intervals and in equality (χ^2 = 0.88; df = 2; p = 0.643) and parallelism (χ^2 = 0.08; df = 1; p = 0.776) tests (Table 1 and Fig. 1), confirm the hypothesis that resistance of SBL to lambda-cyhalothrin is autosomally inherited.

3.3 Dominance of resistance

The degrees of dominance estimated by the Bourguet et al. (2000) decreased with increasing lambda-cyhalothrin doses, indicating an incompletely recessive resistance at the highest doses tested (0.056 and 0.10 µg a.i./larva). However, at the LD_{50} values of heterozygous (0.0016 and 0.0017 µg a.i./larva) the inheritance was an incompletely dominant trait (Fig. 2). By Stone's method, the dominance levels were 0.10 and 0.13 for reciprocal crosses of Lambda-res♀ × Sus♂ and Lambda-res♂ × Sus♀, respectively. These values also indicated that the resistance to lambda-cyhalothrin in SBL is an incompletely dominant trait.

3.4 Survival of SBL strains on soybean leaves sprayed with lambda-cyhalothrin

Larvae of Lambda-res strain fed on soybean leaves sprayed with lambda-cyhalothrin showed significantly (F = 46.86; df = 3, 36; p < 0.0001) higher survival (85%) than heterozygous (60 and 64%) and Sus (8%) strains (Fig. 3). Heterozygous strains had similar survival that feed on lambda-cyhalothrin sprayed-leaves, but showed significantly higher and lower survival rate compared to Sus and Lambda-res strains, respectively. In contrast, larvae from all strains showed a similar survival rate (> 88%) when fed on unsprayed leaves (Fig. 3) (F = 2.27; df = 3, 36; p = 0.10). These results indicated that heterozygous insects had high survival rates at the recommended field dose of lambda-cyhalothrin to SBL in soybean, indicating that in field conditions, the resistance is an incompletely dominant trait.

3.5 Cross-resistance of SBL to other pyrethroids

The Lambda-res strain showed low cross-resistance to deltamethrin and cypermethrin (Table 2). The Lambda-res strain presented a lower resistance ratio for deltamethrin (6.2-fold) compared to another pyrethroid, cypermethrin (22.5-fold). The resistant strain also differed from Sus strain by the equality ($\chi^2 = 77.95$; df = 2; $p < 0.0001$) and parallelism ($\chi^2 = 18.63$; df = 1; $p < 0.0001$) tests for the susceptibility to deltamethrin. However, when exposed to cypermethrin, the Lambda-res and Sus strains differed by the equality test ($\chi^2 = 127$; df = 2; $p < 0.0001$), but not by the parallelism test ($\chi^2 = 3.62$; df = 1; $p = 0.057$), indicating that curves present similar slopes.

3.6 Fitness cost

No differences in the egg period between SBL strains were detected ($F = 0.04$; df = 3, 36; $p = 0.99$) (Fig. 4). However, the larval ($F = 41.41$; df = 3, 36; $p < 0.0001$) and egg-to-adult ($F = 17.18$; df = 3, 36; $p < 0.0001$) periods of the Lambda-res and heterozygous were significantly longer than that of the Sus strain. The pupal period of Lambda-res and heterozygous were shorter than those of Sus strain ($F = 12.84$; df = 3, 36; $p < 0.0001$). No differences in the larval survival of SBL strains were observed ($F = 0.80$; df = 3, 36; $p = 0.51$). In contrast, the egg ($F = 20.16$; df = 3, 36; $p < 0.0001$) and pupal ($F = 6.36$; df = 3, 36; $p = 0.0014$) survival of Lambda-res was significantly lower than heterozygous and Sus strains (Fig. 4). This reduced the number of Lambda-res insects that reached adulthood when compared to the Sus and heterozygous strains ($F = 71.18$; df = 3, 36; $p < 0.0001$). The Lambda-res and Lambda-res♀ × Sus♂ strains had a reduction in the oviposition time when compared to other strains ($F = 5.88$; df = 3, 71; $p = 0.0012$) (Fig. 4). The number of eggs/female was higher for Lambda-res♂ × Sus♀ than Lambda-res (*F* = 3.13; df = 3, 71; *p* = 0.0311); however, was similar among Sus and heterozygous (Fig. 4). These results indicated that the Lambda-res strain oviposited 48% fewer eggs than Sus strain.

Results of fertility life table indicated that Lambda-res♀ × Sus♂ strain presented reproductive rates significantly lower than Lambda-res♂ × Sus♀ and Sus strains (Table 3). According to these results, each Lambda-res female originated 25 females in an average generation time of 34.7 days, while the heterozygous strains produced 209 and 105 females/female in 33 and 35 days, respectively. In the same period, the Sus strain produced 189 females/female. The Lambda-res also showed a relative fitness cost of 0.13, demonstrating a competitive disadvantage compared with the Sus strain, in absence of selection pressure by lambda-cyhalothrin. However, heterozygous had a relative fitness cost of 0.83, presenting no relevant disadvantages compared to Sus strain. These results suggest that the fitness costs of resistance to lambda-cyhalothrin in SBL trend to a recessive trait.

4. Discussion

The survival of Lambda-res larvae on lambda-cyhalothrin sprayed-leaves confirms the presence of phenotypic resistance in SBL to lambda-cyhalothrin. In addition, the Lambda-res strain showed a resistance ratio of 73.3-fold to lambda-cyhalothrin. These results indicate that the resistance to lambda-cyhalothrin in this strain is a genetic characteristic. The resistance is related with the selection pressure caused by the use of pyrethroids against SBL (until 2 sprays/season) on soybean and cotton crops in Brazil. The mixtures of lambda-cyhalothrin with other insecticides (specially neonicotinoids) are also commonly used against stink bugs, and this may exert selection pressure in SBL, because these species infest soybeans at the same time.

In this study, the first characterization of resistance of SBL to lambda-cyhalothrin was reported. However, since 1987 the resistance to SBL has been documented for pyrethroids in the United States (Felland et al., 1990; Thomas and Boethel, 1995; Thomas et al., 1996). The resistance ratio to lambda-cyhalothrin estimated in our study is similar to that of SBL strains resistant to permethrin (70 and 93-fold) (Thomas and Boethel, 1995; Thomas et al., 1996) and deltamethrin (62-fold) (Thomas et al., 1996). However, these resistance ratios are lower than those verified for cypermethrin (145-fold) (Thomas et al., 1996). The resistance to pyrethroids has also been reported in Brazilian populations of *Spodoptera frugiperda* (Smith) to lambda-cyhalothrin (Diez-Rodriguez and Omoto, 2001), *Helicoverpa armigera* (Hübner) to fenvalerate (Durigan et al., 2017), and *Tuta absoluta* (Meyrick) to permethrin, bifenthrin, cypermethrin and deltamethrin (Silva et al., 2015).

Mortality response curves of heterozygous indicated an autosomal inheritance of SBL resistance to lambda-cyhalothrin. Similar inheritance pattern of resistance to pyrethroids has also been observed in *Chloridea virescens* (F.) (Payne et al., 1988), *H. armigera* (Achaleke and Brévault, 2010), and *S. frugiperda* (Diez-Rodriguez and Omoto, 2001). Our results also suggest that the SBL resistance to lambda-cyhalothrin is an incompletely dominant trait. Similar degrees of dominance associated with resistance to pyrethroids was reported in *C. virescens* (Elzen et al., 1994), *Helicoverpa zea* (Boddie) (Brown et al., 1998), and *H. armigera* (Achaleke and Brévault, 2010). Our results indicate that the recommended dose of lambda-cyhalothrin (20 g a.i./ha) against SBL is insufficient to kill all heterozygous larvae, showing that the effective dominance also trends to an incompletely dominant trait. The survival of heterozygous can increase the resistance frequency and make difficult to restore the susceptibility in the absence of selection pressure, because these insects are primarily responsible for dispersing resistant alleles (Tabashnik and Croft, 1982; Roush and McKenzie, 1987). This also explain the low susceptibility of Brazilian populations of SBL to lambda-cyhalothrin-based insecticides.

The Lambda-res strain also presented low cross-resistance to cypermethrin and deltamethrin. This suggest that the mechanism that confers resistance to lambda-cyhalothrin in SBL also affect the toxicity of other pyrethroids. This cross-resistance can be related to the similarity between the structure of the molecules and binding sites of pyrethroids (Carvalho et al., 2013; Dong et al., 2014). Cross-resistance between pyrethroids could result from metabolic detoxification enzymes (cytochrome P450 monooxygenase, GST and esterase) as reported in SBL (Thomas et al., 1996) and *H. armigera* (Martin et al., 2002; Ramasubramanian and Regupathy, 2004; Young et al., 2006; Durigan et al., 2017), but also from mutation at coding region of the voltage-gated sodium channel (Burton et al., 2011; Dong et al., 2014). The occurrence of cross-resistance between pyrethroids can compromise the use of this group of insecticides to control SBL.

The resistance of SBL to lambda-cyhalothrin was also associated with significant fitness costs. Similar fitness costs were documented in *Cydia pomonella* (Linnaeus) and *H. armigera* resistant to deltamethrin and cypermethrin, respectively (Boivin et al., 2001; Djihinto et al., 2012). The presence of fitness costs indicates that removal the control agent from the environmental, the susceptibility of SBL to pyrethroids can be restored, as verified in *H. armigera* resistant to pyrethroids in China and Africa (Wu et al., 2005; Djihinto et al., 2009; Achaleke and Brévault, 2010). Therefore, a reduction in pyrethroid against SBL and the rotation of insecticides with different modes of action can contribute to restore the susceptibility to pyrethroids in SBL. A recent study showed that Brazilian populations of SBL are highly susceptible to several insecticides, such as spinetoram, indoxacarb, thiodicarb, diamides and chlorfenapyr, which can be used in IRM programs (Stacke et al., 2019). The use of other available and effective Integrated Pest Management (IPM) tactics, such as Bt soybean and cotton and baculovirus-based insecticides may also reduce the frequency of resistance to lambda-cyhalothrin in SBL.

In summary, in this study we reported the characterization of SBL resistance to lambda-cyhalothrin and found low cross-resistance between other pyrethroids, demonstrating the potential of evolution of field resistance in this species in Brazil. Future efforts should focus on monitoring the susceptibility of Brazilian populations of SBL to insecticides to identify changes in the susceptibility as a result of repeated exposure to these chemistries. The implementation of IPM programs that integrate multiple control tactics with diverse mortality factors, rather than just relying on wide scale use of single control tactics like insecticides, are also needed to ensure the sustainability of chemical control against SBL and another pest species in soybean and cotton crops in Brazil and other countries.

Author contributions

RFE JVCG and OB conceived and designed the studies; RFE DNG SAH ESB TG BLH DSM selected the resistant colony, performed experiments and collected data; RFE, DSM and OB analyzed the data; RFE BLH DSM JVCG and OB interpreted the results, discussed, and wrote the manuscript; RFE, JVCG and OB coordinated the project; OB acquisition of the financial support for the project leading to this publication. All authors read and approved the final manuscript.

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Table 1. Concentration-mortality (LD; μg a.i. larvae^{-1}) response of SBL strains to lambda-cyhalothrin.

SBL strains	*n*	Slope ± SE^{b}	LD$_{50}$ (95% CI)^{a,b}	LD$_{90}$ (95% CI)^{a,b}	χ^{2c}	df^{d}	RR^{e}
Lambda-res	448	1.34 ± 0.29	0.11 (0.06–0.15) c	0.95 (0.55–3.34) c	5.71	5	73.3
Lambda-res♂ × Sus♀	768	1.48 ± 0.13	0.016 (0.013–0.019) b	0.12 (0.08–0.17) b	6.04	5	10.7
Lambda-res♀ × Sus♂	720	1.38 ± 0.15	0.017 (0.013–0.021) b	0.14 (0.10–0.24) b	6.03	5	11.3
Sus	512	1.27 ± 0.16	0.0015 (0.0007–0.0025) a	0.015 (0.009–0.026) a	4.09	5	-

[a] LD$_{50}$: concentration of insecticide required to kill 50% of larvae in the observation period of 48h. Similarly, LD$_{90}$ is the concentration of insecticide required to kill 90% of larvae tested.

[b] LD$_{50}$ and LD$_{90}$ values designated by different letters within a column are significantly different from each other through non overlap of 95% confidence intervals (CIs).

[c] $P > 0.05$ in the goodness-of-fit test.

[d] Degrees of freedom.

[e] Resistance Ratio (RR) = (LD$_{50}$ of indicated strain)/(LD$_{50}$ of Sus strain).

Table 2. Concentration mortality (LD; µg a.i. larvae^{-1}) response of resistant (Lambda-res) and susceptible (Sus) strains of SBL to deltamethrin and cypermethrin.

SBL strain	*n*	Slope ± SE[b]	LD_{50} (95% CI)[a,b]	LD_{90} (95% CI)[a,b]	χ^{2c}	df[d]	RR[e]
Deltamethrin							
Lambda-res	552	0.92 ± 0.13	0.016 (0.006–0.030) b	0.38 (0.21–0.86) b	3.93	6	6.2
Sus	432	1.31 ± 0.14	0.0026 (0.0018–0.0035) a	0.025 (0.017–0.040) a	3.75	4	-
Cypermethrin							
Lambda-res	528	1.44 ± 0.20	0.081 (0.049–0.112) b	0.633 (0.454–1.053) b	8.89	6	22.5
Sus	494	0.97 ± 0.15	0.0036 (0.0012–0.0073) a	0.075 (0.044–0.15) a	6.32	5	-

[a] LD_{50}: concentration of insecticide required to kill 50% of larvae in the observation period of 48 h. Similarly, LD_{90} is the concentration of insecticide required to kill 90% of larvae tested.

[b] LD_{50} and LD_{90} values designated by different letters within a column are significantly different from each other through non overlap of 95% confidence intervals (CIs).

[c] $P > 0.05$ in the goodness-of-fit test.

[d] Degrees of freedom.

[e] Resistance Ratio (RR) = (LD_{50} of indicated strain)/(LD_{50} of Sus strain).

Table 3. Fertility life table parameters of SBL strains reared on artificial diet.

SBL strain	Fertility life table parameter[a,b]		
	T (days)	R_o (♀ / ♀)	r_m (♀ / ♀*day)
Lambda-res	34.70 ± 0.20 b	25.44 ± 5.67 c	0.09 ± 0.006 c
Lambda-res♂ × Sus♀	33.35 ± 0.13 c	209.39 ± 37.97 a	0.16 ± 0.004 a
Lambda-res♀ × Sus♂	35.04 ± 0.45 ab	105.46 ± 25.87 b	0.13 ± 0.007 b
Sus	32.14 ± 0.18 c	189.48 ± 26.66 a	0.16 ± 0.005 a

[a] T = mean length of a generation (days); R_o = net reproductive rate (females per female per generation); r_m = intrinsic rate of population increase (per day).

[b] Means within a column followed by the same letter are not significantly different (*t*-tests for pairwise group comparisons, $P > 0.05$).

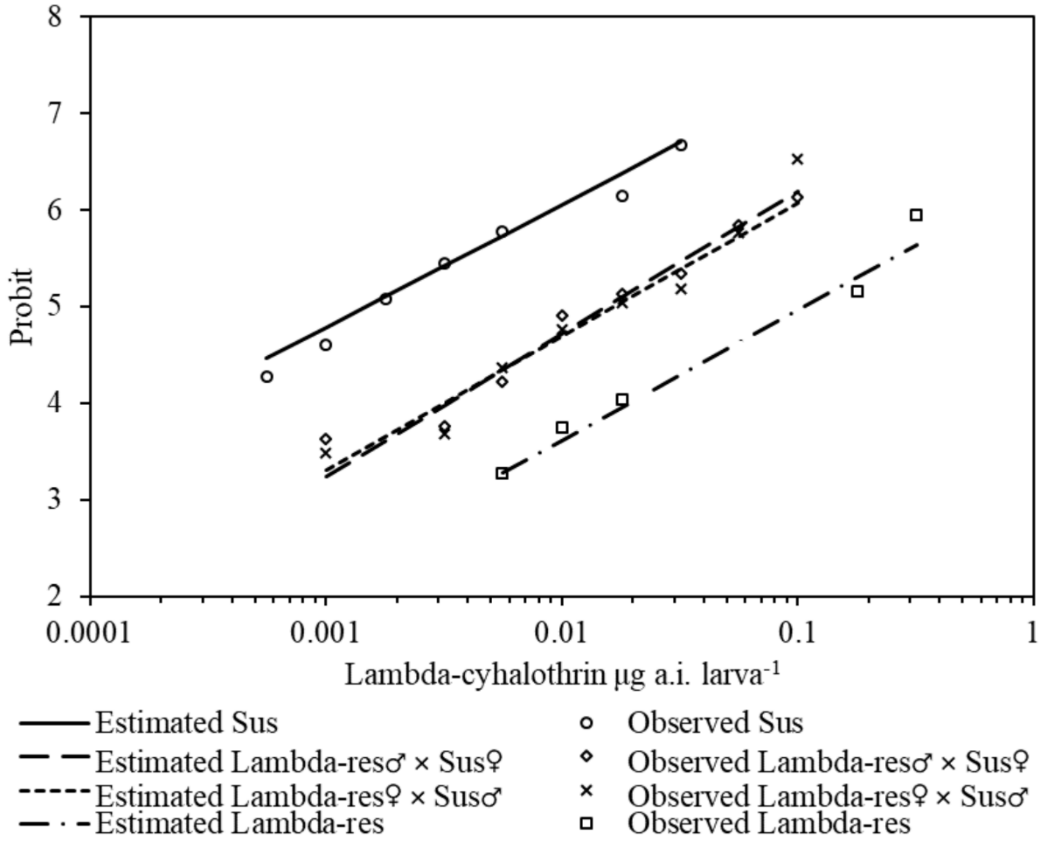

Figure 1. Mortality (Probit) response of SBL strains to lambda-cyhalothrin.

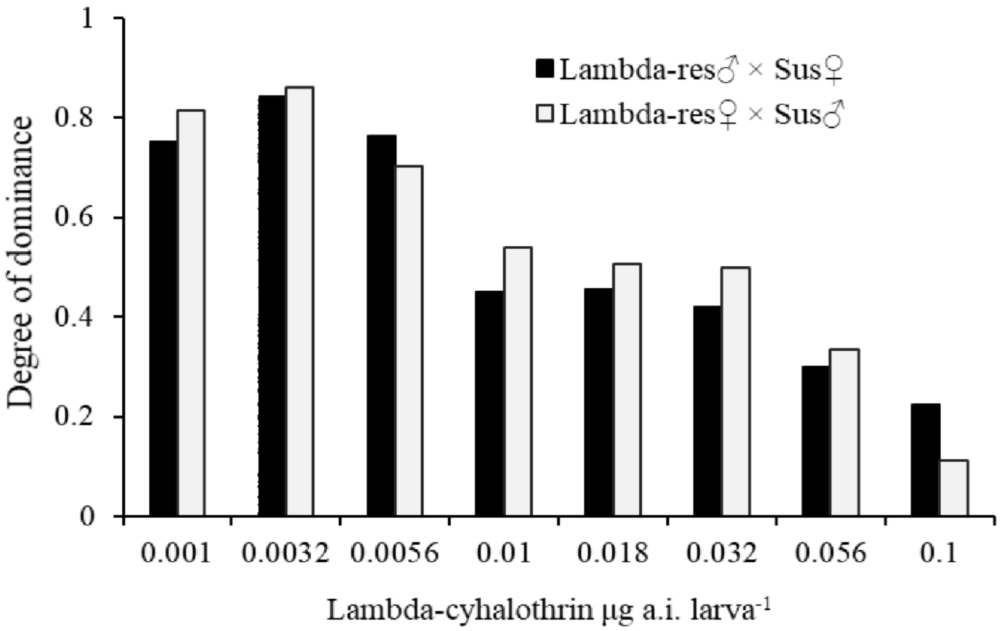

Figure 2. Degree of dominance of resistance to lambda-cyhalothrin in SBL as a function of lambda-cyhalothrin concentration.

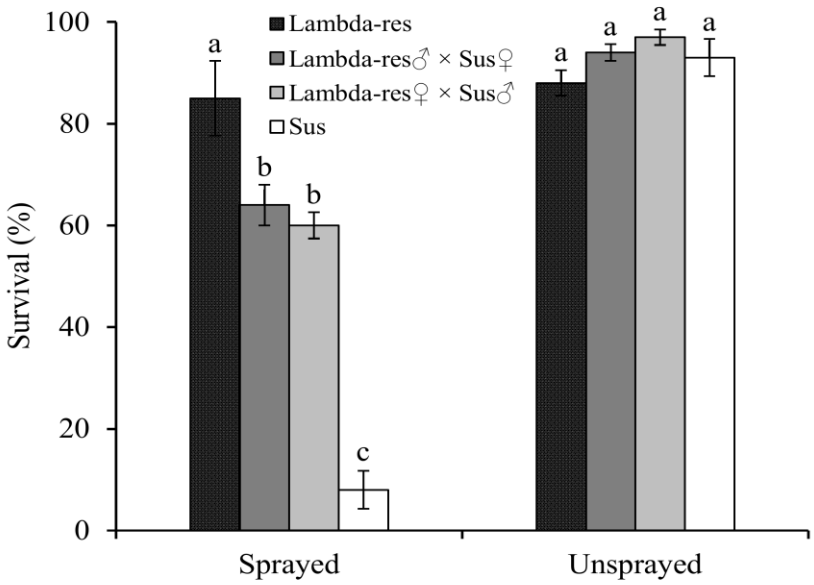

Figure 3. Survival (% ± SE) of third-instar larvae of SBL strains after 2 days on soybean leaves that had either been sprayed with lambda-cyhalothrin or unsprayed. Groups of bars (± SE) with the same letter are not significantly different ($P > 0.05$).

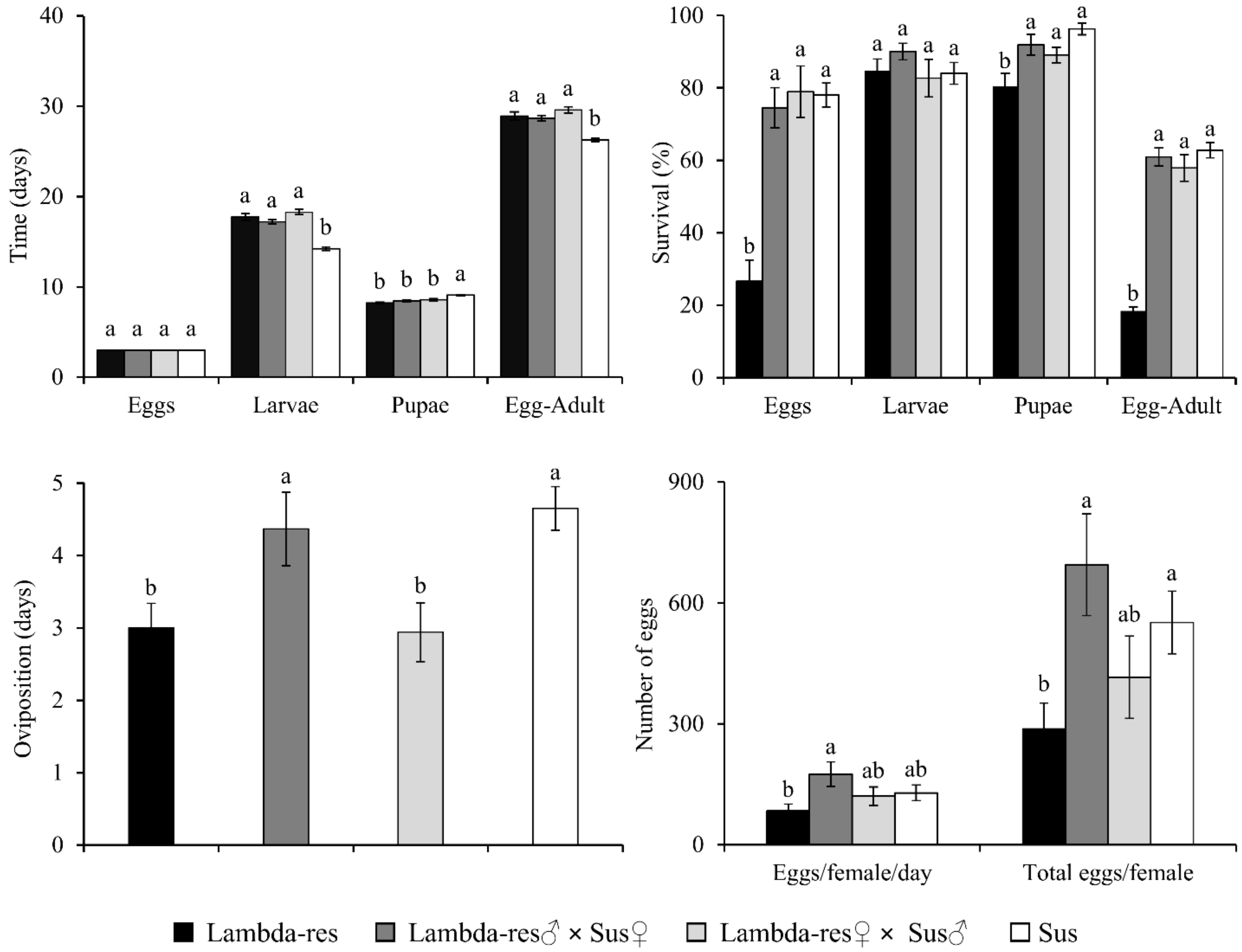

Figure 4. Life history of SBL strains on artificial diet. Groups of bars (± SE) with the same letter are not significantly different ($P > 0.05$).

6 DISCUSSÃO

As populações de *C. includens* apresentaram baixos níveis de resistência aos inseticidas spinetoram, indoxacarbe e tiodicarbe (atuam no sistema nervoso), clorantraniliprole e flubendiamida (atuam no sistema muscular) e ao clorfenapir (atua respiração celular dos insetos). Em contraste, elevados níveis de resistência foram detectados em populações de *C. includens* para lambda-cialotrina (modulador do canal de sódio), metoxifenozide (agonista do receptor de ecdisteroides), novaluron e teflubenzuron (inibidores da biossíntese de quitina). A baixa variação na suscetibilidade das populações brasileiras de *C. includens* ao espinetoram, indoxacarbe, tiodicarbe, clorfenapir, clorantraniliprole e flubendiamida sugere que, atualmente, a frequência de resistência a esses inseticidas é baixa no campo. Isso é comprovado pela alta eficácia de controle de alguns inseticidas, como espinetoram, indoxacarbe e clorfenapir para o controle de *C. includens* durante as safras de 2014-2016 (PERINI et al., 2019).

Em contrapartida, para os inseticidas lambda-cialotrina, metoxifenozide, teflubenzuron e novaluron as populações de *C. includens* demonstraram uma baixa suscetibilidade. A exposição repetida de populações de *C. includens* a esses princípios ativos pode ter favorecido a evolução da resistência. A baixa eficácia da lambda-cialotrina e inibidores da biossíntese de quitina contra *C. includens* também foi relatada em estudos de campo conduzidos ao longo de três anos (2014–2016), nas quais se obteve controle inferior a 45% (PERINI et al., 2019). Esses resultados sugerem que essa espécie evoluiu para resistência a lambda-cialotrina, novaluron e teflubenzuron.

Em laboratório, após três gerações de pressão de seleção, foi selecionada uma população de *C. includens* resistente a lambda-cialotrina. Os bioensaios de caracterização da resistência mostraram que a herança da resistência de *C. includens* à lambda-cialotrina é autossômica e incompletamente dominante. A mesma tendência foi verificada nos ensaios com folhas de soja pulverizadas com a dose comercial de lambda-cialotrina (20 g i.a. ha^{-1}) recomendada para o controle de *C. includens*, em que os heterozigotos apresentaram elevada sobrevivência e a dominância efetiva tendeu a uma característica incompletamente dominante. A sobrevivência dos heterozigotos pode aumentar a frequência dos alelos resistentes no campo, favorecendo a ocorrência de falhas de controle após as pulverizações e retardando o reestabelecimento da suscetibilidade na ausência de pressão de seleção (TABASHNIK; CROFT, 1982; ROUSH; MCKENZIE, 1987).

O estudo do custo adaptativo mostrou que a linhagem Lambda-res teve uma menor taxa de sobrevivência e reprodução do que os indivíduos heterozigotos e suscetíveis. Por outro lado, os heterozigotos têm características semelhantes a linhagem suscetível, indicando que não há custo adaptativo relevante. Esse custo adaptativo associado a resistência de *C. includens* a lambda-cialotrina deve ser explorado no MRI. Esse custo de Lambda-res indica que a retirada do agente de seleção do ambiente, provoca a diminuição dos indivíduos resistentes e consequentemente o reestabelecimento da suscetibilidade aos piretroides no campo. Porém, os indivíduos heterozigotos, principais carregadores dos alelos de resistência, mesmo na ausência da pressão de seleção por lambda-cialotrina, iriam permanecer presentes dentro da população, necessitando assim de um manejo adequado, com a utilização de táticas de controle que causem a mortalidade desses, como a rotação de inseticidas eficientes.

Esse é a primeira caracterização da resistência de *C. includens* a piretroides no Brasil. Essa resistência é uma consequência da pressão de seleção causada pelo uso de piretroides na cultura da soja. Na soja, a mistura de lambda-cialotrina com outros inseticidas (principalmente neonicotinóides) também é comumente usada para o controle de percevejos e isso também exerce pressão de seleção nas populações de *C. includens*, devido ao fato de ambas as espécies ocorrerem em altas infestações no mesmo período de desenvolvimento da soja. Além disso, há evidências de resistência de *C. includens* aos inseticidas reguladores de crescimento, teflubenzuron e novaluron, amplamente utilizados no manejo dessa espécie. Com base nisso, é necessário reduzir e/ou evitar o uso desses ingredientes ativos, bem como, realizar a rotação de diferentes modos de ação para o controle de *C. includens*, evitando a utilização dos mesmos ingredientes ativos dentro da mesma estação de cultivo.

Dentre os inseticidas utilizados para o controle de *C. includens*, o espinetoram, o indoxacarbe, o tiodicarbe, o clorantraniliprole, a flubendiamida e o clorfenapir, ainda apresentam elevada eficácia no controle dessa espécie, dada a baixa razão de resistência verificada (Stacke et al., 2019; Perini et al., 2019), podendo ser usados em rotação no controle dessa praga. Porém, a principal medida de manejo que necessita ser levada em consideração é o monitoramento, saber qual é o nível de infestação existente na lavoura, para assim, baseado no nível de dano econômico e nível de controle, realizar a utilização das táticas de controle no momento adequado, evitando pulverizações desnecessária e assim reduzindo a pressão de seleção. Além disso, o uso de outras táticas de controle disponíveis e eficazes de MIP, como a soja e o algodão Bt, os inseticidas biológicos a base de baculovírus e os parasitoides de ovos, podem contribuir para restaurar a suscetibilidade de *C. includens*, aos piretroides e aos inseticidas reguladores de crescimento.

Desse modo, o uso isolado ou integrado dessas práticas de manejo, pode evitar e/ou retardar a evolução da resistência de *C. includens* para táticas de controle. A não realização dessas medidas de manejo da resistência de *C. incudens*, pode acarretar em sérios prejuízos ao produtor e ao meio ambiente, com elevado custo da aplicação, falhas no controle da praga, aumentos dos danos na cultura e consequentemente a evolução da resistência e a perda de moléculas e opções manejo para essa espécie. Sendo assim, o monitoramento da suscetibilidade do *C. includens*, deve ser realizado com frequência, para identificar as falhas e manter a espécie sob controle.

Os resultados aqui apresentados, são informações relevantes sobre populações de *C. includens* de diferentes regiões produtoras de soja do Brasil e sobre os principais inseticidas utilizados para o controle dessa espécie-praga. Assim como, informações sobre a resistência de *C. includens* à lambda-cialotrina, um inseticida amplamente utilizado na agricultura brasileira. Portanto, o manejo *C. includens* deve considerar os riscos de evolução da resistência a inseticidas, utilizando os inseticidas mais indicados e que apresentam eficiência de controle, apresentados nesse trabalho, combinados com as táticas do MIP e MRI.

7 CONCLUSÃO

As populações brasileiras de *C. includens* são suscetíveis aos inseticidas espinetoram, indoxacarbe, tiodicarbe, clorfenapir, clorantraniliprole e flubendiamida.

As populações de *C. includens* apresentam baixa suscetibilidade aos inseticidas lambda-cialotrina, metoxifenozide, novaluron e teflubenzuron.

A resistência de *C. includens* a lambda-cialotrina é autossômica e incompletamente dominante.

Há custo adaptativo associado à resistência de *C. includens* a lambda-cialotrina.

A resistência de *C. includens* a lambda-cialotrina afeta negativamente a suscetibilidade a deltametrina e cipermetrina.

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