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NAYARA CECÍLIA RODRIGUES COSTA

PARÂMETROS BIOLÓGICOS, COMPORTAMENTAIS E SUSCETIBILIDADE DE *Hypothenemus hampei* À DIAMIDAS

UBERLÂNDIA – MG
2021

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Tese apresentada ao Instituto de Ciências Agrárias da Universidade Federal de Uberlândia como requisito parcial para obtenção do título título de doutor em Agronomia.

Área de concentração: Fitotecnia

Orientador: Prof. DSc. Flávio Lemes Fernandes

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Rodovia BR 050, Km 78, Bloco 1CCG, Sala 206 - Bairro Glória, Uberlândia-MG, CEP 38400-902
Telefone: (34) 2512-6715/6716 - www.ppga.iciag.ufu.br - posagro@ufu.br

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"Nele havia vida, e a vida era a luz dos homens."

João 1, 4.

RESUMO

A broca-do-café (*Hypothenemus hampei*) é a principal praga do cafeeiro e o método químico é o mais utilizado para seu controle, com destaque para o grupo das diamidas. Assim, objetiva-se elucidar parâmetros biológicos, parâmetros comportamentais e a suscetibilidade de *H. hampei* a diamidas. Os bioensaios de efeitos comportamentais foram conduzidos no Laboratório de Entomologia da Universidade Federal de Viçosa (UFV) – Viçosa (MG). A toxicidade, sobrevivência, reprodução larval, resposta comportamental e taxa de respiração foram avaliadas após a exposição ou não dos insetos adultos à clorantraniliprole. Os tratamentos foram as DL_{50} e DL_{90} de clorantraniliprole, e controle. Os bioensaios de linha básica de suscetibilidade, o monitoramento de resistência das populações e o risco de falha de controle foram conduzidos no Laboratório de Manejo Integrado de Pragas da UFV – Rio Paranaíba (MG). As populações foram coletadas em estados produtores de café, de 2016 a 2018. Em dieta artificial de *H. hampei*, aplicou-se os tratamentos das CL_{50} (0,07 mg i.a. mL^{-1}) e CL_{90} (0,57 mg i.a. mL^{-1}) de ciantraniliprole, dose de campo (0,37 mg i.a. mL^{-1}) e controle. O clorantraniliprole foi tóxico para *H. hampei* (DL_{50} = 0,49 mg mL^{-1} e DL_{90} = 1,21 mg mL^{-1}). A sobrevivência foi de 98% em adultos não expostos ao clorantraniliprole, diminuindo para 52% em insetos tratados com DL_{50} e 2% com DL_{90}. Aos 20 dias, a DL_{90} afetou a produção de larvas de *H. hampei*, com o menor número de larvas vivas (0,25), comparado a DL_{50} (2,22 larvas vivas) e ao controle (4,12 larvas vivas). Os adultos do controle percorreram a maior distância (3.871 cm) e ficaram menos tempo de repouso (185 s), enquanto as DL_{50} (3.818 cm e 378 s) e DL_{90} (1.422 cm e 444 s) promoveram ação contrária. A taxa de respiração foi regressiva quando exposta às DL_{90} (0,33 µL de CO_2 h^{-1} $inseto^{-1}$), DL_{50} (0,86 µL de CO_2 h^{-1} $inseto^{-1}$) e ao controle (1,59 µL de CO_2 h^{-1} $inseto^{-1}$). Populações de Campo do Meio (MG), Linhares (ES) e Jaú (SP) foram mais suscetíveis (resistência <2 vezes) ao ciantraniliprole do que populações de Patrocínio (MG) e Londrina (PR) (17 vezes). A frequência de insetos resistentes e o risco de falha de controle ao ciantraniliprole foi baixa e não significativa. A DL_{90} de clorantraniliprole causou efeitos negativos em todos os testes comportamentais. As populações ainda permanecem suscetíveis à ciantraniliprole.

Palavras-chave: Broca-do-café. Controle químico. Inseticidas. Manejo integrado de pragas. Resistência.

ABSTRACT

The coffee borer (*Hypothenemus hampei*) is the main pest of coffee and the chemical method is the most used for its control, especially the group of diamides. That said, this study aimed to understand the biological and behavioral parameters as well as the susceptibility of *H. hampei* to diamides. The behavioral effects bioassays were performed at the Entomology Laboratory of the Federal University of Viçosa (UFV) - Viçosa (MG). Toxicity, survival, larval reproduction, behavioral response and respiration rate were assessed after exposure or non-exposure of adult insects to chloranthranilprole. The treatments of the LD_{50} and DL_{90} of chloranthraniliprole and control were used. Basic susceptibility line bioassays, population resistance monitoring and the risk of control failure were conducted at the Integrated Pest Management Laboratory at UFV - Rio Paranaíba (MG). The populations were collected in coffee-producing states from 2016 to 2018. In the artificial diet of *H. hampei*, the treatments of the LC_{50} (0.07 mg a.i. mL^{-1}) and LC_{90} (0.57 mg a.i. mL^{-1}) of cyantraniliprole, field dose (0.37 mg a.i. mL^{-1}) and control were applied. Chloranthraniliprole was toxic to *H. hampei* (LD_{50} = 0.49 mg mL^{-1} and LD_{90} = 1.21 mg mL^{-1}). The survival rate was 98% in adults not exposed to chlorantraniliprole, 52% in insects exposed to LD_{50} and 2% with LD_{90}. At 20 days, LD_{90} affected the reproduction of *H. hampei* larvae, with the lowest number of live larvae (0.25), compared with LD_{50} (2.22 live larvae) and control (4.12 live larvae). Control adults moved the longest distance (3,871 cm) and spent less time at rest (185 s), while the LD_{50} (3,818 cm and 378 s) and LD_{90} (1,422 cm and 444 s) caused the opposite action. The respiration rate was regressive when exposed to the LD_{90} (0.33 µL of CO_2 h^{-1} $insect^{-1}$), LD_{50} (0.86 µL of CO_2 h^{-1} $insect^{-1}$) and control (1.59 µL of CO_2 h^{-1} $insect^{-1}$). Populations from Campo do Meio (MG), Linhares (ES) and Jaú (SP) were more susceptible (< 2-fold resistance) to cyantraniliprole than populations from Patrocínio (MG) and Londrina (PR) (17-fold). The frequency of cyantraniliprole resistant populations and the likelihood of control failure were low and not significant. The frequency of resistant insects and the control failure likelihood to cyantraniliprole were also low and not significant. The LD_{90} of chlorantraniliprole had negative effects in all behavioral tests. Populations remain susceptible to cyantraniliprole.

Keywords: Coffee berry borer. Chemical control. Insecticides. Integrated pest management. Resistance.

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

SUMÁRIO

INTRODUÇÃO GERAL

A broca-do-café, *Hypothenemus hampei* (FERRARI, 1867) (Coleoptera: Curcolionidae: Scolytidae) é considerada praga-chave da cultura do café (INFANTE *et al*., 2014). *H. hampei* é um inseto monófago, holometábolo e críptico, que se desenvolve no interior dos frutos de café. O ciclo biológico inicia-se pela fêmea, que perfura o fruto até atingir o endosperma, formando uma galeria, onde escava uma câmara para efetuar a postura. Em temperaturas de 22 a 28ºC, o ciclo de vida dura de 23 a 38 dias (ovo: quatro a seis dias, larva: oito a 14 dias, pré-pupa: cinco a oito dias e pupa: cinco a oito dias) com longevidade média dos machos de 78 a 103 dias e de fêmeas de até 156 dias, e capacidade reprodutiva de 119 a 189 ovos (JARAMILLO, 2016).

A reprodução de *H. hampei* apresenta características compatíveis com a teoria da competição local por cópula ("Local Mate Competition – LMC"), devido à biologia dos machos e à razão sexual a favor das fêmeas, sendo obrigados a acasalarem entre irmãos (INFANTE *et al*., 2014; JARAMILLO, 2016) e assegurando um alto nível de endogamia (BARRERA *et al*., 1994; BERGAMIN, 1943; CONSTANTINO *et al*., 2011). A contribuição da endogamia na população da broca-do-café pode ser atenuada pela possibilidade de que fêmeas diferentes possam efetuar posturas no mesmo fruto (BAKER, 1999).

As fêmeas de *H. hampei* atacam no estádio fenológico de maturação de frutos de café de maduros a secos. O ataque causa prejuízos primários a partir da perfuração do fruto, e secundários; subsequentes à lesão criada, se torna um local de entrada exposta à proliferação de fungos e ocorrência de outras pragas que maximizam a queda prematura de frutos, a redução do peso do fruto, o rendimento, a qualidade da bebida, e consequentemente, o valor comercial do produto final (DURÁN *et al.*, 2017; SOUZA et al., 2013). Os prejuízos com a broca-do-café podem chegar a 300 milhões de dólares por ano entre os produtores de café (MOTA *et al.*, 2017; OLIVEIRA *et al*., 2013).

O período crítico para o ataque de *H. hampei* é observado nos meses de novembro a junho, entre a florada e a maturação dos frutos. Os métodos de controle são mais eficientes quando as fêmeas se encontram fora do fruto, normalmente nos meses de novembro e dezembro, período denominado de "trânsito da broca-do-café", entre a floração, chumbinho e expansão de frutos. Entretanto, esse período é curto para se obter o controle efetivo da praga, observando que o alvo se resume apenas às fêmeas, devido à incapacidade de voo dos machos, que permanecem durante todo seu ciclo biológico dentro do fruto (JARAMILLO, 2016). Dessa

forma, os principais métodos de controle são químico, biológico e cultural (colheita eficiente com repasse) (INFANTE *et al.*, 2014; JESCHKE, 2016; SPARKS; NAUEN, 2015).

Diante dessas particularidades, o controle efetivo desse inseto-praga torna-se ainda mais complexo. Associado ao uso indiscriminado de agrotóxicos, é cada vez mais evidente o aumento da dose, o número de aplicações e, eventualmente, a substituição do produto ineficaz por outro (FERNANDES *et al*., 2010; JESCHKE, 2016; SPARKS; NAUEN, 2015). A partir dessas ações, verificam-se perdas de eficiência e seletividade de inseticidas, dificuldades na tecnologia de aplicação, falta de direcionamento do princípio ativo ao alvo e resistência dos insetos-pragas (APRD, 2018; IRAC, 2019).

O inseticida Endosulfan 350 EC (grupo químico: ciclodienoclorado e ingrediente ativo: endosulfan) já foi considerado o inseticida mais eficiente no controle de *H. hampei*, em um levantamento realizado em 30 anos de pesquisa no controle químico da broca-do-café no Brasil, entre 1973 e 2003, em que, de 269 tratamentos levantados, cerca de 21% foram feitos com endosulfan, cuja eficiência variou de 70 a 100% (MANSINGH; RHODES, 1983; OLIVEIRA *et al*., 2003; REIS, 2007). Porém, a sua comercialização no Brasil foi proibida em 2010 devido a sua alta toxicidade humana, animal e ambiental (U.S. EPA, 2010), e seleção de populações de *H. hampei* resistentes (BRUN *et al*., 1989; FFRENCH-CONSTANT *et al*., 1994). Estima-se que a perda de eficiência dos produtos em controlar as pragas gera, em geral, um custo de um bilhão de dólares em produtividade das lavouras (COOK *et al*., 2005).

Atualmente, os inseticidas α-cipermetrina, ciantraniliprole, ciflutrina, clorantraniliprole, clorpirifos, deltametrina, fenpropatrina e tiametoxam têm sido utilizados para controlar *H. hampei*. As diamidas são um grupo químico recente (ingredientes ativos: clorantraniliprole, ciantraniprole, ciclaniliprole, flubendiamida) (IRAC, 2019), com alta eficácia para pragas, seletividade aos inimigos naturais, baixa toxicidade humana, animal e ambiental (JESCHKE, 2016; SPARKS; NAUEN, 2015). Clorantraniliprole e ciantraniprole têm proporcionado o controle da broca-do-café (INFANTE *et al*., 2014; SOUZA *et al*., 2013). Inseticidas desse grupo químico provocam ativação duradoura nos canais de cálcio no retículo sarcoplasmático dos músculos esqueléticos. Os canais de cálcio ativados liberam um excesso de íons cálcio nos filamentos de proteínas e induzem a contração muscular esquelética (NAUEN; STEINBACH, 2016; RODITAKIS *et al*., 2017). Posteriormente, ocorre contração gradual do corpo e morte do inseto (YU, 2014).

Diante disso, o objetivo desta tese é elucidar parâmetros biológicos, comportamentais e suscetibilidade de *H. hampei* a diamidas.

CAPÍTULO I

Chlorantraniliprole–mediated effects on survival, walking abilities, and respiration in the coffee berry borer, *Hypothenemus hampei*

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Chlorantraniliprole–mediated effects on survival, walking abilities, and respiration in the coffee berry borer, *Hypothenemus hampei*

Angelica Plata-Rueda[1], Luis Carlos Martínez[2]*, Nayara Cecília Rodrigues Costa[3], José Cola Zanuncio[4], Maria Elisa de Sena Fernandes[1], José Eduardo Serrão[2], Raul Narciso Carvalho Guedes[4], Flávio Lemes Fernandes[1]

[1] Instituto de Ciências Agrárias, Universidade Federal de Viçosa, 38810-000, Rio Paranaíba, Minas Gerais, Brasil; angelicaplata@yahoo.com.mx; maria.sena@ufv.br; flaviofernandes@ufv.br;

[2] Departamento de Biologia Geral, Universidade Federal de Viçosa, 36570-000, Viçosa, Minas Gerais, Brasil; lc.martinez@outlook.com; jeserrao@ufv.br;

[3] Departamento de Fitotecnia, Universidade Federal de Uberlândia, 38400-902, Uberlândia, Minas Gerais, Brasil; nayaracrcosta@gmail.com;

[4] Departamento de Entomologia, Universidade Federal de Viçosa, 36570-000, Viçosa, Minas Gerais, Brasil; zanuncio@ufv.br; guedes@ufv.br;

*Corresponding author: Luis Carlos Martínez, Departamento de Biologia Geral, Universidade Federal de Viçosa, Avenida Peter Henry Rolfs, Campus Universitário Viçosa, Minas Gerais, Brasil; CEP: 36570-000, Tel: + 55 31 3899-4012; Fax: + 55 31 3899-2108 E-mail: lc.martinez@outlook.com

Abstract

Hypothenemus hampei Ferrari (Coleoptera: Curculionidae) is the main pest of coffee crops, and effective methods for pest management are needed urgently. Bioassays were conducted to assess the effects of the insecticide chlorantraniliprole on *H. hampei* adults. Toxicity, survivorship, larval production, respiration rate, and behavioral responses to six concentrations of chlorantraniliprole were evaluated. Chlorantraniliprole was toxic to *H. hampei* (LD_{50} = 0.49 mg mL^{-1} and LD_{90} = 1.21 mg mL^{-1}). Survivorship was 98% in adults not exposed to chlorantraniliprole, decreasing to 52% in insects exposed to LD_{50} and 2% in insects treated with LD_{90}. *Hypothenemus hampei* showed reduced mobility on insecticide-treated surfaces. The insecticide promoted a decrease in the respiration rate of *H. hampei* for up to 3 h after exposure, altering behavioral responses and locomotor activity. Chlorantraniliprole was shown to have lethal and sublethal effects on *H. hampei* and, thus, can be used rotationally in integrated pest management programs to control of this pest in coffee crops and retard of insect resistance.

Keywords: anthranilic diamides; behavioral response; larval production; respiration rate; survivorship; toxicity

1. Introduction

Hypothenemus hampei Ferrari (Coleoptera: Curculionidae), commonly known as 'coffee berry borer' is the most damaging insect-pest of coffee in the Americas. The insect is native to Africa but has spread to coffee-producing countries in the Americas as a result of the introduction of infested seeds (Damon, 2000). *Hypothenemus hampei* females colonize immature and mature berries of *Coffea arabica* Linnaeus and *Coffea canephora* Linnaeus (Gentianales: Rubiaceae) (Bustillo Pardey, 2006). Adults attack 8–32 week-old coffee berries by tunneling into the endosperm and there deposit their eggs. Consequently, injuries caused by *H. hampei* reduce the yield and quality of the final product, resulting in considerable economic losses for farmers (Moore and Prior, 1988). Injuries caused by *H. hampei* create entry site for phytopathogens, such as *Aspergillus niger* van Tieghem (Trichocomaceae), *Erwinia stewartii* (Smith), *Erwinia salicis* (Day) (Enterobacteriaceae), and *Fusarium solani* (Mart.) (Nectriaceae) (Sponagel, 1994; Damon, 2000; Morales-Ramos et al., 2000).

The use of entomopathogenic fungi or ethanol/methanol traps as control methods, have not been effective against *H. hampei* in Brazilian coffee crops (Silva et al., 2006). As the insect completes its entire life cycle within the seed of the coffee berry, its control is difficult (Baker et al., 1992; Damon, 2000). Because of the high level of infestation and rapid spread of *H. hampei* in Brazilian coffee farms, the use of insecticides has become necessary (Cure et al., 1998). Females lay their eggs and leave the berry at the inter-harvest period to find and colonize other berries; it is in this moment that they can be exposed to chemical agents (Baker et al., 1992). Insecticides such as α-cypermethrin, chlorpyrifos, cyfluthrin, deltamethrin, dieldrin, fenpropathrin, thiamethoxam, and triazophos have been used to control *H. hampei*, but endosulfan is the prefered compound due to its reliably high efficacy (Mansingh and Rhodes, 1983; Damon, 2000; Oliveira et al., 2003). These insecticides can act by contact and/or ingestion and cause neurotoxicity, which may be lethal. Application of insecticides is an

153 efficient method to manage pest populations and reduce coffee seed damage (Oliveira et al.,
154 2003). However, insecticide resistance, particularly to endosulfan, has been reported in *H.*
155 *hampei* (Steichen and Brun, 1994).

156 New insecticides with modes of action different from organochlorines and pyrethroids,
157 are necessary to replace endosulfan and currently used and less effective alternatives. Calcium
158 channels can be a physiological target for pest control because they regulate cell functions that
159 involve muscle contraction and neurotransmitter release (Lahm et al., 2005; Isaacs et al., 2012).
160 Coordinated muscle contraction involves the activation of two distinct classes of calcium
161 channels: voltage-gated channels, which allow the entry of calcium, and ryanodine receptor
162 channels, which regulate the release of internal calcium stores (Cordova et al., 2006).
163 Anthranilic diamides are a novel class of chemical insecticides that act by promoting the release
164 of intracellular Ca^{2+} stores through activation of ryanodine receptors (Isaacs et al., 2012).
165 Within this chemical group, chlorantraniliprole stands out as a broad-spectrum insecticide
166 active against Coleoptera, Diptera, Hemiptera, Lepidoptera, and Thysanoptera (Teixeira et al.,
167 2009; Liu et al., 2012; Su et al., 2012; Hummel et al., 2014; Dale and Borden, 2018).

168 Insecticides can be effective in controlling coffee pests as they rapidly reduce insect
169 populations (Bardner, 1978; Nyambo et al., 1996; Damon, 2000). New insecticides that are
170 achieving registration within the regulatory environment of the United States Environmental
171 Protection Agency include compounds with lower risk to human health and higher toxicity to
172 pests than conventional insecticides (EPA, 2011). The efficiency of chlorantraniliprole was
173 demonstrated in coffee pests (Reis et al., 2014; EFSA, 2015; Zampiroli et al., 2017); however,
174 it is still unknown how this insecticide affects survival, locomotor activity and respiration in an
175 insect as cryptic as *H. hampei*.

In this research, we assessed the effects of the chlorantraniliprole on the survival, locomotor activity, and respiration of *H. hampei* as a means to contribute to the development of new strategies to mitigate insecticide resistance and control this insect pest.

2. Material and methods

2.1. Insects

Coffee berries infested with *H. hampei* were collected manually during the day from a 5-year old coffee farm in Viçosa, Minas Gerais, Brazil (20°45′S 42°52′W) without insecticide exposure. Insects were transferred from the field to the Laboratory of Biological Control of the Federal University of Viçosa for mass rearing. The infested coffee berries were kept in plastic trays (60 cm long × 40 cm wide × 12 cm high) in the dark at 25 ± 1 °C, 70 ± 10% relative humidity, and provided with mature 22-week-old coffee berries. Coffee berries were evaluated daily until adult emergence. Newly emerged 24 h-old *H. hampei* adults without apparent malformations were used in the bioassays.

2.2. Toxicity test

Chlorantraniliprole (350 g L^{-1}, Altacor® OD, Dupont, Alphaville, Brazil) was diluted in 10 mL of distilled water to obtain a stock solution. Six doses of chlorantraniliprole were then prepared and used to determine lethal dose (LD_{25}, LD_{50}, LD_{75}, and LD_{90}): 0.312 mg mL^{-1}, 0.625 mg mL^{-1}, 1.25 mg mL^{-1}, 2.5 mg mL^{-1}, 5 mg mL^{-1}, and 10 mg mL^{-1}. Distilled water was used as negative control. Each solution (0.25 µL) was applied on the thorax of 50 *H. hampei* adults using a 1 µL microsyringe (7001 KH, Hamilton Storage GmbH, Switzerland). Insects were placed individually in glass vials (2.5 cm × 8 cm) with perforated cap for ventilation and absorbent paper, fed *ad libitum* with a coffee berry, and maintained in the dark. The number of dead insects in each vial was counted after 96 h of insecticide exposure.

2.3 Time–mortality bioassay

The time-mortality bioassays to *H. hampei* using the insecticide doses obtained from the toxicity test were carried out to determine the acute/chronic lethal toxicity. Adults of *H. hampei* were exposed to LD_{25}, LD_{50}, LD_{75}, and LD_{90} of chlorantraniliprole, as determined in the toxicity bioassay, but recording mortality every 12 h for 96 h. Exposure procedures, conditions, and number of insects was the same as those described above for the toxicity test.

2.4. Larval production

Coffee berries were exposed to acute/chronic LD_{50} and LD_{90} of chlorantraniliprole following the same procedures of the toxicity bioassay. Each treatment comprised five replicates of 10 insects, following a completely randomized design. After 24 h of exposure, insects were allowed to colonize the coffee berries inside glass vials (2.5 cm × 8 cm). All coffee berries colonized by *H. hampei* were observed at days 1, 5, 10, 15, and 20 using an MX-20 specimen radiography system equipped with a 14-bit digital camera (Faxitron X-Ray Corp., Wheeling, IL, U.S.A.). The location of each individual within the coffee berry was digitally recorded throughout the larval development period, and the number of live larvae per coffee berry was calculated.

2.5. Behavioral responses

Adults of *H. hampei* were placed in a Petri dish arena (90 mm diameter × 15 mm high) lined with filter paper (Whatman no. 1). Then, the inner walls of the Petri dish were covered with polytetrafluoroethylene (Dupont®, Barueri, SP, Brazil) to avoid insect escape. Behavioral response bioassays were conducted in arenas half-treated with 250 µL of chlorantraniliprole dissolved in distilled water (LD_{50} or LD_{90}); dishes treated with distilled water only were used as control. One *H. hampei* adult at a time was released at the center of the insecticide-treated arena (on filter paper) and kept in the Petri dish for 10 min. Twenty-five insects were used for each treatment, following a completely randomized design. For each insect, behavioral

responses (mobility or immobility) within the arena were recorded using a digital camcorder (XL1 3CCD NTSC, Canon, Lake Success, NY, USA) equipped with a 16× video lens (Zoom XL 5.5–88 mm, Canon, Lake Success, NY, USA). A video tracking system (ViewPoint LifeSciences, Montreal, Quebec, Canada) was used to analyze the videos and measure the distance insects walked and the time spent resting on each half of the arena.

2.6. Respiration rate

Respiration rate bioassays were conducted for 3 h after *H. hampei* adults were exposed to chlorantraniliprole (LD_{50} and LD_{90} values), according to the procedures previously detailed in section 2.2. Insects treated with distilled water were used as control. Carbon dioxide (CO_2) production (μL of CO_2 h^{-1} $insect^{-1}$) was measured with a TR3C CO_2 analyzer (Sable System International, Las Vegas, USA) according to methods adapted from previous studies (Plata-Rueda et al., 2017; Fiaz et al., 2018a). An adult of *H. hampei* (female or male) was placed in each respirometry chamber (25 mL) connected to a closed system. After insect acclimation, CO_2 production was measured for 12 h at 27 ± 2 °C. Subsequently, compressed oxygen gas (99.99% pure) was introduced into the chamber at 100 mL min^{-1} for 2 min. The gas flow forces the CO_2 through an infrared reader, which continuously measures the CO_2 held inside the chamber. Before and after the experiment, *H. hampei* adults were weighed on an analytical balance (Sartorius BP 210D, Göttingen, Germany). Fifteen replicates were used for each insecticide treatment and control.

2.7. Statistical analyses

Lethal doses (LD_{25}, LD_{50}, LD_{75}, and LD_{90}) of chlorantraniliprole and their confidence limits were determined by logistic regression analysis of dose–response curves (Finney, 1964). The survival function was estimated by the Kaplan–Meier estimator (log-rank test) using Origin Pro v. 9.1 (OriginLab Corporation, 2013). *Hypothenemus hampei* adults who survived until the end of the experiment were treated as censored data. Larval production and behavioral response

250 data were analyzed by one-way ANOVA, and a Tukey's honestly significant difference (HSD)
251 test was also used for comparison of means at the 5% significance level. Respiration rates of
252 insects exposed to chlorantraniliprole were subjected to two-way analysis of variance (time ×
253 treatment interaction) and Tukey's HSD test ($P < 0.05$). Larval production, behavioral response,
254 and respiration rates were arcsine transformed to secure assumptions of normality and
255 homoscedasticity. The experiments were conducted in a completely randomized design.
256 Toxicity, larval production, behavioral response, and respiration rate results were analyzed
257 using SAS for Windows v. 9.0 (SAS Institute, 2002).
258
259 **3. Results**
260 *3.1. Toxicity*
261 The concentration-mortality model used was suitable ($P > 0.05$) confirming the toxicity
262 of chlorantraniliprole to the coffee berry borer and allowing the estimates of the desired
263 toxicological endpoints for subsequent use (Table 1). A dose–response relation was observed:
264 $LD_{25} = 0.19$ mg mL^{-1}, $LD_{50} = 0.49$ mg mL^{-1}, $LD_{75} = 0.88$ mg mL^{-1} and $LD_{90} = 1.21$ mg mL^{-1}.
265 Mortality remained < 1% in the control group.
266 *3.2. Survival analysis*
267 Analysis of the survival data of *H. hampei* adults exposed to different lethal
268 concentrations of chlorantraniliprole revealed significant differences among treatments (log-
269 rank test; $X^2 = 81.80$; df = 4; $P < 0.001$) (Fig. 1). After 96 h of exposure, survival was greater
270 than 98% in adults that had not been exposed to chlorantraniliprole, decreasing to 79% with
271 exposure to LD_{25}, 52% to LD_{50}, 35% to LD_{75}, and 2% to LD_{90}.
272 *3.3. Larval production*
273 Exposure to chlorantraniliprole affected the number of live *H. hampei* larvae per coffee
274 berry during the 20 days of colonization (Fig. 2). *Hypothenemus hampei* larvae were present in

275 higher numbers in the control and LD_{50} groups but were fewer in the LD_{90} group. The number
276 of larvae was different after 15 days ($F_{2,14} = 13.61$; $P < 0.001$) with 4.21 ± 0.71 in the control,
277 2.72 ± 0.57 in LD_{50}, and 0.22 ± 0.13 in LD_{90}. At the end of the colonization period (day 20),
278 the number of larvae was also different among groups ($F_{2,14} = 10.95$; $P < 0.0001$): 4.12 ± 0.45
279 in the control, 2.22 ± 0.34 in LD_{50}, and 0.25 ± 0.15 in LD_{90} (Fig. 3). Larvae were not found at
280 days 1, 5, and 10 of colonization.

281 *3.4. Behavioral responses*

282 Representative walking tracks of *H. hampei* adults released onto half-treated arenas are
283 shown in Fig. 4. The distance traveled was higher in the control and under the LD_{50} group than
284 under LD_{90} group ($F_{2,23}$ =7.47; $P < 0.015$). Mean distance traveled by *H. hampei* was 3871 ±
285 326 cm in the control, 3818 ± 368 cm in LD_{50} group, and 1422 ± 226 cm in LD_{90} group (Fig.
286 5). The resting period was longer in the LD_{50} and LD_{90} groups than in control ($F_{2,23} = 8.95$, $P <$
287 0.001). The resting period by *H. hampei* was 378 ± 23 s in LD_{50} group, 444 ± 17 s in LD_{90}
288 group, and 185 ± 17 s in the control (Fig. 5).

289 *3.5. Respiration rate*

290 *Hypothenemus hampei* had significantly different respiration rates (μL of CO_2 h^{-1}
291 $insect^{-1}$) when exposed to chlorantraniliprole at LD_{50} or LD_{90}. Respiration rates after 1 and 3 h
292 of insecticide exposure differed significantly ($F_{2,84} = 12.15$; $P < 0.001$). Mean respiration rate
293 was higher in the control (1.59 μL of CO_2 h^{-1}) followed by LD_{50} (0.86 μL of CO_2 h^{-1}), but
294 lower at the LD_{90} (0.33 μL of CO_2 h^{-1}) after 3 h of insecticide exposure. The treatment × time
295 interaction was different between the LD_{90} ($F_{2,44} = 10.36$; $P < 0.001$) followed by LD_{50} ($F_{2,44}$ =
296 5.87; $P < 0.001$) groups but did not differ from that of the control ($F_{2,44} = 0.36$; $P = 0.976$) (Fig.
297 6).

298

4. Discussion

Chlorantraniliprole was toxic to adults of *H. hampei* and had a strong effect by topical application (LD_{50} = 0.49 mg mL^{-1} and LD_{90} = 0.88 mg mL^{-1}). The insecticide caused mortality in *H. hampei* in a dose-dependent manner, as reported for other insects (Jiang et al., 2012; Saglan et al., 2013; Hummel et al., 2014). *Hypothenemus hampei* individuals exposed to high concentrations of chlorantraniliprole (LD_{50} and LD_{90}) displayed muscle contractions and altered locomotor activity. Some individuals suffered paralysis with no signs of recovery when exposed to LD_{90}. These symptoms suggest that chlorantraniliprole is a potent activator of insect ryanodine receptors, causing rapid muscle dysfunction and paralysis in *H. hampei*. In general, topical application of chlorantraniliprole at different concentrations and small volumes was sufficient to cause toxicity in *H. hampei*.

Extended periods of exposure to chlorantraniliprole, from 12 to 96 h, were necessary to induce mortality in *H. hampei*. Survivorship of *H. hampei* is associated with the slow action of chlorantraniliprole, different from that organochlorines and pyrethroids used to control this insect (Mansingh and Rhodes, 1983; Damon, 2000; Oliveira et al., 2003). Anthranilic amides such as chlorantraniliprole are slow-acting molecules that cause moderate topical and ingestion toxicity in pests (Liu et al., 2012; Neoh et al., 2012; Roditakis et al., 2013). A slow-acting insecticide is generally considered essential for the impregnation of plant tissues. In this case, curculionid beetles are able to feed but die later on. Systemic properties of anthranilic diamides can reduce the damage caused to commercial crops by insects (Hannig et al., 2009). Our results showed that *H. hampei* had high mortality when exposed to chlorantraniliprole at LD_{90}, indicating susceptibility to high doses.

The number of *H. hampei* larvae per coffee berry varied throughout the colonization period. The results show that the number of live larvae declined in the LD_{90} group. The action of insecticides throughout the developmental stages has been reported in several insect pests,

affecting intrinsic population growth rate, longevity, survival, and reproduction (Satelle et al., 2008; Hannig et al., 2009; Lai and Su, 2011). Our study suggests that chlorantraniliprole causes larvae mortality and compromises *H. hampei* offspring. In addition, larvae decreased coffee berry consumption, reducing the amount of internal damage to the berry.

The behavioral response assay indicated that chlorantraniliprole had a substantial effect on *H. hampei*. Changes in walking patterns occur as a result of the action of toxic compounds on the nervous system, which either stimulate or reduce insect mobility. Various insect pests show altered behavioral responses when exposed to insecticides; insects are reported to leave toxic environments as soon as they detect toxic compounds (Miller and Gibson, 1994; Plata-Rueda et al., 2017; Fiaz et al., 2018b). Studies show that synthetic insecticides can disrupt the recognition of the host substrate, influencing the olfactory orientation and walking behavior of insects (Thanispong et al., 2009; Germinara et al., 2015; Plata-Rueda et al., 2018). According to the results of the behavioral response bioassay, the odor of chlorantraniliprole was repulsive to *H. hampei*, which can be associated with the insect's respiratory mechanisms. Insecticides are inhaled by insects, entering the spiracles and tracheae during the respiratory process (Wasserthal, 1996; Martínez et al., 2015). Small amounts of insecticide are carried to different tissues through the network of tracheae and tracheoles, thereby reaching their site of action. Our results suggest that *H. hampei* are repelled by chlorantraniliprole, evidenced by the behavioral responses of adults to chlorantraniliprole at LD_{50} and LD_{90} and the high number of insects with altered locomotion, indicating a sublethal effect.

Chlorantraniliprole affected the respiration rate of *H. hampei* up to 3 h after exposure, which probably influenced their behavioral response and locomotor activity. Respiratory rates and body mass of insects are influenced by the energy demands of the physiological functions that are necessary to produce defense mechanisms against insecticides (Pimentel et al., 2007; Kliot and Ghanim, 2012; Plata-Rueda et al., 2018). Low respiration rates result in a high fitness

cost, as resources and energy must be reallocated at the expense of metabolic processes (Kliot and Ghanim, 2012; Martínez et al., 2018). Low respiration rates can also impair muscle activity, leading to paralysis (Pimentel et al., 2007; Kliot and Ghanim, 2012; Plata-Rueda et al., 2018). Inhalation of fumigant insecticides is positively correlated with insect respiration rate (Cotton, 1932; Fiaz et al., 2018a). In this study, *H. hampei* adults exposed to chlorantraniliprole had low respiration rates, which led to fitness costs and energy reallocation from other basic physiological processes. These significant negative effects favor the use of chlorantraniliprole as a systemic insecticide (via contact or ingestion) to control *H. hampei*.

The insecticidal potential of chlorantraniliprole against *H. hampei* was studied. Its toxicity and action as an activator of insect ryanodine receptors might allow the management of *H. hampei* populations and reduce the damage caused by this insect to coffee berries. Our results show that chlorantraniliprole causes high mortality, reduces survivorship and larval production, alters behavioral responses, and lowers the respiration rate of *H. hampei*. Thus, chlorantraniliprole exhibits lethal and sublethal effects on *H. hampei* and can be an alternative to other synthetic insecticides aiding in eventual insecticide resistance management efforts.

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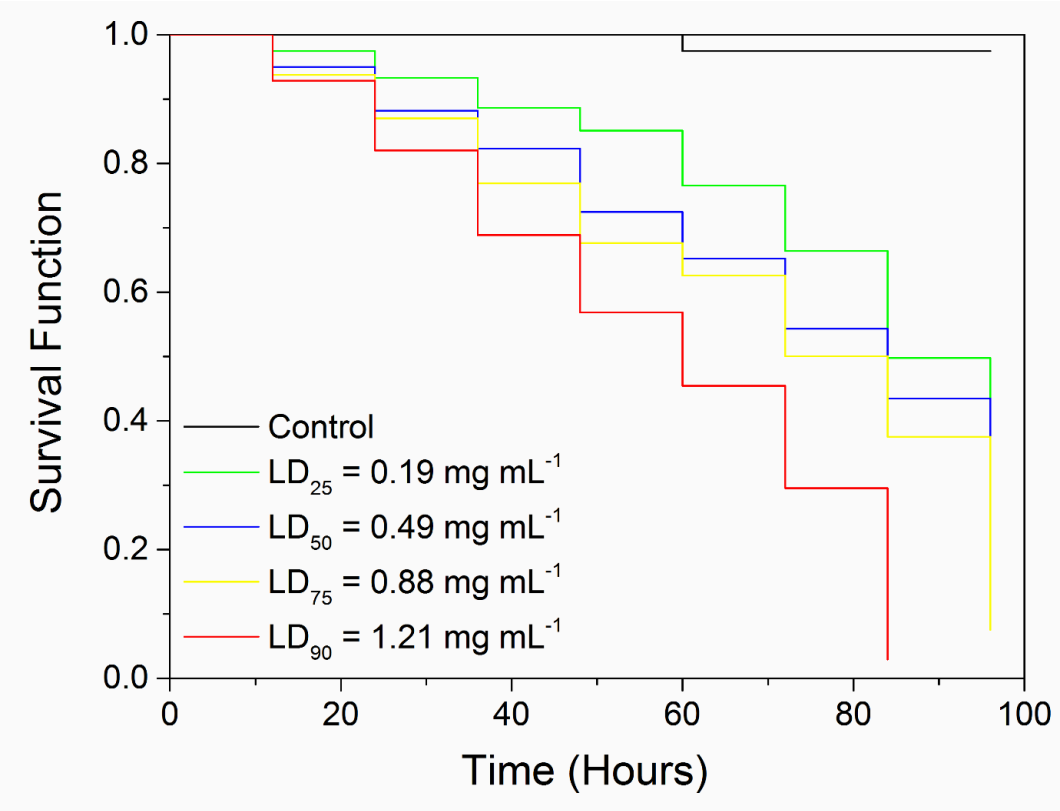
500

Table 1. Lethal doses of chlorantraniliprole against *Hypothenemus hampei* after 24 hours exposure. [1]$LD_{25,50,75\ and\ 90}$, lethal dose causing 25%, 50%, 75% and 90% mortality; [2]EV, estimated value; [3]CI, confidence interval; [4]X^2, Chi-squared value for the lethal doses and fiducial limits based on a log scale with significance level at $P < 0.001$.

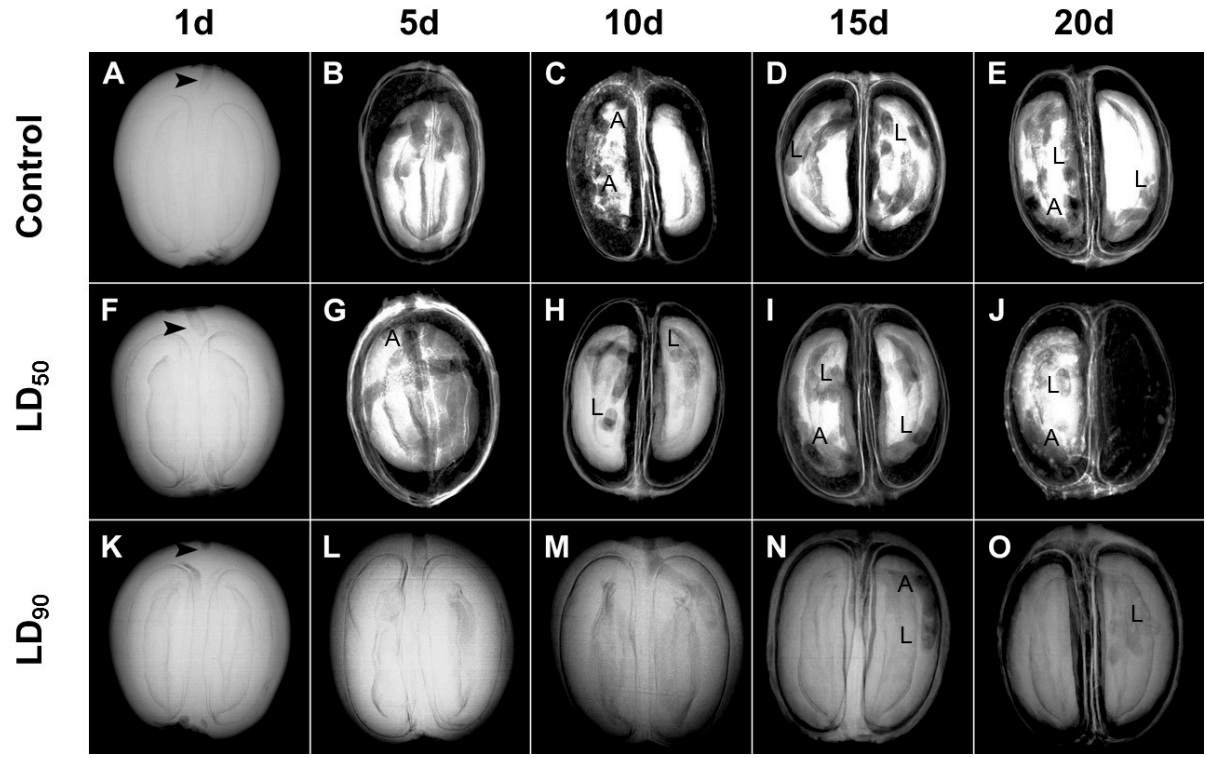
[1]LD	[2]EV (mg mL^{-1})	[3]CI (mg mL^{-1})	[4]X^2
LD_{25}	0.198	0.132 – 0.253	44.66
LD_{50}	0.494	0.448 – 0.592	
LD_{75}	0.880	0.841 – 0.965	
LD_{90}	1.219	1.117 – 1.352	



506

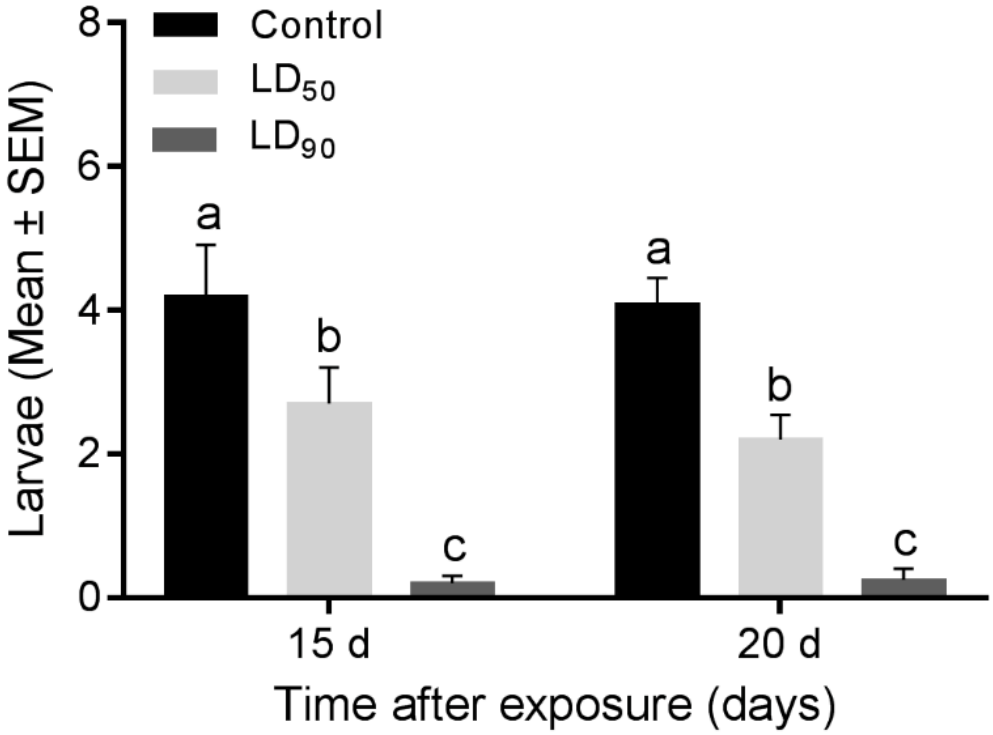
507 **Fig. 1.** Survivorship curves of *Hypothenemus hampei* adults exposure at different lethal doses

508 using the Kaplan-Meier method and compared using the log-rank test ($X^2 = 65.13$; $P < 0.001$).



509

510 **Fig. 2.** Temporal sequence of X-ray pictures showing the number of live *Hypothenemus hampei*
511 (larvae and adults) within single coffee berry colonized after 20 days expose to
512 chlorantraniliprole. Larvae and adults were indicated (L or A, respectively) and arrows denoted
513 initial damages in coffee berry.



514

515 **Fig. 3.** Larvae (Mean ± SEM) of *Hypothenemus hampei* found in coffee berry exposure to

516 chlorantraniliprole (control, LD_{50} and LD_{90} estimated values) for 15 and 20 days. Letters in the

517 treatments indicate significant differences by Tukey's HSD test ($P < 0.05$).

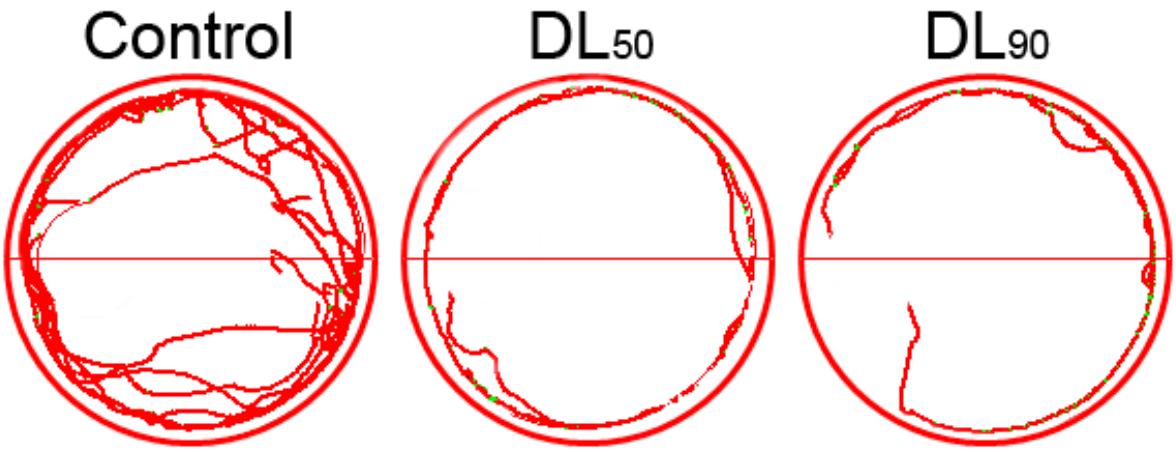
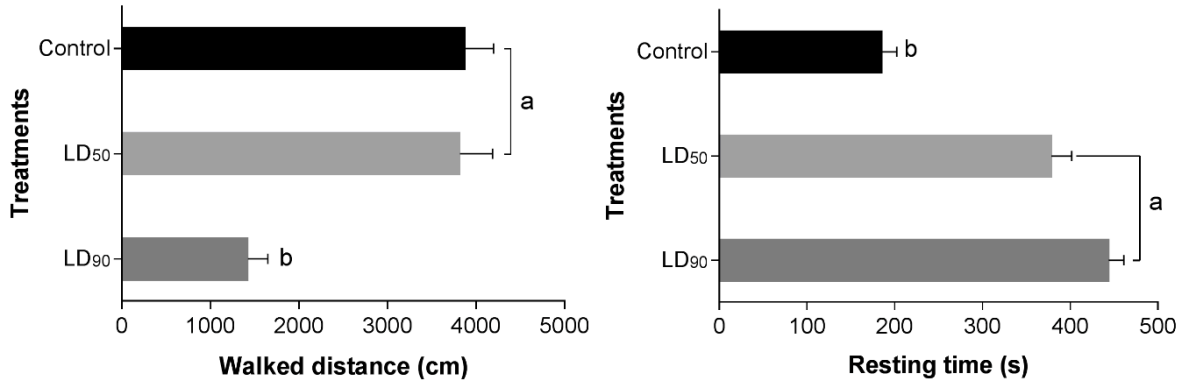


Fig. 4. Representative tracks showing the walking activity of *Hypothenemus hampei* over a 10-min period on paper-filter arenas half impregnated with chlorantraniliprole (upper half of each arena). Red tracks indicate high walking velocity; green tracks indicate low (initial) velocity. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

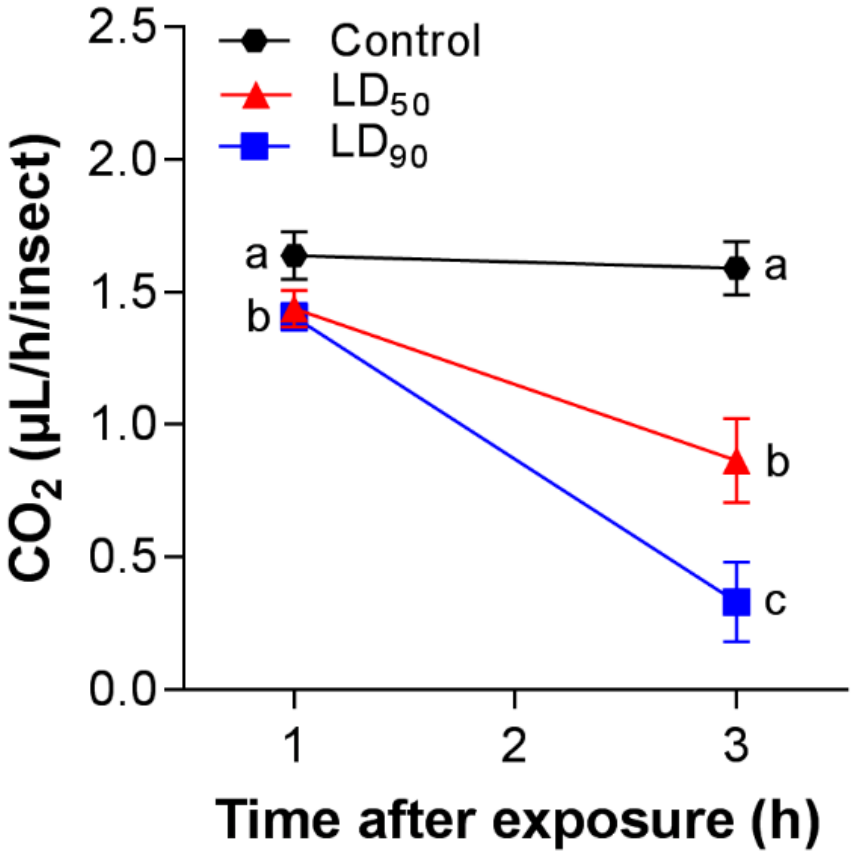


524

525 **Fig. 5.** Distance walked and resting time (Mean ± SEM) of *Hypothenemus hampei* subjected to

526 chlorantraniliprole (control, LD_{50} and LD_{90} estimated values) for 10 min. Letters in the

527 treatments indicate significant differences by Tukey's HSD test ($P < 0.05$).



528

529 **Fig. 6.** Respiration rate (Mean ± SEM) of *Hypothenemus hampei* exposure to

530 chlorantraniliprole (control, LD_{50} and LD_{90} estimated values) for 3 h. Letters in the treatments

531 indicate significant differences by Tukey's HSD test ($P < 0.05$).

CAPÍTULO II

Cyantraniliprole susceptibility baseline, resistance survey and control failure likelihood in the coffee berry borer *Hypothenemus hampei*

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Cyantraniliprole susceptibility baseline, resistance survey and control failure likelihood in the coffee berry borer *Hypothenemus hampei*

Nayara C.R. Costa[a], Eduardo C.M. Picelli[b], Fábio M.A. Silva[a], Alfredo H.R. Gonring[c], Raul Narciso C. Guedes[d], Mariana R. Durigan[b], Flávio L. Fernandes[e,*]

[a]Instituto de Ciências Agrárias, Universidade Federal de Uberlândia, Campus Glória, Rodovia BR 050 Km 78, Uberlândia, 38410-337, Brazil; nayaracrcosta@hotmail.com

[b]FMC Química do Brasil Ltda., Rodovia PLN 145, 943, Bairro Boa Esperança, 13148-080, Paulínia-SP, Brazil; eduardo.picelli@fmc.com; fabio.silva2@fmc.com

[c]Corteva Agrisciences, Rodovia SP-147 km 71.5 s/n, Bairro - Pederneiras, Mogi Mirim, SP, 13801-540, Brazil; alfredo.h.goring@corteva.com

[d]Departamento de Entomologia, Universidade Federal de Viçosa, Campus Viçosa, 36570-900, Brazil; guedes@ufv.br

[e]Instituto de Ciências Agrárias, Universidade Federal de Viçosa, Campus Rio Paranaíba, Rodovia BR 354 Km 310, Rio Paranaíba, 38810-000, Brazil; flaviofernandes@ufv.br

*Corresponding author

Running Title: Susceptibility of *Hypothenemus hampei* to cyantraniliprole

Abstract

Cyantraniliprole was recently registered for controlling the coffee berry borer *Hypothenemus hampei*, the main coffee pest in the world. In this study, baseline determination and resistance monitoring to cyantraniliprole were carried out in Brazilian populations of *H. hampei*. Evaluations were carried out for three years with representative field-collected populations from nine coffee-producing states in Brazil, using artificial diet containing the insecticide. The likelihood of control failure due to cyantraniliprole resistance was also determined. Populations from Campo do Meio, Linhares and Jaú were more susceptible (< 2-fold resistance) to cyantraniliprole than populations from Patrocínio and Londrina (17-fold). Nonetheless, the frequency of cyantraniliprole resistance insects was low and not significant throughout the regions survey and the likelihood of control failure was negligible. Therefore, cyantraniliprole remains an important management tool against the coffee berry borer without current problems of control failure. However, enough field variation in susceptibility to cyantraniliprole exists justifying attention and careful management of this insecticide to prevent quick development of insecticide resistance in populations of this insect pest species.

Keywords: Chemical control; Coffee pest species; Diamide insecticide resistance; Risk of control failure; Integrated pest management

1. Introduction

Modern agriculture advocates that pest control with insecticides is economically, socially, and environmentally appropriate, and technically sustainable. The proper use of insecticides can reduce applications, pest control costs, hazards to environment and non-target organisms, as well as improve control efficiency (Jeschke, 2016). When using chemical control, efficiency is considered suitable when pest control is high and sustained allowing the insecticides to remain competitive in the market (Sparks, 2013; Jeschke, 2016).

The chemical control of agriculture pest species around the world includes the use of groups of insecticides such as neonicotinoids, pyrethroids, organophosphates, and carbamates, which are the most widely used. From 2004 to 2014, 11 insecticides were launched in the market, with three different modes of action (Sparks and Nauen, 2015; Jeschke, 2016). Among these products, diamides are highly effective against pests, selective to natural enemies, and exhibits low environmental persistence, low toxicity to human and other non-target organisms (Sparks and Nauen, 2015; Jeschke, 2016; Machado et al., 2019). Diamides are ryanodine receptor modulators and cause mortality via calcium release in muscle cells (Selby et al., 2013).

Cyantraniliprole is a representative compound of the diamide insecticides that is efficient in the control of a few coffee pests, such as the false spider mite *Brevipalpus phoenicis* [(Geijskes, 1939) (Acari: Tenuipalpidae)], the red broad mite *Oligonychus ilicis* [(McGregor, 1917) (Acari: Tetranychidae)] (Reis et al., 2014), and the coffee berry borer *Hypothenemus hampei* [(Ferrari, 1867) (Coleoptera: Scolytidae)] (Souza et al., 2013). This insecticide stands out for its broad spectrum of control, exhibiting control efficacy against the fall armyworm *Spodoptera frugiperda* [(J. E. Smith, 1797) (Lepidoptera: Noctuidae)] (Hardke et al., 2011) and the boll weevil *Anthonomus eugenii* [(Cano, 1894) (Coleoptera: Curculionidae)] in the USA (Caballero et al., 2015), the green peach aphid *Myzus persicae* (Sulzer, 1776) and the cotton aphid *Aphis gossypii* [(Glöver, 1877) (Hemiptera: Aphididae)] in Europe (Foster et al., 2012),

618 and the oriental fruit fly *Bactrocera dorsalis* [(Hendel, 1794) (Diptera: Tephritidae)] in China
619 (Zhang et al., 2015).

620 Cyantraniliprole is effective against the coffee berry borer *H. hampei*, the key coffee pest
621 species in the world (Damon, 2000; Souza et al., 2013; Infante et al., 2014). This is especially
622 important since the main insecticide commercialized against this species, the cyclodiene
623 endosulfan, was phased out in 2010. This active ingredient was considered the most effective
624 treatment against *H. hampei*. Despite that, toxicity to non-target organisms and environmental
625 persistence (U.S. EPA, 2010), as well as the detection of resistant populations of the coffee
626 berry borer (Brun et al., 1989; Ffrench-Constant et al., 1994), justified the interruption of
627 endosulfan commercialization.

628 The first reports of insecticide resistance in insect pest species dates back to 1914 and
629 since then, there has been an increasing number of reports of resistance to new chemical groups
630 with the initial reports occurring 2–20 years after a product has been launched in the market
631 (Metcalf, 1955; O'Brien, 1967). The speed at which cyantraniliprole resistance can evolve in
632 coffee borer populations highlights the need to monitor pest resistance (Wang et al., 2018),
633 especially after the introduction of this insecticide in the market. It is estimated that the cost
634 associated with control failure by a given pesticide is around $1 billion (Cook et al., 2005). Pest
635 resistance monitoring is the first step for mitigation, contributing for development of resistance
636 management strategies. In order to achieve that, the establishment of pest susceptibility baseline
637 is crucial (Robertson et al., 2007; Teixeira and Andaloro, 2013).

638 The establishment of baseline susceptibility curves for the coffee berry borer to
639 cyantraniliprole is essential to monitor the problem through time allowing quick and reliable
640 diagnoses of the pest susceptibility to this insecticide. Understanding the impact of selection
641 pressure on the risk of developing resistance is necessary for the detection of eventual field
642 control failure and essential for comparisons among pest populations coming from different

643 locations (Teixeira and Andaloro, 2013). The baseline curves are established from
644 concentration-mortality bioassays and allow the establishment of discriminatory concentrations
645 and detection of field variation in susceptibility among field populations of the pest species to
646 a given insecticide (Storch et al., 2008; Foster et al., 2012; Caballero et al., 2013). Thus, the
647 aim of this paper was to determine the susceptibility baseline curves and to monitor the response
648 of Brazilian populations of the coffee berry borer to the insecticide cyantraniliprole. The risk
649 of control failure with cyantraniliprole use due to resistance to this insecticide was also
650 estimated.
651

652 **2. Materials and methods**

653 *2.1. Insects and insecticides*

654 Ninety-seven field-populations of the coffee berry borer infesting *C. arabica* and *C.*
655 *canephora* were collected from coffee berries from 2016 to 2018 (Table 1). All locations have
656 significant coffee production (Coltro et al., 2012). On each location, 5 kg of bored berries were
657 randomly collected, packed in cardboard boxes and taken to the Integrated Pest Management
658 Laboratory of the Federal University of Viçosa in Rio Paranaíba, Minas Gerais, Brazil (UFV-
659 CRP). The brocade berries were immersed in a 5% solution of sodium hypochlorite for 60 s,
660 dried on paper towels, and packed separately in polyvinyl chloride (PVC) tubes (10 cm diameter
661 x 25 cm height) for fermentation and adult emergence. Each PVC tube had lids and small
662 openings (3 mm) covered with voil to allow O_2 exchange. Each PVC tube received 350–400
663 ripe coffee berries and was inspected every other day to collect 30–120 insects per tube for use
664 in the bioassays (Hirose and Neves, 2002). Subsequently, adult females were transferred to
665 gerbox boxes (6 cm diameter x 2 cm height; 24 cells) containing artificial diet (Giraldo-
666 Jaramillo and Parra, 2018) and maintained in growth chambers set at 25 ± 1 °C, 65 ± 5 %
667 relative humidity (RH), and 12:12 h light:dark until the emergence of the F1 generation.

668 *2.2. General*

669 Only females fully sclerotized and mated at least 3 h prior the bioassays were used. They
670 were removed from the boxes containing artificial diet with the aid of tweezers and transferred
671 for 5 mL vials (Damon, 2000; Pardey, 2006). The insects were maintained without food or
672 water access until the beginning of the bioassays. The insecticide used was cyantraniliprole (oil
673 suspended concentrate, 100 g L^{-1}, Benevia®, FMC, Campinas, São Paulo, Brazil), and the
674 method used in the bioassays was that of Gonring et al. (2019). In this method, slabs of artificial
675 diet (2 x 2 x 1 cm) were cut and transferred to 24-cell gerboxes (Corning, New York; cell
676 diameter: 15.4 mm and volume of 4.5 mL), treated with a water suspension containing the
677 insecticide (0.25 mL per cell), and left to dry in the dark for 8 h. Then, 20 borer female adults
678 (8–10 days of age) were individually transferred to the treated surface. Each cell containing 20
679 insects constituted a replicate, and a total of four replicates was used and maintained in
680 environmental chamber set at 24 ± 1 °C, 65 ± 3 % RH, and 12:12 h light:dark. After eight days
681 of exposure, insect mortality was determined under a stereomicroscope with 40x magnification
682 (SZ, Olympus, Tokyo, Japan). Insects were considered dead if they failed to respond when
683 touched with a fine hair brush. Treatments were arranged in a completely randomized design.

684 *2.3. Baseline concentration-mortality curve*

685 This bioassay aimed at determining the relative toxicity of chlorantraniliprole to
686 representative field-populations of the coffee borer and subsequent estimation of relevant
687 toxicological endpoints. The treatments were increasing concentrations of cyantraniliprole,
688 diluted in distilled water, and control (with only water) to assess natural mortality. Previous
689 tests were carried out with 11 populations to determine the range of cyantraniliprole
690 concentrations for use to obtain a mortality range between 5 and 99% (Table 2). The
691 concentrations of cyantraniliprole used ranged from 0.001 to 2.45 mg a.i. mL^{-1}. The data from
692 all surveyed populations was pooled to obtain overall estimates and selection of suitable

693 diagnostic concentration (LC_{90}; Table 2) for monitoring coffee borer resistance to
694 cyantraniliprole (Caballero et al., 2013).

695 *2.4. Resistance monitoring and control failure likelihood*

696 Field populations of the coffee berry borer were collected in 2016, 2017 and 2018 for
697 resistance monitoring encompassing a total of 97 populations sampled and tested from nine
698 coffee-producing Brazilian states (Table 1). These insect populations were also used to assess
699 the risk of cyantraniliprole control failure due to the resistance to this compound. The methods
700 for both bioassays, resistance monitoring and estimation of control failure likelihood followed
701 the same methods, but using different diagnostic concentrations – the pooled susceptibility
702 baseline estimate for the former (0.57 mg a.i. mL^{-1}; or the LC_{90}), and the registered label rate
703 for the latter. Four replicates with 20 adult females were used for each population, year and
704 type of bioassay with cyantraniliprole, always using a completely random experimental design
705 and proper control treatments (and replicates) where only water was used to allow correction
706 for natural mortality (Abbott, 1925). The overall bioassay methods were those already
707 described for the susceptibility baseline study because they were recognized as the most
708 sensitive and reliable (Gonring et al., 2019).

709 The estimates of control failure likelihood (CFL; or risk of control failure) were obtained
710 from the mortality results observed with the use of the registered label rate of cyantraniliprole
711 for the coffee berry borer in Brazil (0.37 mg a.i. mL^{-1}) (MAPA, 2020). The formulae used was
712 that of Guedes (2017), where CFL = 100 - [observed mortality x 100] ÷ expected mortality
713 considered as 80% following MAPA (1995). Negative values of CFL indicate negligible risk
714 of control failure

715 *2.5. Statistical analysis*

716 Mortality was corrected by the natural (control) mortality (Abbott, 1925), and subjected
717 to Probit analysis (Proc Probit, SAS Institute, 2012). The resistance ratio at LC_{50} (RR_{50}) was

718 determined by diving the LC_{50} of a field population by that of the susceptible one (laboratory
719 standard susceptible population), and the 95% confidence internal for the ratio was estimated
720 and considered significant if the value 1 was not included (Robertson et al., 2007). The data
721 from the resistance monitoring survey and the control failure likelihood were subjected to
722 unilateral Z test at 95% confidence level with correction for continuity (Roush and Miller,
723 1986). Bonferroni correction was used to correct the *P*-values.
724
725 **3. Results**
726 *3.1. Baseline susceptibility curve*
727 The natural mortality of adult females of the coffee berry borer remained lower than 5%
728 in the bioassays carried out attesting the suitability of the methods used. Furthermore, the low
729 χ^2 -values and high P-values obtained with the probit model (< 15,85 and > 0.05 respectively)
730 indicate the suitability of the analyses allowing the proper estimates of the desired toxicological
731 endpoints.
732 The cyantraniliprole baseline data of the coffee borer populations are presented in Table
733 2. The baseline data for 11 field populations did show significant variation. The concentration
734 x mortality curves ranged from 0.01 to 0.17 mg a.i. mL^{-1} for LC_{50} and 0.16–1.55 mg a.i. mL^{-1}
735 for LC_{90}. The most susceptible populations to cyantraniliprole at the LC_{50} were Linhares,
736 Campo do Meio and Jaú (Table 2). The least susceptible populations were Londrina
737 (Laboratory; PR) and Patrocínio (MG), which presented the highest estimated LCs (LC_{50}= 0.17
738 and LC_{90}= 1.55 mg a.i. mL^{-1}; and LC_{50}= 0.17, and LC_{90}= 0.70 mg a.i. mL^{-1}, respectively) (Table
739 2). Resistance ratios (RR_{50}) ranged from 1 to 17-fold. Populations from Campo do Meio,
740 Linhares and Jaú had the lowest RR50 values (1, 2 and 2-fold, respectively), while the highest
741 values (17- fold) were observed in populations from Patrocínio and Londrina (Laboratory; PR).
742 According to the pooled data obtained for each field population, the discriminatory

743 concentration was 0.57 mg a.i. mL^{-1} (LC_{90}) of cyantraniliprole and this concentration was used
744 for resistance monitoring when using artificial diet bioassays (Table 2).

745 *3.2. Multi-year monitoring and failure control*

746 Cyantraniliprole exhibited high efficacy against the surveyed populations of the coffee
747 berry borer between 2016 and 2018 with mortality at the diagnostic concentrations ranging
748 from 80 to 100% (Table 3 e 4). Such variation was small across years for the frequency of
749 resistant individuals (Table 3 and Fig. 1), as was the control failure likelihood, which was
750 negligible (Table 4).

751

752 **4. Discussion**

753 The coffee berry borer has been little studied regarding insecticide resistance. A likely
754 reason is the limited number of insecticides effectively used against this species with the
755 dominance of the BHC and lindane early on, and latter prevalence of endosulfan. The latter
756 with reported cases of resistance detected since the late 1980's (Brun et al., 1989). Nonetheless,
757 the phasing out of endosulfan led to the registration and use of cyantraniliprole as an alternative
758 pest management tool justifying the concern with the potential development of resistance to
759 this diamide insecticide.

760 In the present study, 97 coffee borer populations were exposed to cyantraniliprole to
761 determine susceptibility baseline curves for resistance monitoring purposes. The results suggest
762 a similar cyantraniliprole susceptibility among Brazilian populations of the coffee borer with
763 few exceptions where the level of resistance reached 10 and 17-fold levels. The relative
764 uniformity of response may be due to the behavior and genetics of the species, which exhibits
765 little genetic diversity (Benavides et al., 2005), what may slow down the development of
766 insecticide resistance in populations of this species. Such low genetic variability is due to the
767 prevalence of mating between siblings within the coffee berry leading to high levels of

768 endogamy (Damon, 2000; Constantino et al., 2011). Therefore, the relative uniformity of
769 response to the insecticide is not a surprise and allowed the establishment of a robust diagnostic
770 concentration based on the estimated LC_{90} for monitoring cyantraniliprole resistance among
771 populations of the coffee berry borer.

772 Cyantraniliprole was released in the Brazilian market only recently (i.e., in 2015)
773 (MAPA, 2015, 2020). In order to register a pesticide in Brazil, a minimum threshold of
774 mortality is required - 80% (MAPA, 1995). Therefore, the use of the label rate against the coffee
775 borer should lead to a minimum acceptable level of mortality of 80% providing basis for
776 determining the risk of control failure with this insecticide. Cyantraniliprole resistance was not
777 significant among the coffee borer populations tested and, as consequence, the risk of control
778 failure with this insecticide was negligible.

779 Studies of cyantraniliprole resistance were carried out in different countries and showed
780 that susceptibility levels to this insecticide were also high. Examples include *M. persicae*, *A.*
781 *gossypii* (Foster et al., 2012) and *Trialeurodes vaporariorum* [(Westwood, 1856) (Hemiptera:
782 Aleyrodidae)] (Moreno et al., 2018) in Europe, *Leptinotarsa decemlineata* [(Say, 1824)
783 (Coleoptera: Chrysomelidae)] in Canada (Scott et al., 2014), *A. eugenii* (Caballero et al., 2015)
784 and *Listronotus maculicollis* [(Kirby, 1837) (Coleoptera: Curculionidae)] (Koppenhöfer et al.,
785 2018) in the USA, *Helicoverpa armigera* [(Hübner, 1809) (Lepidoptera: Noctuidae)] (Bird,
786 2016), and *M. persicae* (Little and Umina, 2017) in Australia, *B. phoenicis* and *O. ilicis* in Brazil
787 (Reis et al., 2014).

788 The lack of significant problems of cyantraniliprole resistance so far, may be due to the
789 still relatively recent use of this compound. It's mode of action may also be a contributor
790 because it also changes insect behavior by sequentially delaying locomotion, feeding,
791 respiration, reproduction and survival rates (Selby et al., 2013; Plata-Rueda et al., 2019).
792 Nonetheless, other diamides under use for a bit longer are already exhibiting problems of

793 insecticide resistance, as the case with chlorantraniliprole in putative white fly species and the
794 South America tomato pinworm (Biondi et al., 2018; Dângelo et al., 2018; Guedes et al., 2019),
795 to name a few examples.

796 Coffee borer populations exposed to cyantraniliprole under laboratory conditions at the
797 LC_{50} (0.67 mg a.i. mL^{-1}) and LC_{90} (1.71 mg a.i. mL^{-1}) showed that control peaked 20 days after
798 exposure to the lowest concentrations, which demonstrated its progressive and residual action.
799 After 3 h of exposure at the highest concentration, some individuals exhibited decreased
800 locomotion, followed by paralysis without exhibiting signs of recovery (Plata-Rueda et al.,
801 2019). Regardless, the efficacy was high allowing for selection under continued exposure to
802 cyantraniliprole, as expected for insecticides in general and even under sublethal exposure
803 (Guedes et al., 2017). Therefore, cyantraniliprole resistance and associated control failure is not
804 a current problem among populations of the coffee berry borer, but may became one if
805 resistance management strategies were neglected.

806 The behavior and genetics of the coffee borer, together with the data obtained from the
807 bioassays performed here, suggest that using cyantraniliprole-based insecticides can be an
808 effective alternative for use in rotation with insecticides of different chemical groups. This
809 strategy should be particularly effective if integrated with additional control methods that are
810 part of an IPM (integrated pest management) program and accompanied by frequent evaluation
811 of insecticide efficacy.

812

813 **Author statement**

814 Nayara Costa: Methodology, Validation, Formal analysis, Investigation. Eduardo Picelli:
815 Methodology, Validation. Fábio Silva: Methodology, Validation. Alfredo Gonring:
816 Methodology, Validation. Raul Guedes: Conceptualization, Validation, Review, Editing.
817 Mariana Durigan: Conceptualization, Validation, Review. Flávio Fernandes:

Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known conflicting interest.

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977 **Table 1.** Sampling sites and their geographical coordinates for the field populations of the
978 coffee berry borer *Hypothenemus hampei*.

Number of populations collected/year			State	County	Geographic coordinates	
2016	2017	2018				
1	1	1	Bahia	Luís Eduardo Magalhães	12° 03′ 54″ S	45° 54′ 12″ W
1	1	1	Distrito Federal	Brasília	15° 56′ 34″ S	47° 56′ 28″ W
1	1	1	Espírito Santo	Jaguaré	18° 52′ 45″ S	40° 06′ 20″ W
1	1	1		Linhares	19° 23′ 09″ S	40° 03′ 59″ W
1	-	-		Vermelho Novo	21° 02′ 00″ S	42° 16′ 01″ W
1	1	1	Goiás	Ipameri	17° 40′ 48″ S	48° 11′ 36″ W
3	2	1	Minas Gerais	Araguari	18° 36′ 38″ S	48° 11′ 58″ W
1	1	-		Araxá	19° 35′ 17″ S	46° 56′ 39″ W
1	-	-		Campo do Meio	21° 06′ 27″ S	45° 49′ 50″ W
1	-	-		Campos Gerais	21° 14′ 15″ S	45° 45′ 29″ W
1	-	-		Carmo do Paranaíba	19° 00′ 03″ S	46° 18′ 33″ W
5	1	1		Coromandel	18° 12′ 47″ S	47° 07′ 29″ W
1	-	-		Cristais	20° 51′ 19″ S	45° 31′ 07″ W
1	-	-		Guapé	20° 51′ 27″ S	45° 57′ 18″ W
1	-	-		Guaraciaba	20° 34′ 03″ S	43° 00′ 23″ W
2	-	1		Ibiá	19° 29′ 07″ S	46° 32′ 38″ W
1	-	-		Indianópolis	19° 01′ 35″ S	48° 04′ 06″ W
2	2	1		Monte Carmelo	18° 42′ 21″ S	47° 30′ 18″ W
1	-	-		Paracatu	17° 10′ 32″ S	46° 52′ 18″ W
1	1	1		Patos de Minas	18° 34′ 21″ S	46° 30′ 59″ W
5	1	1		Patrocínio	18° 54′ 42″ S	46° 59′ 42″ W
-	-	1		Perdizes	19° 20′ 03″ S	47° 17′ 36″ W
3	1	1		Presidente Olegário	18° 22′ 39″ S	46° 25′ 08″ W
5	1	2		Rio Paranaíba	19° 12′ 27″ S	46° 30′ 23″ W
1	-	-		Sacramento	19° 51′ 09″ S	47° 26′ 34″ W
1	-	-		Tiros	19° 00′ 56″ S	45° 57′ 42″ W
1	1	-		Três Pontas	21° 21′ 34″ S	45° 30′ 36″ W
1	2	-		Unaí	16° 19′ 31″ S	46° 54′ 21″ W
-	-	1		Varjão de Minas	18° 22′ 14″ S	46° 01′ 55″ W
1	-	1	Paraná	Londrina (Laboratório)	23° 18′ 03″ S	51° 10′ 11″ W
2	1	1		São José da Boa Vista	23° 54′ 23″ S	49° 39′ 12″ W
1	1	1	Rio de Janeiro	Varre-Sai	20° 57′ 23″ S	41° 54′ 15″ W
1	1	1	Rondônia	Rolim de Moura	11° 52′ 37″ S	61° 47′ 32″ W
1	1	1	São Paulo	Franca	20° 31′ 50″ S	47° 24′ 06″ W
-	1	1		Garça	22° 12′ 44″ S	49° 39′ 16″ W
1	-	-		Jaú	22° 18′ 05″ S	48° 34′ 32″ W
52	23	22				

979 The use of hyphen (-) indicates that no population was collected in a given year until the
980 emergence of the F_1 generation.

Table 2. Relative toxicity of cyantraniliprole to Brazilian populations of the coffee berry borer *Hypothenemus hampei*.

State	Population	Number of insects	Concentration (95% FL) mg a.i. mL^{-1}		Slope ± SE	[a]χ^2 (degree of freedom)	*P*-value	[b]Resistance ratio at LC_{50} [RR_{50}] (95% CI)
			LC_{50}	LC_{90}				
Espírito	Jaguaré	800	0.05(0.04-0.06)	0.40(0.30-0.52)	6.87±0.20	11.81 (8)	0.16	5(2.31-6.23)*
Santo	Linhares	880	0.01(0.01-0.02)	0.82(0.74-0.88)	0.74±0.01	13.76 (9)	0.09	1(0.18-1.64)
	Vermelho Novo	720	0.05(0.04-0.06)	0.19(0.08-0.28)	2.05±0.03	7.36 (7)	0.06	5(1.13-6.65)*
Minas Gerais	Araguari	880	0.10(0.09-0.12)	0.60(0.51-0.69)	1.68±0.04	15.85 (9)	0.07	10(4.67-14.32)*
	Campo do Meio	640	0.02(0.01-0.02)	0.16(0.10-0.30)	1.27±0.01	8.71 (6)	0.19	2(1.13-4.84)
	Guapé	720	0.06(0.05-0.07)	0.35(0.25-0.46)	1.64±0.02	13.63 (7)	0.06	6(4.21-9.21)*
	Ibiá	640	0.08(0.06-0.10)	0.49(0.38-0.54)	1.64±0.03	7.68 (6)	0.26	8(3.42-12.67)*
	Patrocínio	720	0.17(0.15-0.19)	0.70(0.42-0.95)	2.08±0.02	6.38 (7)	0.50	17(11.45-19.11)*
	Três Pontas	800	0.05(0.04-0.06)	0.40(0.32-0.48)	1.45±0.01	10.63 (8)	0.22	5(3.21-6.21)*
Paraná	Londrina (Laboratory)	880	0.17(0.12-0.24)	1.55(1.05-1.74)	1.33±0.03	4.30 (9)	0.51	17(10.23-20.11)*
São Paulo	Jaú	640	0.02(0.01-0.03)	0.65(0.51-0.77)	0.84±0.01	4.91 (6)	0.18	2(0.98-3.12)
	[c]Pooled population data	8320	0.07(0.06-0.09)	0.57(0.42-0.57)	1.96±0.05	9.92 (102)	0.27	3(2.03-4.78)*

[a]Chi-square (χ^2) value at $P > 0.05$ indicate suitable fit to the probit model, whose slope (±SE) is indicated; log transformation was used for the probit model fitting. [b]An asterisk (*) following the resistance ratio at lethal concentration (LC_{50}) indicate significant difference from the standard susceptible population when the confidence interval does not include the value 1, following Robertson et al. (2007). [c]Pooled data to all populations following Carabello et al. (2016).

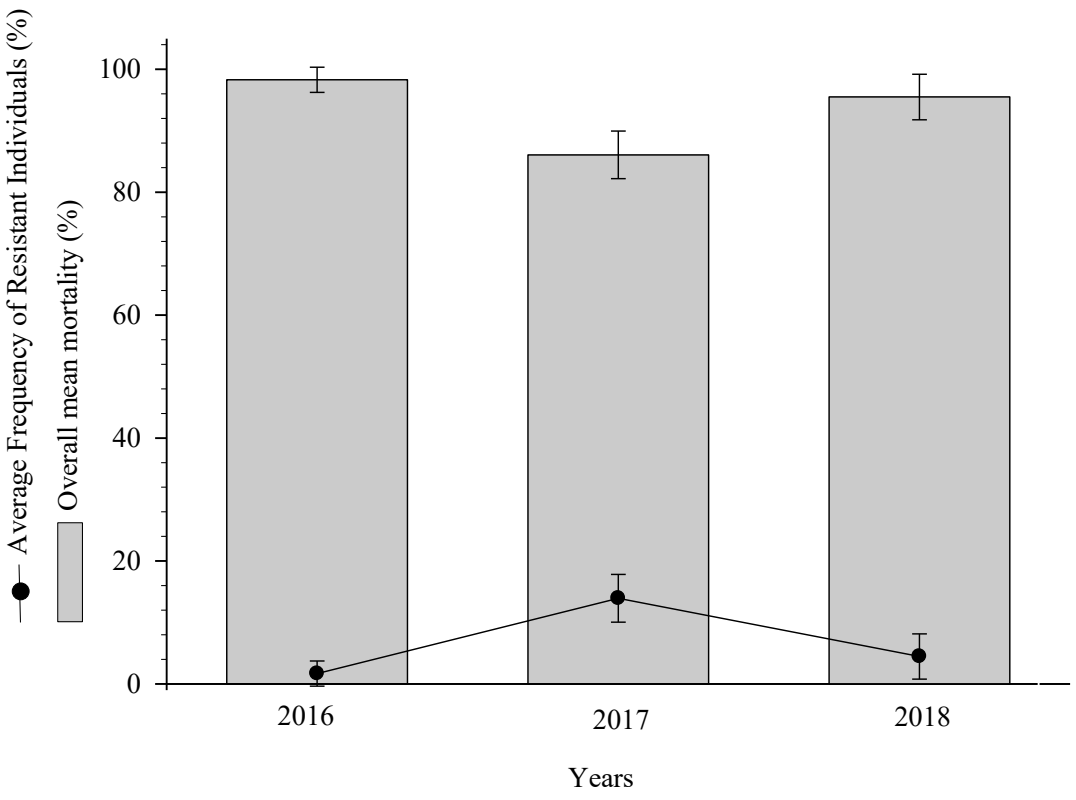
Table 3. Mortality of females of coffee berry borer *Hypothenemus hampei* subjected to the diagnostic concentration of cyantraniliprole (0.57 mg a.i. mL^{-1}) to detected resistant populations when the mortality significantly differs from the expected 90% based on the Z-test with correction for continuity and Bonferroni's adjustment. No significant difference was detected.

2016		2017		2018	
Population	Mortality (%)	Population	Mortality (%)	Population	Mortality (%)
Araguari 1	90.5	Araguari 1	90.2	Araguari 1	97.5
Araguari 2	99.4	Araguari 2	80.5	Coromandel 1	89.4
Araxá	100	Araxá	85.2	Ibiá	95.6
Campos Gerais	100	Coromandel	90.7	Monte Carmelo	96.8
Carmo do Paranaíba	96.1	Garça	85.4	Patos de Minas	99.7
Coromandel 1	99.2	Monte Carmelo 1	80.0	Patrocínio	87.5
Coromandel 2	98.7	Monte Carmelo 2	85.6	Perdizes	96.1
Coromandel 3	100	Patos de Minas	90.2	Presidente Olegário	97.7
Coromandel 4	99.5	Patrocínio 1	80.2	Rio Paranaíba 1	96.4
Coromandel 5	100	Presidente Olegário	85.9	Rio Paranaíba 2	97.2
Cristais	99.4	Rio Paranaíba	90.1	Varjão de Minas	97.0
Franca	99.7	Três Pontas	90.4	-	-
Guaraciaba	99.4	Unaí 1	85.4	-	-
Ibiá	99.0	Unaí 2	85.3	-	-
Indianópolis	98.6	-	-	-	-
Monte Carmelo 1	96.4	-	-	-	-
Monte Carmelo 2	100	-	-	-	-
Paracatu	99.5	-	-	-	-
Patos de Minas	98.1	-	-	-	-
Patrocínio 1	100	-	-	-	-
Patrocínio 2	99.0	-	-	-	-
Patrocínio 3	96.0	-	-	-	-
Patrocínio 4	95.1	-	-	-	-
Presidente Olegário 1	95.5	-	-	-	-
Presidente Olegário 2	96.3	-	-	-	-
Presidente Olegário 3	98.7	-	-	-	-
Rio Paranaíba 1	100	-	-	-	-
Rio Paranaíba 2	98.2	-	-	-	-
Rio Paranaíba 3	100	-	-	-	-
Rio Paranaíba 4	96.4	-	-	-	-
Rio Paranaíba 5	95.7	-	-	-	-
Sacramento	99.9	-	-	-	-
São J. da Boa Vista 1	98.4	-	-	-	-
São J. da Boa Vista 2	98.2	-	-	-	-
Tiros	99.1	-	-	-	-
Unaí	100	-	-	-	-

991 **Table 4.** Efficacy of the cyantraniliprole bale rate (and respective control failure likelihood)
992 against the coffee berry borer *Hypothenemus hampei* subjected to the insecticide label rate (0.37
993 mg a.i. mL^{-1}). No significant departure from the expected minimum mortality threshold (i.e.,
994 80%) was observed based on the Z-test with correction for continuity and Bonferroni's
995 adjustment.

2016		2017		2018	
Population	Mortality (%)	Population	Mortality (%)	Population	Mortality (%)
Araguari 1	100[0.0]	Araguari 1	88.1[0.0]	Araguari 1	90.3[0.0]
Araguari 2	100[0.0]	Araguari 2	95.4[0.0]	Coromandel 1	88.5[0.0]
Araxá	100[0.0]	Araxá	89.4[0.0]	Ibiá	95.5[0.0]
Campos Gerais	98.2[0.0]	Coromandel	94.2[0.0]	Monte Carmelo	93.2[0.0]
Carmo do Paranaíba	95.3[0.0]	Garça	97.4[0.0]	Patos de Minas	90.1[0.0]
Coromandel 1	95.8[0.0]	Monte Carmelo 1	95.3[0.0]	Patrocínio	95.1[0.0]
Coromandel 2	98.6[0.0]	Monte Carmelo 2	92.0[0.0]	Perdizes	91.4[0.0]
Coromandel 3	98.4[0.0]	Patos de Minas	98.4[0.0]	Presidente Olegário	98.3[0.0]
Coromandel 4	100[0.0]	Patrocínio 1	95.6[0.0]	Rio Paranaíba 1	100[0.0]
Coromandel 5	99.5[0.0]	Presidente Olegário	96.8[0.0]	Rio Paranaíba 2	100[0.0]
Cristais	100[0.0]	Rio Paranaíba	100[0.0]	Varjão de Minas	100[0.0]
Franca	99.5[0.0]	Três Pontas	100[0.0]	-	-
Guaraciaba	100[0.0]	Unaí 1	95.3[0.0]	-	-
Ibiá	95.1[0.0]	Unaí 2	100[0.0]	-	-
Indianópolis	98.0[0.0]	-	-	-	-
Monte Carmelo 1	99.0[0.0]	-	-	-	-
Monte Carmelo 2	98.9[0.0]	-	-	-	-
Paracatu	100[0.0]	-	-	-	-
Patos de Minas	89.5[0.0]	-	-	-	-
Patrocínio 1	100[0.0]	-	-	-	-
Patrocínio 2	100[0.0]	-	-	-	-
Patrocínio 3	100[0.0]	-	-	-	-
Patrocínio 4	100[0.0]	-	-	-	-
Presidente Olegário 1	100[0.0]	-	-	-	-
Presidente Olegário 2	100[0.0]	-	-	-	-
Presidente Olegário 3	100[0.0]	-	-	-	-
Rio Paranaíba 1	100[0.0]	-	-	-	-
Rio Paranaíba 2	100[0.0]	-	-	-	-
Rio Paranaíba 3	100[0.0]	-	-	-	-
Rio Paranaíba 4	100[0.0]	-	-	-	-
Rio Paranaíba 5	100[0.0]	-	-	-	-
Sacramento	98.5[0.0]	-	-	-	-
São J. da Boa Vista 1	98.5[0.0]	-	-	-	-
São J. da Boa Vista 2	95.4[0.0]	-	-	-	-
Tiros	97.1[0.0]	-	-	-	-
Unaí	96.4[0.0]	-	-	-	-

996



997

998 **Fig. 1.** Overall mean mortality and average frequency of resistant individuals (%; ± SEM) of
999 *Hypothenemus hampei* to cyantraniliprole, from 2016 to 2018, in field populations of Brazilian
1000 coffee-producing.

CONSIDERAÇÕES FINAIS

O comportamento e a genética de *H. hampei*, juntamente com os dados obtidos nos bioensaios realizados, sugerem que o uso de inseticidas à base de clorantraniliprole e ciantraniliprole podem ser uma alternativa eficaz para o controle químico dessa praga. A toxicidade e o modo de ação das moléculas do grupo químico das diamidas podem permitir o manejo equilibrado das populações de *H. hampei* e reduzir os danos causados por esse inseto aos frutos do café.

O clorantraniliprole possui potencial inseticida contra *H. hampei*, apresentando alta mortalidade de insetos e redução da sobrevivência, produção larval, resposta comportamental e taxa de respiração, principalmente na CL_{90}. O clorantraniliprole exibiu efeitos letais e subletais em broca-do-café. O ciantraniliprole, bem como o clorantraniliprole, também exibiu alta eficiência contra as populações pesquisadas de *H. hampei* entre 2016 e 2018. A resistência ao ciantraniliprole e a falha de controle associada não são um problema atual entre as populações de broca-do-café, embora possam se tornar, se as estratégias de manejo da resistência forem negligenciadas.

Ambos os ingredientes ativos podem ser uma ferramenta no uso em rotação com inseticidas de diferentes grupos químicos. Essa estratégia deve ser particularmente eficaz se integrada a métodos de controle adicionais que fazem parte de um programa de manejo integrado de pragas (MIP) e acompanhada por avaliação frequente da eficácia do inseticida, auxiliando em eventuais esforços de manejo da resistência a inseticidas.

O controle sustentável é necessário para preservar a molécula inseticida. O processo de descoberta e desenvolvimento de novas moléculas inseticidas tem se tornado cada vez mais caro e complexo. Nesse contexto, é fundamental que os inseticidas sejam usados de maneira racional na agricultura. Para tanto, se faz necessário a expansão do conhecimento sobre os modos de ação de inseticidas, efeitos comportamentais, fatores e mecanismos envolvidos na evolução da resistência, manejo da resistência e suscetibilidade associados ao MIP. O conjunto dessas ferramentas são indispensáveis para o controle de pragas, principalmente visando a aplicações reduzidas, com menor impacto social e ambiental, a partir de adoções de técnicas de monitoramento, controle e aplicação. A implementação dessas ações tende a agregar valor comercial aos grãos, além de contribuir com os pilares de respeito e importância para com a sociedade e o mundo.

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