SALMO DE MELO DAVÍ JÚNIOR

RESISTANCE OF INSECTICIDE-TREATED COFFEE BERRIES OF DIFFERENT VARIETIES TO THE PENETRATION OF *Hypothenemus hampei* (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE)

Dissertation presented to the Federal University of Uberlândia, as part of the requirements of the Graduate Program in Agronomy - Master's Degree, to obtain the title of "Master".

Supervisor

Prof. Dr. Flávio Lemes Fernandes

UBERLÂNDIA
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ATA DE DEFESA - PÓS-GRADUAÇÃO

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Iniciando os trabalhos o presidente da mesa, Dr. Flávio Lemes Fernandes, apresentou a Comissão Examinadora e o candidato, agradeceu a presença do público, e concedeu ao Discente a palavra para a exposição do seu trabalho. A duração da apresentação do Discente e o tempo de arguição e resposta foram conforme as normas do Programa.

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Aos meus pais, Salmo e Matildes, que muitas vezes se doaram e renunciaram aos seus sonhos, em prol dos meus.

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"Agora, pois, ergamo-nos, finalmente. A escritura nos desperta dizendo: Esta é a hora de levantar-nos do sono"

São Bento de Nursia

SUMMARY

ABSTRACT

DAVÍ JÚNIOR, SALMO DE MELO. **Resistance of fruits of coffee varieties to the penetration of Hypothenemus hampei (Coleoptera: Curculionidae: Scolytinae) under the action of insecticides**. 2020. 26f. Dissertation (Masters in Agronomy) - Federal University of Uberlândia, Uberlândia.

The control of *Hypothenemus hampei*, the coffee berry borer beetle, is difficult as the insects infest the interior of the berry and are thus protected from agrochemicals. In this context, coffee varieties with an increased penetration time by *H. hampei* can help control this pest as *H. hampei* tends to be exposed for longer. Therefore, this study aimed to determine the time taken by *H. hampei* to entirely penetrate berries of different coffee varieties and whether insecticides have any influence on the penetration time. Thus, 5 berries of 27 coffee varieties in the green phenological stage were introduced in rubber caps, with the berry crown exposed. Copulated *H. hampei* females were released on the berries, and the penetration time was counted. From this experiment, seven varieties were selected for another bioassay, with insecticides being sprayed on the berries. Female *H. hampei* were released on the berry, and the penetration time was assessed. Finally, a free-choice test to verify *H. hampei* food preference was performed, using the same seven coffee varieties. The insects took longer to penetrate the Arara, Catuaí Vermelho IAC 144, and Guará coffee-variety berries. Moreover, all coffee varieties treated with the insecticide cyantraniliprole showed an increased penetration time by *H. hampei*. Most coffee varieties treated with insecticides showed a prolonged penetration time by *H. hampei* compared to the same untreated ones, except for the chlorpyrifos insecticide in the Catuaí IAC 144 and IAC 62 varieties. Additionally, *H. hampei* showed no feeding preference among the different tested coffee varieties.

KEY WORDS: *Coffea arabica*; Chemical control; Food preference.

RESUMO

DAVÍ JÚNIOR, SALMO DE MELO. **Resistência de frutos de variedades de café a penetração de *Hypothenemus hampei* (Coleoptera: Curculionidae: Scolytinae) sob a ação de inseticidas**. 2020. 26f. Dissertação (Mestrado em Agronomia) – Universidade Federal de Uberlândia, Uberlândia.

O Manejo de *Hypothenemus hampei*, broca-do-cafeeiro, é complicado pelo fato de que os insetos infestarem o interior do fruto e, assim, ficarem protegidos da ação de agroquímicos. Neste caso, variedades de café que retardam o tempo de penetração da broca-do-café podem auxiliar no controle desta praga, pois o inseto tende a ficar exposto por mais tempo. Diante disso, o objetivo do trabalho é verificar o tempo de penetração de *H. hampei* em variedades de café e o efeito de inseticida no tempo de penetração. Assim, cinco frutos de 27 variedade de café na fase chumbão foram introduzidos em tampas de borracha, com a coroa do fruto exposta. A broca foi liberada sobre os frutos, contabilizando o tempo para a sua penetração. Após, foram selecionadas sete variedades para realizar outro bioensaio com a aplicação de solução inseticida sobre os frutos. Em seguida, uma fêmea da broca-do-café foi liberada sobre o fruto, e contabilizado o tempo de penetração do inseto no fruto. Por fim, foi verificado a preferência alimentar, com as mesmas sete variedades. Os insetos levaram mais tempo para penetrar em frutos das variedades Arara, Catuaí Vermelho IAC 144 e Guará. O inseticida Ciantraniliprole inibiu a entrada da broca-do-café nos frutos de todas as variedades testadas. A maioria dos frutos das variedades tratadas com inseticidas proporcionou maior tempo de penetração da broca-do-café comparados aos não tratados, com exceção ao inseticida clorpirifós nas variedades Catuaí IAC 144 e IAC 62. Não houve diferença estatística no teste de preferência da broca-do-café nas diferentes variedades testadas.

PALAVRAS – CHAVE: *Coffea arabica*; Controle químico; Preferência alimentar.

INTRODUCTION

The coffee borer beetle, *Hypothenemus hampei* (Coleoptera: Curculionidae: Scolytinae), is considered one of the most aggressive pests that attack coffee berries. *H. hampei* pierces the crown region, penetrates the seed, and feeds on its endosperm (ALBA-ALEJANDRE, ALBA-TERCEDOR; VEGA, 2018; GUIDE et al., 2018; INFANTE, PÉREZ; VEGA, 2014; VEGA et al., 2015). *H. hampei* continues its biological cycle within the seed: females lay from 1 to 3 eggs a day, the larvae hatch and pupate, and then adults emerge (ROMERO; CORTINA, 2007). Mating occurs inside the seed between siblings; after copulation, the females leave the damaged berry to feed and lay their eggs in different, undamaged berries (ARISTIZÁBAL et al., 2016; CEJA-NAVARRO et al., 2015). The time it takes for the coffee berry borer beetle to penetrate the fruit, which is essential information for pest management, is still poorly understood. The time *H. hampei* takes to fully penetrate berries can vary among coffee varieties, depending on the chemical, anatomical, and physiological characteristics of the fruit. *H. hampei* spends most of its life within the coffee fruit (INFANTE, PÉREZ; VEGA, 2014).

The berries' anatomical variations, such as the diameter of the crown, may influence the penetration behavior of *H. hampei* (MACHADO et al., 2017). Chemical characteristics are also important determinants of insect behavior (BRUCE; PICKETT, 2011), especially that of the females, which can sense the different volatile compounds released by attacked and intact fruit and differentiate among them (JARAMILLO et al., 2013; BRASSIOLI-MORAES et al., 2019). Coffee berries from different varieties may interact with insecticide molecules, which can interfere with the insect's biology; insects might take longer to penetrate the fruit or even die in the effort. This interaction has been seen in species such as peach (*Prunus persica* L.) and sweet pepper (*Capsicum annuum* L.) that have fruits that accumulate insecticide residues differently in their surface (AHLAWAT ET AL., 2019; ŽUNIĆ ET AL., 2020). Thus, the interaction of coffee fruit with insecticides can lead to differences in the penetration rate of the coffee borer beetle in the fruit and help to understand this insect behavior in fruit of different coffee varieties.

H. hampei management is mostly performed with organophosphate and pyrethroid insecticides, the residues of which remain on the surface of the fruit and can be toxic to humans and other non-target organisms present in the environment (MEKONEN, AMBELU; SPANOGLE, 2015). In this context, Integrated Pest Management relies on molecules that are increasingly selective, less toxic to humans, with low residual value in food and are highly effective in pest control, thus being considered as model molecules for the development of more effective insecticides (JECKLER, 2016). Diamide, for example, is part of a chemical group with high insecticidal potential. This insecticide acts on Ca^{2+} channels (ryanodine receptors), causing muscle paralysis and feeding inhibition, being marginally toxic to mammals and selective to natural enemies (SHAD; SHAD, 2019). Chlorantraniliprole and cyantraniliprole are examples of insecticides based on diamide that caused mortality and reduced *H. hampei* motor and respiratory activities in several studies (GONRING et al., 2019; NAKAO; BANBA 2015; NAKAO; BANBA, 2016; PLATA-RUEDA et al., 2019). Another broad-spectrum insecticide, Metaflumizone, belongs to the semicarbazone group presenting very low acute and chronic toxicity to mammals (HEMPEL et al., 2007; TAKAGI et al., 2007).

Therefore, this study aimed to evaluate the time taken by *H. hampei* females to penetrate in berries of different coffee varieties and whether insecticides could interfere in this process. Ultimately, the results will be used to identify coffee varieties with berries that are resistant to *H. hampei* penetration, as well as select new insecticides that can be associated with these borer-resistant varieties.

MATERIAL AND METHODS

The study was conducted at the Integrated Pest Management Laboratory (*Laboratório de Manejo Integrado de Pragas*–LMIP) at the Federal University of Viçosa, Rio Paranaíba *Campus*. Three bioassays were performed to assess berry resistance to *H. hampei* and different insecticides. In the first bioassay, *H. hampei* penetration time was determined in berries of several coffee varieties, without insecticides. In the second bioassay, berry preference by *H. hampei* females was assessed among the tested coffee varieties. The third bioassay followed the same procedure used in bioassay 1, with the application of insecticides on the coffee berries. All bioassays were performed in a completely randomized design.

Berries of 27 varieties of arabica coffee were collected from a coffee crop located at the Abaeté dos Mendes farm in the municipality of Rio Paranaíba–MG, coordinates 19°08' S and 46°08' O. The varieties were: Acauã, Acauã Novo, Arara, Asabranca, Bem te vi Vermelho (19/17 and 19), Yellow Bourbon IAC J9, Catiguá MG2, Catuaí Amarelo IAC 17, Catuaí Amarelo IAC 62, Catuaí Amarelo IAC Caratinga, Catuaí Vermelho IAC 144, Catuaí Vermelho IAC 99, Catucaí Vermelho 20/15, Catucaí Vermelho 24/137, Catucaí Amarelo 2SL, Guará, IAC 125 RN, IPR 100, IPR 103, IPR 107, MGS Aranãs, MGS Paraíso 2, Mundo Novo 379–19, Oeiras MG 6851, Rubi–MG 1192, Saíra, and Topázio MG 1190. The berries were harvested manually in the green phenological stage. Insecticides had not been applied in the crop nor the nearby plots. Approximately 100 fruit of each variety were collected and stored in 2 L plastic bags. After harvesting, the berries were sent to the LMIP and maintained under refrigeration (10° C) to delay fermentation, before the bioassays start.

Hypothenemus hampei adults were collected from dried berries from commercial arabica coffee crops (*Coffea arabica*), Catuaí Vermelho IAC 144, at Abaeté dos Mendes farm, located in the municipality of Rio Paranaíba, MG, coordinates 19°08' S and 46°08' W. Collected berries were then packed in plastic bags and sent to the laboratory.

Hypothenemus hampei females were removed from the collected berries by making a longitudinal cut in the fruit, 1-2 millimeters below the crown, and using a stylus to extract the insects without harming them. After the insects were removed, they were placed in a Petri dish (90 × 15 mm) for later use.

Bioassay 1–Assessment of penetration time in berries without insecticide

Five berries of each variety (in the green phenological stage) were introduced in rubber caps of penicillin flasks, with the crown upwards and arranged equidistantly in a circle in a Petri dish (90 × 15 mm). Each berry was considered an experimental unit. After removing the insects from the berries and keeping them fasting for 2 hours, one *H. hampei* female per fruit was released on the berry's crown and observed; when the female started to perforate the crown, the time for penetration into the fruit was computed. Penetration time was considered to be the period the insect drilled the berry until it was not possible to observe any part of the insect's body horizontally to the position of the berry's crown. ANOVA was used to determine significant differences between the groups, and means were compared with the Scott-Knott cluster test at 5% probability and significance level.

Bioassay 2–Free-choice preference test

This bioassay aimed to identify *H. hampei* feeding preference. Seven coffee varieties (Arara, Catuaí Vermelho IAC 62, Catuaí Amarelo IAC Caratinga, Catuaí Vermelho IAC 144, IPR 100, IPR 107, and Saíra) were selected from bioassay 1. The Scott-Knott test was used to determine the coffee varieties. Two varieties were chosen because *H. hampei* took longer to penetrate the berry (the highest average among all groups), and one coffee variety from each of the significantly different groups regarding penetration time.

Four arenas were prepared, one per Petri dish (150 × 15 mm), with the selected seven varieties of coffee (Arara, Catuaí IAC 62, Catuaí IAC Caratinga, Catuaí IAC 144, IPR 100, IPR 107, and Saíra). In this bioassay, Styrofoam was used as a base; seven holes were made in the arenas' outer rims, and an EVA sheet was placed and fixed at the Styrofoam top to obtain a smooth surface and prevent *H. hampei* from entering the holes. One berry of each of the seven varieties was inserted in each orifice. Subsequently, ten females of *H. hampei* were released on the center of the arena, one at a time.

The insects were released into the arena using a paintbrush. Once one insect was released, the Petri dish was covered and left on the bench until the insect started perforating one of the berries. Once drilling started, the perforated berry (along with the insect) was replaced by another berry of the same variety. Then, another insect was released in the arena. The process was repeated with ten insects in each of the four prepared arenas. The collected data were analyzed to determine whether they met the assumptions of analysis of variance, and they were subjected to ANOVA and Tukey's test at 5% significance level.

Bioassay 3 - Assessment of penetration time in berries with insecticide

This bioassay was conducted similarly to bioassay 1, and insecticides were sprayed on coffee berries. Seven coffee varieties (Arara, Catuaí IAC 62, Catuaí IAC Caratinga, Catuaí IAC 144, IPR 100, IPR 107, and Saíra) were selected from bioassay 1. Two varieties from the group with the best results (longest time by *H. hampei* females to penetrate the berries) were selected, along with one variety from each statistically different mean groups according to the Scott-Knott grouping. Thus, it was possible to determine the penetration time of the insect in the fruit in the presence of insecticides.

For this, 20 berries per selected variety were placed in Petri dishes (90 × 15 mm). The experiment followed a simple factorial design (seven varieties × four insecticides + control group), with five replicates; each berry was considered an experimental unit. The chosen insecticides were acetamiprid + bifenthrin (UPL do Brasil Indústria e Comércio de Insumos Agropecuarios SA; dose: 200 g ha^{-1}, 50 g ia ha^{-1} acetamiprid associated to 50 g ia ha^{-1} bifenthrin), chlorpyrifos (NUFARM Indústria Química e Farmacêutica SA; dose: 1.5 L ha $^{-1}$, 720 mL ia ha^{-1}), cyantraniliprole (DuPont do Brasil SA, dose: 1.5 L ha^{-1}, 150 g ia ha^{-1}), and metaflumizone (BASF SA, dose: 2 L ha^{-1}, 480 mL ia ha $^{-1}$). All insecticides were diluted in distilled water to obtain the label rate for the coffee berry borer, and 1 mL was applied directly over the coffee fruit, 15 cm away, using an aerograph airbrush (MP–1003, Wimpel) calibrated at a pressure of 40 Psi. All applications were performed in the laboratory at room temperature where the berries remained for 30 min for complete drying. Then, a female *H. hampei* was released on the berry, and the penetration time was assessed. The evaluation was conducted for up to ten hours—considering the moment the insect started the penetration process until its completion. The Petri dishes were kept on a laboratory bench under the same conditions that of insecticide application on the berries. The obtained data were analyzed to determine whether they met the assumptions of analysis of variance, and if so, they were subjected to analysis of variance and Tukey's test at 5% significance.

RESULTS AND DISCUSSION

In bioassay one, we evaluated how long female *H. hampei* would take to completely penetrate berries from different coffee varieties without any insecticide application. The coffee varieties with a longer penetration time by *H. hampei* were: Arara (5.7 h), Guará (5.6 h), and Catuaí Vermelho IAC 144 (5.4 h) (Figure 1). The penetration time of the coffee berry borer in these three varieties was approximately twice as long as the penetration time in the Catiguá MG2, IPR 103, IPR 107, and MGS Ananãs varieties. The IPR 103, IPR 107, and MGS Ananãs varieties showed shorter *H. hampei* penetration times: 2.8, 2.9; and 3.0 h, respectively. Although all coffee berry borer were collected from IAC 144 in the field, this variety was among the three in which the insect took the longest to penetrate the berries (Figure 1) completely.

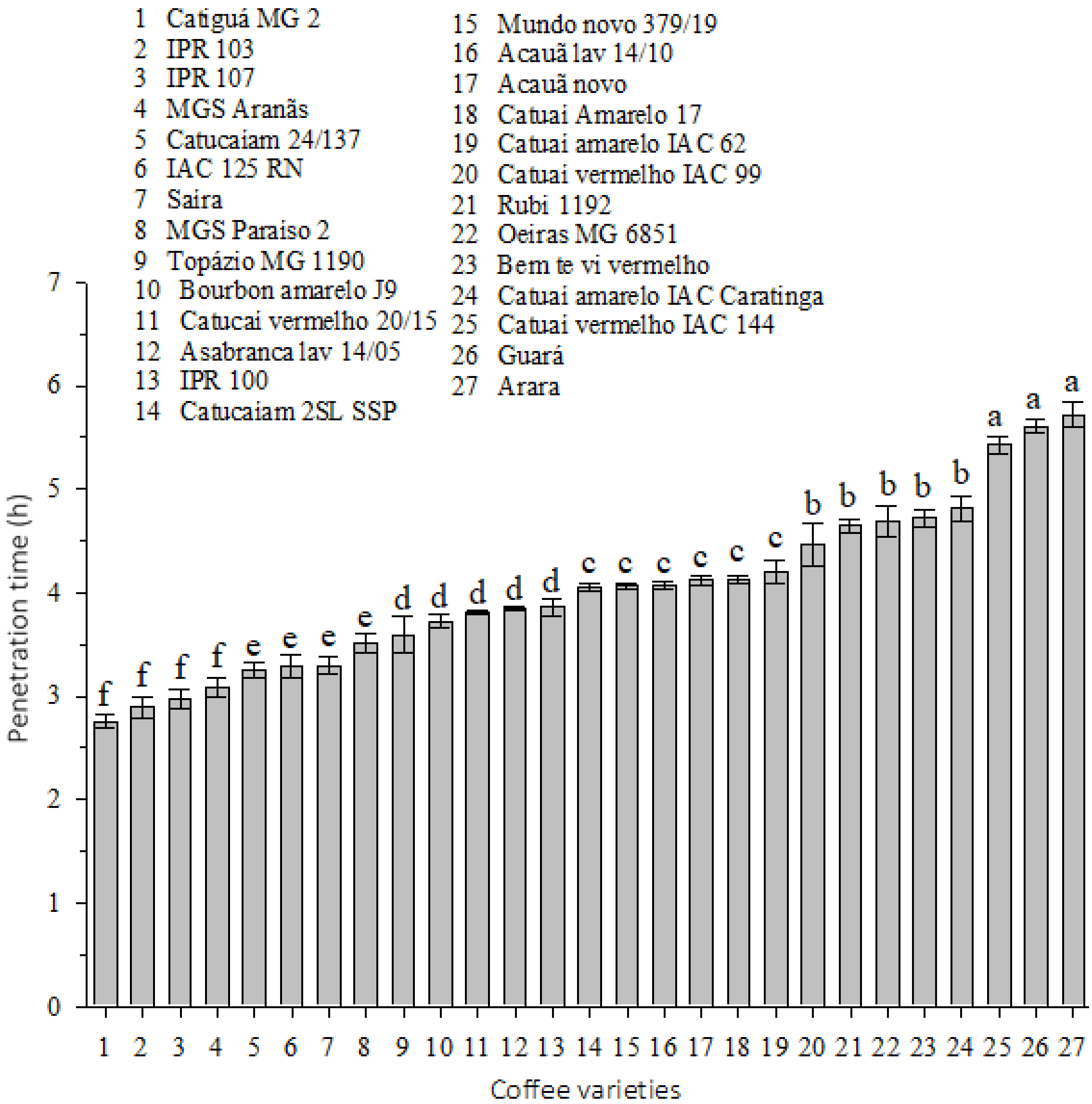

Figure 1 - Penetration time (hours) (average ± standard error) of *Hypothenemus hampei* females in *Coffea arabica* varieties.

In bioassay 2 (free-choice preference test), no differences were found between the averages of the varieties evaluated ([F: 6.28] = 0.8519; P = 0.5415; CV = 79.23%).

In bioassay 3 (evaluation of penetration time in berries with insecticide), we verified that most varieties treated with insecticides affected *H. hampei* females. The insects showed longer penetration in the insecticide-treated berries than in the untreated ones (control group) (Table 1), except for the Catuaí IAC 144 and IAC 62 varieties sprayed with chlorpyrifos. We observed that the insect penetration time was independent of insecticide application since the penetration time was changed with the application of the products. However, this occurred similarly in all tested varieties, causing an increase in penetration time in almost all of them; the insecticide cyantraniliprole inhibited the entry of *H. hampei* in all tested berries (Table 1). The insecticides metaflumizone and acetamiprid + bifenthrin increased the *H. hampei* penetration time in the Arara (7.9 h and 7.8 h) and Catuaí IAC 144 (7.7 h and 7.6 h) varieties, followed by chlorpyrifos in Arara (6.2 h) (Table 1).

Table 1. Average penetration time (hours) of *Hypothenemus hampei* females in insecticide-treated coffee berries.

Treatment	[1]Coffee variety						
	Arara	[2]IAC144	[2]IAC 62	[2]IAC Caratinga	IPR 100	IPR 107	Saíra
Control	5.7Ac	5.4Ab	4.2Cc	4.8Bc	3.9Cd	3.0Dd	3.3Dc
Cyantraniliprole	N.p.	N.p.	N.p.	N.p.	N.p.	N.p.	N.p.
Chlorpyrifos	6.2Ab	5.7Bb	4.5Cbc	5.5Bb	4.5Cc	3.6Dc	4.5Ca
Acet.+Bif.	7.8Aa	7.6Aa	5.8Ca	6.2Ba	5.4Cb	4.3Db	4.1Db
Metaflumizone	7.9Aa	7.7Aa	4.6DEb	5.6Cb	6.2Ba	4.8D	4.3Eab
CV (%)				3.90			

Acet.+bif. = Acetamiprid + bifenthrin; Np = Non-penetration; [1]Average of characteristics followed by the same uppercase letters in the rows and lowercase letters in the columns do not differ by the Tukey test ($P > 0.05$);[2] Catuaí varieties.

The disparity in *H. hampei* penetration time in different coffee varieties may be associated with the intrinsic genetic characteristics of each coffee variety. Genetic variability affects plant characteristics such as productivity and fruit maturation cycle (SANTIN et al., 2019), drink quality (BARBOSA et al. 2019; LEMOS et al., 2020), produced secondary metabolites (BECERRA et al., 2019), and berry crown diameter (MACHADO et al., 2017). Thus, the extended penetration time by *H. hampei* in Arara, Guará and Catuaí Vermelho IAC 144 varieties may be linked to genes that regulate pericarp characteristics that might hinder *H. hampei*, increasing the time females would take to drill into the berry.

Indeed, the increase in the penetration time by *H. hampei* provides producers an advantage as the insects will be exposed for a longer period. Thus, integrated pest management techniques can be more effective. The Arara, Catuaí Vermelho IAC 144 and Guará varieties, with the longest penetration times, are highly recommended varieties, mostly when other methods effective in controlling *H. hampei* are used, such as the application of entomopathogenic fungi (EDGINGTON et al., 2000), with an 88% mortality rate; further, only 5% of the surviving insects are able to drill into the berry and reach the seed endosperm (MOTA et al., 2017). Notably, these methods can be even more efficient when applied to varieties with the studied characteristic.

In addition to exposure to entomopathogenic fungi, entomopathogenic nematodes can infect *H. hampei* females that stay on the berry's surface longer. This can be achieved because some nematode isolates can show approximately 50% virulence for adults of *H. hampei* (GUIDE et al., 2018), which could be even higher if these nematodes were to be applied to coffee varieties crops that hinder *H. hampei* drilling abilities. These isolates were shown to resist up to 48 hours after exposure to cyantraniliprole (GUIDE et al., 2018). This compatibility is useful because as *H. hampei* takes longer to penetrate the berry, the nematodes can infect the female in this period when the insect is exposed, drilling the berry.

When considering the addition of insecticides, the effect was very positive as the increase in the penetration time by *H. hampei* in all evaluated varieties was significant. This increase in time reveals a possible change in the insect's biological parameters since the application of chemical compounds may affect behavior, especially feeding (CELESTINO et al., 2015), as some insecticides directly affect the digestive system, consequently decreasing the mass gaining capacity of the insects. Thus, longevity and fertility rate of *H. hampei* might have been reduced. In this context, the results reported by Dastranj et al (2018) corroborate with this hypothesis, as they found a decrease by 30% and 17%, respectively, in the body mass of *Plutella xylostella* (Lepidoptera: Plutellidae) and *Pieris rapae* (Lepidoptera: Pieridae) larvae.

Insecticides can cause indigestion through ingestion and contact. In the short term, mortality from ingestion is much more significant. When associated (ingestion and contact, whether direct or tarsal contact only), there is an additive effect and insecticide's toxicity becomes more effective due to the chronic effect of insecticides, causing more mortality (REZÁC, ŘEZÁČOVÁ; HENEBERG, 2019).

The insecticide cyantraniliprole inhibited the entrance of the coffee berry borer, thus altering its behavior. The same modification was found by Joseph et al (2020). The authors observed that the insecticide reduced pest feeding, being very effective in the coffee berry borer management. The efficiency in the control of insect pests by cyantraniliprole is supported by studies with other crops, such as rice and strawberries. Cyantraniliprole was considered more effective compared to chlorpyrifos, bifenthrin, and chlorantraniliprole; it was also considered a low-risk insecticide, both in toxicological and environmental terms (JOSEPH et al., 2020; MAO et al., 2019; RENKENA et al., 2020).

Another feature that is worth mentioning regarding cyantraniliprole is its ability to cause damage and changes in DNA, which can cause desired changes in the biological parameters and behavioral characteristics of insects (QIAO et al., 2019), such as decreased feeding and female fertility rate. However, when it comes to chemical control, insects developing resistance to certain active ingredients is a concern. Studies already reported insects developing resistance to insecticides such as chlorantraniliprole and chlorpyrifos (Mallott et al., 2019; WANG, LOU; SU, 2019), the latter not controlling *H. hampei* as effectively as metaflumizone. No insect resistance to metaflumizone has been reported (SUN et al., 2019), despite the fact that this insecticide is not as effective as abamectin or azadirachtin (AMIZADEH et al., 2019).

No food preference was found among *H. hampei* insects in the selected coffee varieties, agreeing with these results by Sara et al (2010). Targeted studies aiming at the identification of volatiles emitted by different varieties are necessary (BRASSIOLI-MORAES et al., 2019), as they provide significant results to determine the preference of *H. hampei* for different coffee varieties.

CONCLUSIONS

The Arara, Catuaí Vermelho IAC 144, and Guará varieties obtained the best results for the penetration time of *H. hampei* in coffee berries, with 5.7 h, 5.6 h and 5.4 h, respectively.

The insecticide cyantraniliprole inhibited *H. hampei* drilling in berries of all tested varieties. Moreover, berries from most varieties treated with insecticides increased penetration time by the coffee berry borer beetle compared to the untreated ones, except for chlorpyrifos in the Catuaí IAC 144 and IAC 62 varieties.

Additionally, no feeding preference of the coffee berry borer was observed among the different varieties tested.

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