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Attractiveness of semiochemists to *Hypothenemus hampei*

Atratividade de semioquímicos a *Hypothenemus hampei*

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Brazilian Journal of Development

ABSTRACT
The alcohol-based volatile compounds have been used as attractive in the management of coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari, 1867) (Coleoptera: Curculionidae: Scolytinae). However, some of these compounds are highly toxic to humans and the environment, as is the case with methanol. Then, the objective of this study was to verify the attractiveness of semiochemicals mixed with ethanol to CBB in olfactometer and to determine a possible substitute for methanol in the mixture with ethanol in the capture of the CBB in the field. The laboratory tests were performed on an olfactometer, with the compounds: cinnamaldehyde; eugenol; citral; ethyl acetate; acetic acid; geraniol; isopropyl alcohol; butyl alcohol and the methanol. The mixtures that stood out most in the olfactometer tests were used in the field experiment. These compounds were used in transparent traps made with 2 L PET bottles, with rectangular side opening (20 x 15 cm) to capture of CBB. The ethanol: cinnamaldehyde and ethanol: methanol mixtures attracted a greater number of CBB in the olfactometer tests. The ethanol: methanol mixture provided a better efficiency in the capture of CBB in all periods of field evaluations.

Keywords: Coffee, volatile compounds, pest management.

RESUMO
Os compostos voláteis a base de álcoois têm sido usados como atrativos no manejo da broca-do-café, *Hypothenemus hampei* (Ferrari, 1867) (Coleoptera: Curculionidae: Scolytinae). No entanto, alguns destes compostos apresentam alta toxicidade ao homem e ao meio ambiente, como é o caso do metanol. Deste modo, o objetivo deste estudo é verificar a atratividade de semioquímicos em mistura com etanol a *H. hampei* em olfatômetro e determinar um possível substituto para o metanol na mistura com o etanol na captura da broca-do-café em campo. Os testes em laboratório foram realizados em olfatômetro, com os compostos: cinamaldeído; eugenol; citral; acetato de etila; ácido acético; geraniol; álcool isopropílico; álcool butílico e o metanol. As misturas que mais se destacaram nos testes em olfatômetro foram utilizadas no experimento de campo. Estes compostos foram utilizados em armadilhas transparentes confeccionadas com garrafas PET 2 L, com abertura lateral retangular (20 x 15 cm) para captura de *H. hampei*. As misturas etanol:cinamaldeído e etanol:metanol atraíram um maior número de broca-do-café em olfatômetro; a mistura etanol:metanol propiciou uma melhor eficácia na captura da broca-do-café em todas as épocas de avaliações em campo.

Palavras-chave: Café, compostos voláteis, manejo de pragas.

1 INTRODUCTION

The volatile compounds produced by plants play an important role in helping insects to locate hosts (KNOLHOFF; HECKEL, 2014; TOSH; BROGAN, 2015). The most insects use olfactory stimuli to locate host plants. In scolytins, such as *Dendroctonus armandi* (Tsai; Li) and *D. valens* (LeConte, 1860) the olfactory stimuli have been used successfully in monitoring (CHEN et al., 2015; KELSEY; WESTLIND, 2017).

In the case of the coffee berry borer (CBB), *Hypothenmeus hampei* (Ferrari, 1867) (Coleoptera: Curculionidae: Scolytinae), the olfactory stimuli have helped in the development of new insect management strategies. It is known that *H. hampei* uses volatile compounds emitted by the fruits, mainly alcohols, to locate them (GIORDANENGO et al.,1993; MATHIEU et al., 1997). The ethanol mixed with methanol are widely used in traps for population monitoring and management of the CBB (DUFOUR; FRÉROT, 2008; PEREIRA et al., 2012; ARISTIZÁBAL et al., 2015).

However, although methanol is a volatile compound, it has high toxicity to humans and the environment, which may imply its use in the management of *H. hampei*. Therefore, the search for new volatile compounds that present the same efficiency of this alcohol in the insect's attractiveness and that present low toxicity is of paramount importance. In this context, the essential oils (EOs) are very promising due to their low toxicity to humans and animals (ISMAN, 2008). Besides that, the active ingredients of these compounds are biodegradable, and don't stay in the environment for long periods (HÜTER, 2011).

The essential oils, volatile compounds produced from the secondary metabolism of some plants (HÜSNÜ et al., 2007), are formed by several molecules with known biological activities, such as: the cinnamaldehyde (CHENG et al., 2008) main active component of cinnamon essential oil (*Cinnamomum* sp), which is used as a trap to capture the *Holotrichia* (Coleoptera: Scarabaeidae: Melolonthinae) (LI et al., 2013); the eugenol, primary compound of clove oil; geraniol, marjoritary monoterpenoid of rose oil, both used to capture the *Phyllopertha hortícola* (Linnaeus, 1758) (Coleoptera: Scarabaeidae) and *Popillia japônica* (Newman, 1841) (Coleoptera: Scarabaeidae: Rutelinae) (RUTHER; MAYER, 2005; CHEN et al., 2014); and the ethyl acetate, found in ripe coffee fruits, used to capture *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) (AL-SAOUDA, 2013). Despite the attractiveness of these compounds for some coleopterans, there are few studies that assess their attractive potential for *H. hampei*.

Thus, the present study aimed to verify the efficiency of volatile compounds in the attractiveness of *H. hampei* mixed with ethanol in an olfactometer and to determine a possible substitute for methanol in the mixture with ethanol to capture the CBB in the field.

2 MATERIALS AND METHODS

2.1 ATTRACTIVENESS OF VOLATILE COMPOUNDS TO *Hypothenemus hampei* IN OLFACTOMETER

The experiment was conducted in the entomology laboratory in the *Núcleo de Desenvolvimento Tecnológico e Científico em Manejo Fitossanitário de Pragas e Doenças* (NUDEMAFI) in the *Centro de Ciências Agrárias e Engenharias da Universidade Federal do Espírito Santo* (CCAE-UFES).

Brazilian Journal of Development

The *H. hampei* adult females used in the tests were taken from the breeding established in the entomology sector of CCAE-UFES. The insects were kept at 25 ± 1°C, 65 ± 5% of relative humidity (RH), in plastic boxes (15 x 30 x 5 cm) with cover, containing fruit on only one side of the box, for collecting insects on the free end. The collection was made with suction of small insects, adapted to a vacuum pump (DALVI; PRATISSOLI, 2012). The females were individualized and kept fasted for at least 12 hours, before testing.

The tests were carried out on olfactometers made with a round plastic gerbox with four arms, attached to a square wooden base with four coolers, one on each side, with a distance of 16 cm to the arms of the olfactometer. The transport of the air of the treatments, to the central arena of the olfactometer, was done through a silicone hose (8 cm long) connected to the orifice of the central arena and the lateral arena, with the aid of the cooler. In each side arena, an opening was made covered with Voil fabric, in order to assist in the dispersion of compounds to the central arena. The tests were carried out in an air-conditioned room (25± 2°C and 65± 5% RH).

Five mated females of *H. hampei* were inserted in the central arena of each olfactometer, with the help of a fine-tipped brush, observing the behavior and movement. Immediately, after insertion of females, the olfactometer cover, with several holes, was placed together with a Voil fabric. The entrance of the insect in one of the arms of the olfactometer with permanence for one minute was registered as “choice”. The insects that remained immobile for three minutes were replaced, as described by Wang et al. (2016). The observation was made visually and timed for a 30 minute shift.

After each repetition, the olfactometers were washed with a hexane solution and placed to dry. The positions of the compounds were changed to avoid possible interference in the response of insects. The experiments were carried out between 2 pm and 6 pm, which corresponds to the period of greatest activity and flight of the insect (SILVA, et al., 2014). In each side arena of the olfactometer, a moistened cotton was placed with the respective compounds. All new compounds tested were 99.8% pure, confirmed by gas chromatography analysis. In all tests, the proportion used in mixtures with ethanol was 1:1. The reference mixture used was Ethanol (E): Methanol (M), with (99.9% and 100% of purity), respectively.

The compounds used in the mixture with ethanol were:

Test 1 - Ethanol: Cinnamaldehyde, Ethanol: Eugenol, Ethanol: Citral and Ethanol: Methanol;

Test 2 - Ethanol: Ethyl acetate, Ethanol: Acetic Acid, Ethanol: Geraniol and Ethanol: Methanol;

Test 3 - Ethanol: Isopropyl alcohol, Ethanol: Butyl alcohol, Ethanol: Cinnamaldehyde: Acetic Acid and Ethanol: Methanol;

Test 4 - Ethanol: Cinnamaldehyde, Ethanol: Methanol: Cinnamaldehyde, Ethanol: Cinnamaldehyde: Acetic Acid and Ethanol: Methanol.

The experimental design was completely randomized with 10 repetitions. The data were analyzed using the Dunn non-parametric test, at the 5% probability level, in the statistical software R (Pacote dunn.test) (R Development Core Team, 2019).

2.2 OLFACTORY RESPONSE OF *Hypothenemus hampei* TO SEMIOCHEMICALS IN THE FIELD

The experiments were carried out in a *Coffea arabica* field cultivar 'Catuaí Vermelho', 1.70 x 2.70 m spacing, located in the Celina district, municipality of Alegre, (latitude 20° 45' 50.28'' S and longitude 41° 35' 30.72'' W), Espírito Santo, Brazil, altitude of 700 m at sea level.

Transparent traps made with 2 L PET bottles, with rectangular side opening (20 x 15 cm), were used to capture the *H. hampei* because they have low cost (FERNANDES et al., 2015).

The traps were attached to bamboo poles 1.5 m above the ground as described by Uemura-Lima et al (2010), with galvanized wire number 12. Inside each trap, a 12 ml glass bottle was fixed with string, containing the chemical compounds. The bottle was sealed with a plastic screw cap, with a 2 mm diameter hole in the center, to release the attraction. Deep in the trap, 120 mL of water with 5% neutral detergent was added to capture the insects. After collections, the insects were taken to the laboratory for counting.

The mixtures that stood out in the attractiveness of the CBB, in laboratory, were used with a mixture volume of 12 mL. The solutions were prepared in the *Núcleo de Desenvolvimento Tecnológico e Científico em Manejo Fitossanitário de Pragas e Doenças* (NUDEMAFI).

The trap openings were positioned towards the center of the coffee line, to allow the odorous plume of the attraction to disperse between the rows (FERNANDES et al., 2014). 50 traps throughout the area were distributed equidistant (15x15m), over 10 entirely randomized blocks, with five traps per block evaluated every two weeks. Then, the plots were subdivided over time.

The experimental data were collected in the 2017/2018 harvest, in the following phenological stages of coffee: small green (November), expansion (December), grain formation (January / February / March) and maturation (April).

The data were subjected to analysis of variance and the means were compared using the Tukey test at a significance level of 5% in the statistical software R (ExDes.pt package) (R DEVELOPMENT CORE TEAM 2019).

3 RESULTS AND DISCUSSION

3.1 ATTRACTIVENESS OF VOLATILE COMPOUNDS TO *Hypothenemus hampei* IN OLFACTOMETER

In Test 1, the responses of H. hampei females in an olfactometer showed preference for the mixture Ethanol: Cinnamaldehyde, with the value of 2.1±1.45 CBB, differing significantly (Kruskal-Wallis chi-squared = 11.61; $p < 0.05$) of mixtures Ethanol:Eugenol and Ethanol:Citral, with the values of 0.7±0.82 and 0.3±0.48 CBB, respectively. However, there was no difference in relation to the mixture Ethanol: Methanol, whose value was 1.0±0.82 CBB (Table 1).

Table 1. *Hypothenemus hampei* adults attracted by volatile compounds in olfactometer in the Test 1.

Attractive	**CBB[1] Number**
Ethanol: Cinnamaldehyde	2.1±1.45a*
Ethanol: Methanol	1.0±0.82ab
Ethanol:Eugenol	0.7±0.82b
Ethanol:Citral	0.3±0.48b

[1] CBB is coffee berry borer.
* Values followed by the same letter do not differ at the 5% probability level by the Dunn test.

In Test 2, there was no significant difference (Kruskal-Wallis chi-squared = 2.60; $p > 0.05$) between the mixtures Etanol: Ethanol: Ethyl acetate; Ethanol: Acetic Acid; Ethanol: Geraniol; and Ethanol: Methanol, whose values were: 0.4±0.70; 1.0±1.05; 0.6±1.07 and 0.9±1.10 CBB, respectively (Table 2).

Table 2. *Hypothenemus hampei* adults attracted by volatile compounds in olfactometer in the Test 2.

Attractive	**CBB[1] Number**
Ethanol: Ethyl acetate	0.4±0.70a*
Ethanol: Acetic Acid	1.0±1.05a
Ethanol: Geraniol	0.6±1.07a
Ethanol: Methanol	0.9±1.10a

[1] CBB is coffee berry borer.
* Values followed by the same letter do not differ at the 5% probability level by the Dunn test.

In Test 3, the number of CBB showed a significant difference (Kruskal-Wallis chi-squared = 5.87; $p < 0.05$) just between the mixtures Ethanol: Methanol (1.3±1.57 CBB) and Ethanol: Cinnamaldehyde: Acetic Acid (0.2±0.63 CBB) (Table 3).

Table 3. *Hypothenemus hampei* adults attracted by volatile compounds in olfactometer in the Test 3.

Attractive	CBB[1] Number
Ethanol: Isopropyl alcohol	0.5±0.53ab*
Ethanol: Butyl alcohol	0.5±0.71ab
Ethanol: Cinnamaldehyde: Acetic Acid	0.2±0.63b
Ethanol: Methanol	1.3±1.57a

[1] CBB is coffee berry borer.
* Values followed by the same letter do not differ at the 5% probability level by the Dunn test.

In Test 4, the *H. hampei* females showed preference for the mixture Ethanol: Cinnamaldehyde, with the value of 2.0±0.82 CBB, differing significantly (Kruskal-Wallis chi-squared = 20.30; $p < 0.05$) of the mixtures Ethanol: Cinnamaldehyde: Acetic Acid (0.5±0.70 CBB) and Ethanol: Methanol: Cinnamaldehyde (0.5±0.53 CBB). However, there was no significant difference in relation to the mixture Ethanol: Methanol, which presented 1.6±0.70 CBB (Table 4).

Table 4. *Hypothenemus hampei* adults attracted by volatile compounds in olfactometer in the Test 4.

Atrativo	CBB[1] Number
Ethanol: Cinnamaldehyde	2.0±0.82a*
Ethanol: Methanol	1.6±0.70a
Ethanol: Cinnamaldehyde: Acetic Acid	0.5±0.70b
Ethanol: Methanol: Cinnamaldehyde	0.5±0.53b

[1] CBB is coffee berry borer.
* Values followed by the same letter do not differ at the 5% probability level by the Dunn test.

The *H. hampei* females were significantly more attracted to the mixtures Ethanol: Cinnamaldehyde and Ethanol: Methanol. These results suggest that the volatile compound Cinnamaldehyde has the potential to replace Methanol. Besides that, this compound has low toxicity to humans and the environment (ISMAN, 2008), which makes it a promising alternative to be used in traps in the phytosanitary management of CBB.

The attractiveness of the mixture Ethanol: Cinnamaldehyde to *H. hampei*, may be related to the fact that this compound is an aldehyde, which is one of the main volatiles produced by the fruits of *Coffea arabica* and *C. canephora*, and one of those responsible for the olfactory response of CBB (MATHIEU et al., 1996; WARTHEN et al., 1997). Besides that, this compound has a striking aroma, since it is widely used in the fragrance industries (COCCHIARA et al., 2005; LÓPEZ-MATA et al., 2017). Several studies have reported the efficiency of the Cinnamaldehyde in traps as an attraction of other species of coleoptera, such as *Astylus variegatus* (Germar, 1824) (Coleoptera: Melyridae), *Bruchus rufimanus* (Bohemann, 1833) (Coleoptera: Chrysomelidae) and *Holotrichia oblita* (Faldermann, 1835) (Coleoptera: Scarabaeidae) (VENTURA et al., 2007, BRUCE et al. 2011, LI et al., 2013).

3.2 OLFACTORY RESPONSE OF *Hypothenemus hampei* TO SEMIOCHEMICALS IN THE FIELD

There was interaction between the factors product and evaluation period ($F = 11.86$; $p < 0.05$) (Table 5).

Table 5. *Hypothenemus hampei* adults caught in traps with attractive mixtures in biweekly evaluations.

	Product	
Evaluation Period	**Ethanol: Methanol**	**Ethanol: Cinnamaldehyde**
30/11 (small green)	26.00±3.07Aa*	5.30±0.51Bbc
15/12 (expansion)	23.20±1.08Aab	16.00±0.74Ba
30/12 (expansion)	13.20±1.49Acd	8.55±0.64Bb
15/01 (grain formation)	9.44±0.80Ad	4.20±0.77Bcd
30/01 (grain formation)	7.80±0.94Ad	4.20±0.26Bcd
15/02 (grain formation)	14.82±0.97Acd	2.80±0.47Bcd
02/03 (grain formation)	12.8±0.97Acd	2.80±0.47Bcd
17/03 (maturation)	11.20±1.62Acd	2.00±0.43Bcd
02/04 (maturation)	12.23±1.49Acd	2.00±0.16Bcd

* Averages represented by equal uppercase letters in the lines and equal lowercase letters in the columns do not differ at the level of 5% probability by the Tukey test.

In all evaluation periods, there was a greater capture of *H. hampei* in the mixture Ethanol: Methanol ($F = 23.67$; $p < 0.05$). In this mixture, the biggest catches occurred on 11/30 (small green) and 15/12 (expansion) with 26.00±3.07 and 23.20±1.08 CBB, differing significantly from the other evaluation periods ($F = 32.14$; $p < 0.05$). In the mixture Ethanol: Cinnamaldehyde, the biggest capture occurred on 12/15 (expansion) with 16.00±0.74 CBB, differing significantly from the other evaluation periods ($F = 32.14$; $p < 0.005$).

The smaller numbers of *H. hampei* caught in traps with Ethanol: Cinnamaldehyde in relation to the reference mixture Ethanol: Methanol may be related to the fact that this compound has presented low volatility in the environmental conditions presented, because the vapor pressure of cinnamaldehyde is lower than that of methanol (DOLLIMORE, 1999). This is because the size of the cinnamaldehyde molecule is larger than that of methanol, onsequently the interactions are stronger, which can lead to slower evaporation (RUSSEL, 1994).

In the mixture Ethanol: Methanol, the highest CBB densities occurred on 11/30 (small green) and 15/12 (expansion). In the mixture Ethanol: Cinnamaldehyde, the highest CBB densities occurred on 15/12 (expansion). These phenological stages of the coffee fruit, that had the highest catches, correspond to the periods of greatest pest transit in the crop, when the CBB leave the fruits that were on the ground in the previous harvest and fly in search of new fruits (DAMON, 2000). Higher catches in these periods were also observed in other studies on the peak of the CBB in the coffee crop (PEREIRA et al., 2012).

4 CONCLUSION

The mixtures Ethanol: Cinnamaldehyde and Ethanol: Methanol attracted a greater number of CBB in an olfactometer.

The mixture Ethanol: Methanol provided a better efficiency in the capture of the CBB in all periods of field evaluations.

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