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# Response of *Hypothenemus hampei* (Coleoptera: Curculionidae) to semiochemicals and blends using baited traps in coffee fields

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**Abstract**

*Hypothenemus hampei* (Ferrari) is an important pest of coffee fields all around the world. The understanding of the synthetic attractive volatiles is essential for the development of appropriate strategies for its integrated management. The olfactory response of this insect to ethylene (ETL) and ethyl acetate (EA) was investigated in experimental coffee (*Coffea canephora* 'Conilon') fields in Rondonia, Brazil, using baited traps with single compounds and combined with alcohols, i.e., ethanol:methanol (ET:MT, 1:1). Baited traps were placed along plant rows within the coffee field using a randomized block design. Collected insects were properly separated, counted and identified using a stereomicroscope in the laboratory. Traps baited with EA captured a higher number of *H. hampei* compared to control traps, suggesting its effect as possible attractant. Despite that, the combination of EA with single alcohols, i.e., EA:ET (1:1) and EA:MT (1:1) and then ET:MT (1:1), i.e., EA:ET:MT (1:1:1) did not increase beetle capture compared with currently used *H. hampei* attractants, i.e., ET:MT (1:1). Other experiments will be performed to improve bait performance using different EA to ET:MT ratios.

**Keywords**: Coffee berry borer; attractants; ethylene; ethyl acetate, alcohols.
**Abbreviations**: ETL_ethylene; EA_ethyl acetate; ET_ethanol; MT_methanol.IPM_integrated pest management; SA_selected attractant; Ctrl_control; HIPV_herbivore-induced plant volatile.

## Introduction

The understanding of chemical signaling between *Hypothenemus hampei* (Coleoptera: Curculionidae) and its host plant *Coffea* spp. (Gentianales: Rubiaceae), is still limited (Jaramillo et al., 2013). Herbivore beetles use volatiles emanating from their host plants as a cue to locate them. Thus, the role of these semiochemicals is essential for pest control. Studies on the species *Coffea arabica* L. and *Coffea canephora*, Pierre ex A. Froehner, revealed presence of several odorant compounds in higher amounts in *C. canephora* (Mendesil et al., 2009) and also *H. hampei* preference for this species (Guerreiro and Mazzafera, 2003). Thus, testing the attraction of *H. hampei* to different plant compounds has a great potential to improve integrated pest management (IPM), since attractive traps that accurately estimate data on the population density of pests are important for more sustainable and successful decision making (Fernandes et al., 2015).

Currently in coffee, the most attractive semiochemicals to trap *H. hampei* are the alcohols ethanol (ET) and methanol (MT), widely used in baited traps for the integrated management of this important pest (Aristizábal et al., 2015). However, when the coffee fruit is full ripen, the semiochemicals composition also contains esters. Among the esters found in ripe coffee fruits, ethyl acetate (EA) stands out with a relatively high concentration compared to other chemical compounds (Ortiz et al., 2004).

Other studies also revealed high levels of EA in coffee fruits due to the fermentation process (De Melo Pereira et al., 2015). In general, EA is a natural volatile compound present in fruits (Paul and Pandey, 2014), and also in Rubiaceae flowers (Duarte et al., 2016). This compound is also reported attractive to some coleopteran species, including curculionids (Al-Saouda, 2013). In addition, other groups of insects are attracted to EA emanating from decomposed fruit tissues, such as dipterans (Drosophila) (Cha et al., 2012). Moreover, El-Shafie and Faleiro (2017) reported that volatile compounds, resulting from fermentative fruit processes such as EA, are attractive to several species of insects and are potentially relevant to IPM.

Other compounds, such as the phytohormone ethylene (ETL), play an important role in insect and plant interaction, since many species of insects use this compound as a semiochemical (Groen and Whiteman 2014) and it was also attractive to curculionids (González and Campos, 1995; Campos and Peña, 1995). Besides, colonizing females of *H.*

*hampei* initiate oviposition during fruit ripening (Cure et al., 1998), which is the phenological stage that plant produces the greatest amount of ETL (Pereira et al., 2005).

Therefore, new possible attractive compounds not currently used in traps were tested in the field to investigate their attractiveness and the possible synergism with the attractants that have already been proven effective in attracting *H. hampei*.

**Results**

***Evaluation of possible attractive compounds ethyl acetate (EA) and ethylene (ETL)***

EA collected more insects when compared to control traps (Fig 4), during preliminary evaluation in April/2015 when presumably attractive compounds were tested. However, the combination of all attractants and possible attractive compounds in a single trap (EA+ETL+ET:MT) did not result in a significant increase in the capture of *H. hampei*, compared to the mixture of ET:MT (1:1) (Fig 4).

The mean number (log) of *H. hampei* in traps placed within coffee plants collected during pre-harvest period (April/2015) was 3.13 (SE 0.32), using the ET:MT (1:1); 2.62 (SE 0.32) with EA+ETL+ET:MT; 0.66 (SE 0.32) with EA, 0.14 (SE 0.32) with ETL and 0.12 (SE 0.36) with the control (Fig 4).

***Combining ethyl acetate (EA) with alcohols ethanol (ET) and methanol (MT)***

The synergistic effect of the non-usual semiochemical presenting the highest average number (log) of *H. hampei* in the previous experiments, i.e., EA, was evaluated in different combinations with ET and MT alcohols at a 1:1 ratio during both harvest and pos-harvest period.

The average number of collected *H. hampei* (SE 0.19) using ET:MT was 5.08 during the harvest period (Fig 5a) and 4.46 (SE 0.12) in the post-harvest period (Fig 5b). However, when EA was added to ET or MT during harvest, the average number of *H. hampei* was 2.89 (SE 0.19) and 4.00 (SE 0.19), respectively, for the ET:EA (1:1) and MT:EA (1:1) (Fig 5a) and 2.08 (SE 0.19) and 3.20 (SE 0.19), respectively, for the ET:EA (1:1) and MT:EA (1:1) during pos-harvest (Fig 5b).

The combination of EA with both ET and MT, i.e., ET:MT:EA (1:1:1), also did not increase insect attraction compared to ET:MT during harvest and pos-harvest (Fig 5a and 5b) ($p > 0.05$).

**Discussion**

In the experiments with the new possible attractive compounds, ETL did not significantly attract *H. hampei*, compared to control traps (unbaited) (Fig 4). The high ETL peaks mainly occurred at the beginning of the coffee fruit ripening process (Pereira et al., 2005). This result suggests that the most attractive volatiles for *H. hampei* are found when fruits are already in advanced ripening process. Previous studies that have dealt with volatile compounds emanating from ripe coffee fruits found that alcohols, esters and aldehydes stand out as the compounds with the highest concentrations (Ortiz et al., 2004; De Melo Pereira et al., 2015). This probably explains the preference of *H. hampei* for the alcohols ET and MT and to a lesser extent for the ester EA, compared to ETL.

The low capture levels of *H. hampei* using ETL as bait (Fig 4) suggests that this compound is not attractive to this species under our experimental conditions. Actually, ETL has other ecological functions acting as an herbivore-induced plant volatile (HIPV) attracting insects considered natural enemies or even as a repellent for other insect species (Groen and Whiteman, 2014). In this sense, ETL plays an important role in induction of volatiles in plants (War et al., 2011) when plants are attacked, and parasitoids or other members of the third trophic level use induced volatiles as cues to find the feeding herbivore (Kahl et al., 2000). Interestingly, ETL traps in the present work captured a high number of potential natural enemies of *H. hampei* (data not published), such as the ants *Camponotus blandus* (McClure et al., 2008); *Crematogaster evallans* (Boscardin et al., 2012), *Pseudomyrmex gracilis* (Rivas-Arancibia et al., 2014) and *Brachymyrmex* sp. (Cividanes 2002).

We also do not rule out the possibility that ETL is actually a repellent compound for *H. hampei*, resulting from a strategy developed by this insect to avoid its natural enemies, since herbivorous insects behavior is determined by complex processes resulting from trophic interactions (Clavijo McCormick, 2016). Therefore, assessing the potential attractiveness of ETL to insects that are natural enemies, e.g., ants, may result in information relevant to the integrated management of *H. hampei*, since this potential management tool have not been successfully incorporated into control programs (Castro et al., 2017).

Trap baits with EA attracted *H. hampei* compared to unbaited traps control (Ctrl) (Fig 4). However, the combination of alcohols and possible attractants in a single trap, and also the different combinations of EA with ET and MT (1:1) did not result in a significant increase in insect capture (Fig 5a and 5b). Although EA exerts an interchangeable effect with ET and other alcohols on the olfactory response of other coleopteran species (Lin and Phelan, 1991), the use of EA at the same ratio in all the combinations, evaluated in this study, was probably unable to increase *H. hampei* attractiveness compared to ET:MT. Also, Girón-Pérez et al. (2009) revealed that the production of ET in plant substrates is higher than EA over time, suggesting that the ratio of the ester EA in the mixture should probably be lower in relation to the alcohols ET and MT. Perhaps other compounds should be added to potentiate the synergistic effect among the volatiles found in greatest proportions in the mature fruits, i.e., aldehydes (Sulaeha et al., 2017).

EA produced by plants is also mentioned as a volatile compound that acts synergistically with aggregation pheromones from Curculionidae (Reddy and Guerrero, 2004), but the methodological design of the present study does not allow us to investigate this hypothesis.

Our present data corroborate the use of ET and MT as effective bait to capture *H. hampei* in coffee fields in Rondonia. Thus, new studies with possible attractants need to include this alcoholic mixture, due to its synergistic effect with other volatile compounds from coffee using baited traps in the field (Leite, 2016).

The present results indicate that further works are needed to elucidate whether other EA ratios combined with known attractants can increase the capture of *H. hampei* using

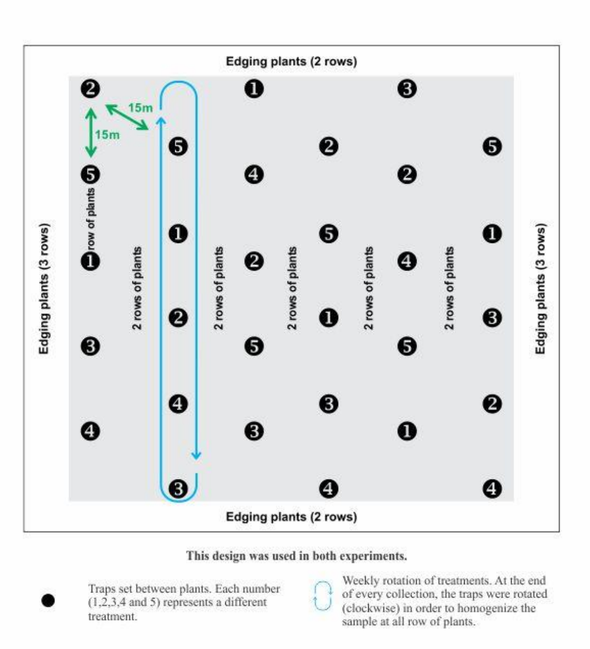


**Fig 1**. Trap distribution design in the experimental area. Each sequence of duly randomized traps was installed in a row of plants, represented by blocks. Among the blocks, two rows of plants were delimited with the purpose to cause the border effect. In each block, a repetition of each evaluated treatment was installed. The distances between all the traps (15x15m) were delineated to avoid interference between treatments.

(a) (b)

**Fig 2.** Trap with different semiochemicals for evaluation of synergic effect during the preliminary experiment. (**a**) Collection of insects captured from the baited trap recipient; (**b**) Counting of insects captured in each trap with their respective semiochemical using an automatic counter and stereoscopic magnifying glass, evaluated in the field at the Entomology Laboratory of Embrapa Rondonia.


(a) (b)

**Fig 3. (a)** Trap with different semiochemicals for evaluation of synergic effect during the preliminary experiment. (1) ethylene (ETL); (2) ethanol:methanol (ET:MT, 1:1) and (3) ethyl acetate (EA); **(b)** Trap installed in field at 1.2 m high.

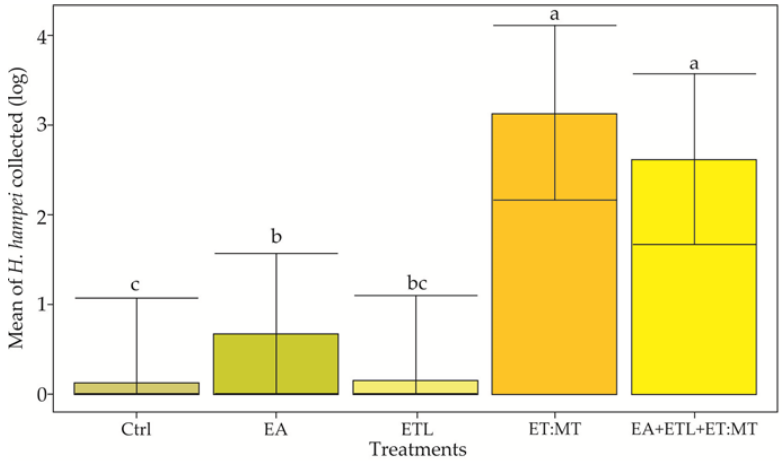


**Fig 4**. Mean number (log) of collected *H. hampei* using the different attractants in the coffee plantation during a preliminary evaluation in April/2015. Control (Ctrl), ethyl acetate (EA), ethylene (ETL), ethanol:methanol (ET:MT, 1:1) and EA+ETL+ET:MT. The averages in the columns followed by the same letter did not differ significantly (p > 0.05).

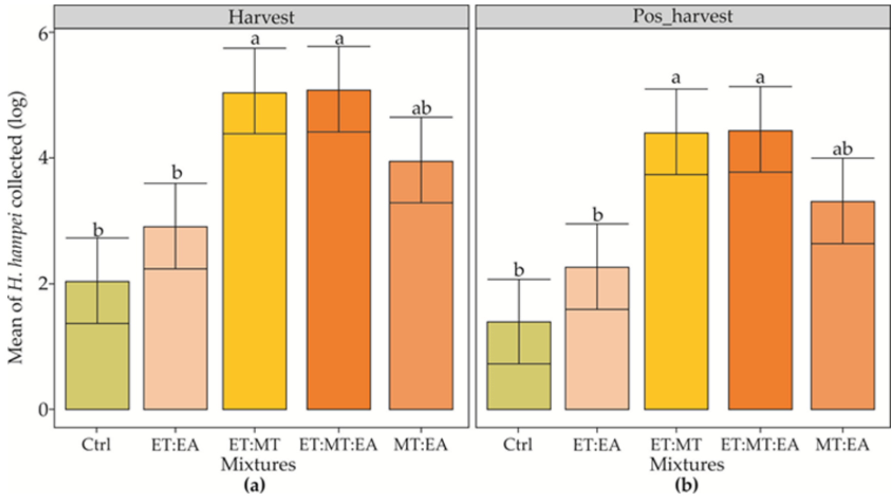


**Fig 5**. Mean number (log) of collected *H. hampei* using ethyl acetate (EA) combined with ethanol (ET) and methanol (MT). (**a**) *H. hampei* collected over 10 weeks during the insect's high population period (Harvest, May to July/2015), in the traps with different mixtures (1:1) of EA with ET and MT ± SE; (**b**) *H. hampei* captured over 10 weeks during the insect's low population period (Post-harvest, July to October/2015), in traps with different blends (1:1) of EA with ET and MT ± SE.

baited traps and improve monitoring or control tools used for this important insect.

**Materials and Methods**

***Experimental area***

Experiments were conducted in the Experimental Field of the Brazilian Agricultural Research Corporation (Embrapa Rondonia) located in the municipality of Porto Velho, Brazil, at latitude 8°48'01.47"S and longitude 63°51'03.61"W.

The climate of the region was Am by classification of Köppen (rainy tropical) with rainy summer (October to May) and dry winter (June to September). According to the weather conditions, average monthly temperatures range from 30ºC in summer to 17ºC in winter. The average annual precipitation is 2,200 mm, with rainy season from October to May and the dry season from June to September.

A coffee field of *Coffea canephora* 'Conilon - BRS OuroPreto' variety was used in full production. The tillage was planted on December 20, 2008. The plants were distributed in the area in single rows, spacing 2.0 m (between plants) by 3.0 m between rows, resulting in 1,666 plants per hectare. The plants were pruned for production in July 2013, and from the emergence of new shoots, five (5) stems were maintained per plant. The coffee trees were cultivated in dry conditions, without irrigation, and the fertilizations were carried out following the technical recommendations for the culture in the region (Marcolan et al 2009). The experimental area was cultivated without the use of agrochemicals, and comprised 16 rows with approximately 640 plants. Edging plants were excluded from the study (Fig 1).

***Traps and experimental design***

Impact traps made with two-liter polyethylene (PET) bottles with a 12x9 cm rectangular side opening. The traps were painted red on the inside and outside. Glass vials (10 ml) with rubber caps were used as attractant diffusers. Seven milliliters of the evaluated compounds were placed in each diffusor. The rubber caps were drilled in order to insert metal straws with 3.8 mm diameter openings for the volatiles to exit the vials. Vials containing attractants were replaced weekly after each collection and the traps were rotated clockwise (Fig 1).

After impact on the trap wall, the insects fell into the collection recipient which was filled with water mixed with 10% ethylene glycol (J.T.Baker ®) and 1% neutral detergent, where they died by drowning. Samples were collected weekly using filter paper (Fig 2a). After collection, the insects were properly separated by treatments, packaged and taken to the laboratory for sorting and counting (Fig 2b).

Traps distribution in the blocks followed a completely randomized experimental design. Five traps were installed by blocks with their proper treatments. Each block (plant row) represented a repetition of each evaluated treatment and separated by three plant rows. This way, a total of 30 (6x5) equidistant (15x15m) traps were distributed throughout the six blocks (Fig 1). For each block, traps were installed between the plants at a height of 1.2 meters (Fig 3b) with a distance of 15 meters to avoid trap overlap effect as outlined by previous studies (Dufour and Frérot, 2008; Shou-An and Shue-Jie, 2013).

***The attractants: treatments***

ETL ($C_2H_4$) production and volatilization from the diffusers resulted from the mix between the phytoregulator etephon, 2-chloroethylphosphonic acid (Ethrel® 240) and distilled water plus sodium hydroxide (NaOH) according to the method described through a technical communication from Embrapa Instrumentation (Calbo et al., 2010).

The sources of the remaining compounds, i.e., ET, MT, and EA were $CH_3CH_2OH$ (99.8% P.A.), $CH_3OH$ (99.5% P.A.) [QuímicaModerna®], and $CH_3COO_2H5$ (99.5% P.A.) [Neon®], respectively. Experiments with combined attractants and possible attractants were performed using a 1:1 ratio.

***Preliminary test of ethylene (ETL) and ethyl acetate (EA)***

During the pre-harvest season (April/2015) the *H. hampei* response to synthetic ETL and EA was previously observed. For these experiments, traps with pure and combinations of compounds were used. Five (05) treatments were defined: [i] Control = distilled water (Ctrl); [ii] Ethyl acetate (EA); [iii] Ethylene (ETL); [iv] Ethanol and Methanol = (ET:MT, 1:1) and [v] EA + ETL + (ET:MT, 1:1), i.e., all attractants placed in the same trap using individual vials for each compound, except for ET:MT, 1:1 (Fig. 3a).

***Combining a selected attractant (SA) with ethanol (ET) and methanol (MT)***

An attractive compound was selected based on the result obtained in the previous evaluation with ETL and EA, i.e., SA. The experiments were performed during ten weeks defined during the [i] harvest (May to early July) and [ii] post-harvest periods (late July to October) to correlate with the high and low population densities of the pest, respectively. The following treatments were delimited for this experimental stage with the SA: [i] Control; [ii] ET:SA; [iii] ET:MT; [iv] ET:MT:SA and [v] MT:SA.

The ratios of all the treatment mixtures were 1:1, i.e., 3.5ml of each attractant was mixed in a single diffuser, totaling 7ml of mixture in each vial (ET:EA or MT:EA). In the treatment with the ternary combination (ET:MT:EA), 3ml of each attractant was placed in the same diffuser vial, totaling 9 ml of mixture in the vial. In the eventual selection of ETL, was remained in a single diffuser to evaluate the different combinations with ET and MT, i.e., two diffusers would be used for traps.

***Statistical analyses***

Mean number of collected *H. hampei* was transformed using log (xi +1). The transformed data were fitted to linear mixed effect models using the normal probability distribution and checked through residue analysis. Later, the Tukey HSD posteriori test (El Keroumi et al., 2012) was used for pairwise comparison between the different combinations of attractants. All analyses were performed using the free statistical software R (R Development Core Team 2015).

**Conclusion**

This is the first report of using ETL and EA in bait traps for *H. hampei* in coffee fields. Among the two possible attractive compounds evaluated, i.e., ETL and EA, only EA attracted significantly more *H. hampei* compared with unbaited traps (Ctrl). Despite that, different combinations of EA were tested in the field. The alcohols ET and MT at a ratio of (1:1) did not increase the efficiency of these attractants. New experiments using different ratios of EA to the alcohols are needed to elucidate its role in *H. hampei* attraction.

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