



Ethylene as a synomone to ants and wasps (Insecta: Hymenoptera) at a *Coffea canephora* (Pierre) plantation

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Abstract. Ethylene is a volatile phytohormone that plays an important role in the physiological processes of coffee plants. However, the role of this compound as a semiochemical in the tritrophic interaction involving *Hypothenemus hampei*, *Coffea canephora*, and hymenopteran insects remains unknown. Ethylene-baited traps were used in a coffee plantation in the experimental field of Embrapa Rondônia in the municipality of Porto Velho, Rondônia state, Brazil. The experiment was conducted during the fruit maturation period (March and April). Ethylene did not attract *H. hampei* compared to control traps (distilled water) but was significantly attractive to hymenopterans suggesting that it acts as a synomone.

Keywords: Coffee berry borer; Integrated Management; Natural enemies; Semiochemical.

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The population fluctuation of *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae) depends on the physiology of its host, i.e., plants of the genus *Coffea* (BOTELHO *et al.* 2021). A study reported that the populations of *H. hampei* and the parasitoid *Cephalonomia stephanoderis* Betrem (Hymenoptera: Bethyridae) increased significantly at the beginning of the fruit maturation period (between March and April) (DE SOUZA *et al.* 2014).

Ethylene plays an important role in physiological and phenological processes, including flowering and fruit ripening (PEREIRA *et al.* 2005; SÁGIO *et al.* 2013; SÁGIO *et al.* 2014). The highest production of ethylene is achieved during flowering and at the initiation of maturation of coffee fruits (PEREIRA *et al.* 2005). In this phenological phase of coffee development, the female colonizers of *H. hampei* increase their flight activity in search of new fruits to bore and deposit eggs. In this context, it is known that the phytohormone ethylene acts as a semiochemical in the insect-plant interaction because many insect species use this compound as a signal for finding their hosts or prey (GROEN & WHITEMAN 2014). In addition to being synthesized by the plant to perform physiological functions, ethylene is used as a defense against the attack of herbivorous insects and pathogenic microorganisms (BROEKGAARDEN *et al.* 2015).

Despite the lack of information about the role of ethylene in the olfactory behavior of the coffee berry borer, it is known that this compound attracts some species of Coleoptera of the Curculionidae family (CAMPOS *et al.* 1994; GROEN & WHITEMAN 2014). Moreover, modern integrated pest management tactics use ethylene for the attraction and maintenance of natural enemies of pests in and around plantations (RODRIGUES-SAONA *et al.* 2012). Among the broad range of insect orders that are natural enemies of pests, Hymenoptera is important for the control of pest insect populations (PEREIRA *et al.* 2015). Species of the order Hymenoptera belonging to the families Bethyridae, Braconidae, Eulophidae, and Formicidae are used in the biological control of *H. hampei* (BUSTILLO *et al.* 2002). Studies regarding the richness of ant species in coffee plantations highlighted the importance of the quality of crop management for the maintenance and preservation of these insect species in plantations and reported the dominance of species of the genus *Solenopsis*, the generalist feeding habits of which enable them to prey on *H. hampei* (DIAS *et al.* 2008).

Ethylene-baited traps used for the capture of *H. hampei* were evaluated during the period of coffee fruit maturation (*Coffea canephora*) in the experimental field of Embrapa Rondônia located in the municipality of Porto Velho, Rondônia state, Brazil. The traps were analyzed in a pesticide-free plot wherein 16 rows were planted with approximately 500 plants spaced at 3.0 and 2.0 m. Impact traps made from 2 L polyethylene bottles with a lateral rectangular opening measuring 12 and 9 cm were used in the experiments. Glass vials (10 mL) with rubber stoppers were used as diffusers, and a small hole was made in each vial for the insertion of metal straws with a diameter of 3.8 mm, through which the volatile compounds were released. Overall, 7 mL of a mixture containing the phytohormone ethephon (2-chloroethylphosphonic acid; Ethrel® 240), sodium hydroxide (NaOH), and distilled water was added to each diffuser for the production and volatilization of ethylene, according to the method described by CALBO *et al.* (2010). A mixture of ethanol

and methanol (Química Moderna®) at a ratio of 1:1 and concentration of 99.8% and 99.5%, respectively, was used as a control.

The traps were equidistantly distributed in six blocks (row of plants) following a completely randomized experimental design. Three traps with their respective treatment compounds were installed per block: ethylene, ethanol-methanol, and distilled water (control). Each block represented a repetition of each evaluated treatment. Each trap was installed between the plants at a height of 1.2 m and a distance of approximately 20 m between the traps, according to the methodological design of DUFOUR & FRÉROT (2008).

The insect collection containers of the traps were filled with water, detergent (1%), and ethylene glycol (J. T. Baker®) (10%), and the insects that were caught in the containers were collected weekly. Following the collection, the insects were transported to the laboratory for sorting and counting and separated through treatment. The traps were rotated within each block after the weekly collection procedure for sample homogenization. Collections were conducted for 9 weeks, from March 6 to May 8, 2015. The data were square-root transformed (SQRT) and subjected to analysis of variance and the Scott-Knott mean test ($p < 0.05$). For population-level analysis, the data underwent a logarithmic transformation by determining the regression of $\log Y$ and $\log X$ using the least squares method.

The average number of insects captured during the study period demonstrated that ethylene did not attract *H. hampei* compared to control traps (distilled water) but was determinately attractive to hymenopterans (Figure 1). The capture of insects of the order Hymenoptera attracted by ethylene was importantly increased when the fruits began to ripen and began to actually decrease only the sixth week onward (Figure 2A). In contrast, during the same period, only a few specimens of *H. hampei* were caught in the ethylene-baited traps. However, from the sixth week onward, some coffee berry borers were caught in the ethylene-baited traps (Figure 2A). In this period, the evaluation of the ethanol-methanol-baited traps confirmed the presence of *H. hampei* in the crop because their capture was increased throughout the sampling period, including the fruit maturation period, indicating the presence of colonizing females in search of suitable fruits (Figure 2B). This capture pattern was not observed for hymenopteran insects with alcohol-baited traps (Figure 2B). We also observed a decrease in the capture of hymenopterans in the alcohol-baited traps from the sixth week onward (Figure 2B).

Our results indicate that ethylene can attract insects considered natural enemies of *H. hampei*. The relatively large number of insects that were caught at the beginning of the fruit maturation period using ethylene baits may be due to the strategy developed by this species to use this volatile compound as a synomone for seeking and finding hosts or prey. The decrease in the number of insects captured from the sixth week onward can be explained by the absence of *H. hampei* in the fruits because the fruits were not suitable for the deposition of eggs by colonizer females. However, the damage caused by plant-eating insects induces the synthesis of more ethylene by the plants (BROEKGAARDEN et al. 2015). For this reason, during the fruit maturation period, the population density of hymenopteran insects may increase in this environment for two reasons: a) the production of ethylene by the plant due to the attack by *H. hampei* and b) the presence of many host fruits suitable for colonization.

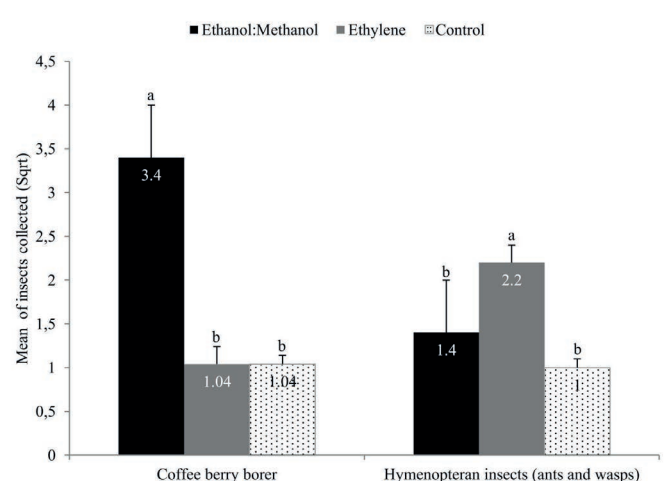


Figure 1. Average number of coffee berry borers caught in the baited traps containing ethanol:methanol, ethylene, or distilled water (Control) during the fruit maturation of *Coffea canephora*. The data refer to collections conducted at Embrapa experimental farm in Porto Velho Rondônia. Data transformed into the square root of the $Y + 1.0 - \text{SQRT}(Y + 1.0)$. Averages followed by different letters indicate statistically significant differences using the Scott-Knott mean test ($p < 0.05$).

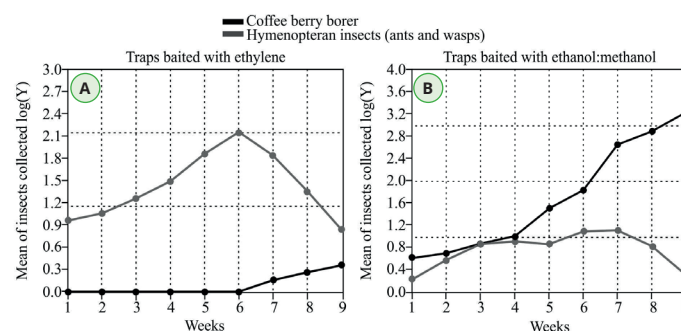


Figure 2. (A): Average number of coffee berry borers and hymenopteran insects collected during a nine-week follow-up in ethylene-baited traps (A) and in ethanol-methanol-baited traps. The collections occurred during the period of fruit maturation (March to May) in coffee (*Coffea canephora*) plantations at the experimental field of Embrapa in Porto Velho, Rondônia.

The results also suggest a repellency effect of ethylene on the *H. hampei* population and that this phytohormone is used by several plant species as a repellent (GROEN & WHITEMAN 2014). This characteristic may be a consequence of the strategy of these insects to avoid encounters with their natural enemies. Therefore, the excess water in coffee fruits during plant maturation may not be the only factor that prevented the boring of fruits by *H. hampei* at this phenological stage (DE SOUZA et al. 2015). Ethylene in the fruits may function as a signal for the pests to leave the fruit until it is more suitable and the insects are less vulnerable to attack by natural enemies.

Although ethylene is an important semiochemical in interactions that involve more than one trophic level in the environment, this study is the first to evaluate the role of ethylene in the behavior of *H. hampei* and its natural enemies. Our results could serve as the foundation for future studies on this compound and the chemical ecology of this pest. In this context, *H. hampei* may have elicitors that trigger a metabolic response in the plant for the production of ethylene.

The results presented here suggest that ethylene is involved in the foraging behavior of the natural enemies of the coffee berry borer. This study was conducted to test the hypothesis that ethylene functions as a kairomone for pest insects. However, our results indicated that it is more likely that this compound served as a synomone to ants, wasps, and C.

canephora.

The complex trophic interactions between *H. hampei*, its natural enemies, and coffee plants still need to be understood. Therefore, additional studies on the role of ethylene in the integrated management of *H. hampei* are necessary. Moreover, chemical, phenological, and ecological studies are essential to clarify the behavior of insects of economic importance to coffee production.

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