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Commercial extract of coffee (*Coffea arabica* L.) and mango essence as potential attractants for *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)

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Abstract. Various host compounds have been investigated to produce an alternative attractant for monitoring and controlling pest insects. Several studies evidenced that volatile molecules released by coffee and mangoes attracted both male and female *Ceratitis capitata* (Wiedemann), a significant pest in fruit crops. In this study, we tested the effectiveness of the commercial glycolic extract of coffee (*Coffea arabica* L.) and a commercial mango essence (*Mangifera indica* L.) to attract *C. capitata*. We identified the main volatile compounds (VOCs) emitted from such products. The experiments were conducted in field cages and showed that the glycolic extract of coffee attracts both sexes of *C. capitata*, while the mango essence attracts only males. After seven days, the pH of the coffee extract and mango essence did not change. These results indicate that attractants based on commercial fruit extracts and essences can be an option for integrated pest management of the Mediterranean fruit fly in orchards.

Keywords: Mediterranean fruit fly; medfly; pests; semiochemicals; Trypetinae.

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Currently, Brazil ranks third in fruit-producing countries, producing 44.3 million tons of fresh fruits in 2020 (Bornal *et al*. 2021; Kist *et al*. 2021). However, in terms of exports, the country occupies only the 27th position (Bornal *et al.* 2021). The presence of fruit flies and the number of pesticide residues is one of the most significant impasses in expanding the export market for fresh fruits (Malavasi & Zucchi 2000; Carvalho *et al*. 2019).

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is one of the most important pests of fruit production causing severe damage to fruits due to their biological cycle (Malavasi & Zucchi 2000). These insects attack a wide range of host plants, and in Brazil, there are records of infestation in 115 plant species in 31 plant families. The highest number of hosts of this fly are Myrtaceae (25 species) and Rutaceae (20 species) (Zucchi & Moraes 2022).

Monitoring fruit flies in agrosystems to evaluate their population level and detect the presence of exotic and quarantine species allows the implementation of control measures, contributing to the success of integrated pest management (Kogan 1988). Traps associated with attractant compounds are essential in control programs to detect, suppress, and eradicate pests (Drew & Yuval 1999; Carvalho 2005; Alves *et al*. 2014; Goldshtein *et al*. 2017).

The commercial attractant compounds used to monitor *C. capitata* are protein hydrolysates and pheromones (specific for males). Unfortunately, these compounds are expensive and not reasonably available for small farmers. Thus, alternatives, such as molasses, fermented products, and brewer's yeast, have been tested in the field (Lemos *et al*. 2002; Carvalho 2005; Candia *et al*. 2018; Santana *et al*. 2019).

Studies have suggested that odor from coffee (*Coffea arabica* L.) fruits and leaves are potential attractants for male and female of *C. capitata* at different ages and physiological states (Vargas *et al*. 1995; Prokopy & Vargas 1996; Prokopy *et al*. 1997; Warthen *et al.* 1997; Prokopy *et al.* 1998). Additionally, volatile molecules, such as esters and terpenes, released by mangoes activated behavioral and electrophysiological responses in both sexes of *C. capitata*, which indicates that attractants can be developed using these compounds (Cossé *et al.* 1995; Hernández-Sánchez *et al.* 2001).

Natural plant extracts contain different molecules produced and released by plants, including fruit semiochemicals that attract fruit flies (Prokopy & Vargas 1996; Prokopy *et al.* 1997; Dubey 2011; Pavela 2016). Essences are artificial products that simulate the smell and

taste of fruits and other vegetables (Schultz *et al.* 1967). The association of food attractants with host plant compounds can improve the attractiveness of fruit flies. For example, ANAMED® (ISCA) is a fruit fly attractant with fruit plant extracts and feeding stimulants in the composition (Borges *et al.* 2021).

Due to the importance of plant volatile compounds in the plant-insect interaction (Aluja & Prokopy 1992; Miyazaki *et al*. 2018), the use of extract and essence from host plants can be used alone or in association with other compounds in traps for the monitoring and control of adults of the Mediterranean fruit fly. In this paper, we evaluated the potential of commercial coffee extract and mango essence to capture males and females of *C. capitata* in a field cage bioassay and characterized the volatile compounds from both products.

MATERIAL AND METHODS

Insects. The experiments were conducted using the medfly colony maintained (for more than 20 years) at the Laboratório de Ecologia Comportamental de Insetos from Instituto de Biologia, Universidade Federal da Bahia (Salvador, Brazil) (13º00'04" S, 38º30'29" W), using methodologies adapted from the entomology laboratories of Food and Agriculture Organization - FAO/IAEA (Seibersdorf, Austria) and Centro de Energia Nuclear na Agricultura - CENA/USP (Vera *et al.* 2006; Mastrangelo *et al.* 2021). The flies were held in cages (32 × 21 × 19 cm) and provided with water *ad libitum* and a diet based on hydrolyzed protein and sugar (1:3), following Silva-Neto *et al*. (2012). The eggs laid by the medfly females in screen cage were collected periodically and transferred to Petri dishes containing a yeast-based artificial diet (Carvalho *et al*. 1998). We kept the Petri dishes with larvae in plastic trays (40 × 25 × 6 cm) containing vermiculite. After seven days, the pupae were collected, and the adults emerged after 7-8 days. Cages were maintained at a constant temperature (25 ± 1 °C) and relative humidity (80 ± 10%) under 12 h light: dark photoperiod. Illumination was provided by fluorescent lamps at approximately 2000 lux.

Attractive Compounds. The undiluted attractive compounds studied were the commercial Glycolic Extract of Coffee, *Coffea arabica* L. (Peter Paiva - Exclusividades Artesanais) and a commercial mango essence (Bio Tae Ind. e Com. de Insumos Farmacêuticos, Cosméticos e Alimentícios Ltda ME). Water was used as negative control, and commercial hydrolyzed protein 5% as positive control.

Field Cage Tests

Glycolic Extract of Coffee. The experiments were conducted at the external area of the Instituto de Biologia at the Universidade Federal da Bahia (Salvador, Brazil). Fifty sexually mature medfly couples (5-7 days old) were released in a rectangular field cage (2.30 × 1.50 × 2.30 m) with one potted of *Eugenia uniflora* L. (Myrtaceae) tree (30 cm) in the center of the cage to simulate natural conditions (adapted from Rousse *et al*. 2005). All experiments were carried out in the morning, starting at 8-9 am.

The first set of experiments compared the attraction of glycolic extract of coffee (100%) against water (control) and the second one compared hydrolyzed protein 5% (Bio Controle Ltda.) against the coffee extract. In both sets of experiments, two McPhail traps filled with 150 mL of each treatment were placed inside the cage. Five independent replications for each experiment were conducted.

Each solution's potential to capture medflies was evaluated based on the number of insects captured by each treatment. Captured flies were removed from the trap and counted at 8, 24, and 48 hours after the beginning of the experiment to avoid possible odor modification caused by the decomposition of dead insects, following the methodology of Rousse *et al*. (2005).

Mango Essence. We followed the same methodology used for the coffee extract tests to evaluate the attraction of *C. capitata* (males and females) to the mango essence. In this experiment, three McPhail traps were placed inside the cage, and Mango essence was tested simultaneously against water and hydrolyzed protein 5%.

Effect of time on the pH of the attractants. An experiment was conducted to investigate if the pH of the extract of coffee and mango essence changes after seven days. In monitoring programs for fruit flies, the attractants compounds are kept for seven days in the field (Dias *et al*. 2018), and pH alterations can affect the attractiveness of some fruit fly species (Heath *et al*. 1994, 2009). The pH of five microliters of each compound and the control (hydrolyzed protein 5%) were compared at zero and seven days under semi-field conditions. The measurements were conducted using the pH meter Tecnal TEC-53.

Characterization of Volatile Organic Compounds (VOCs)

Extraction of VOCs. To extract the volatile compounds of extract of coffee, mango essence, and hydrolyzed protein, we used a solid-phase microextraction technique in headspace mode (HS-SPME) with a manual sampler. A quantity of 10 mL of the sample was put in a sealed 20 mL glass vial, and the extraction was performed by placing the vial into an aluminum heating block (4 cm in height by 14 cm in diameter) on a temperature-controlled heating plate at 80 °C and 300 rpm agitation. The volatile organic compounds (VOCs) were extracted using fiber Carboxen/PDMS of 75 µm (Supelco, Bellefonte, USA) (adapted from Mesquita *et al*. 2018) previously conditioned according to the manufacturer's indication. After the extraction period (20 min), the fiber was inserted into the gas chromatograph injector for 3 min at 250 °C for desorption of the VOCs.

GC-MS Analysis. The volatile compounds in the samples were detected using a gas chromatograph coupled to a mass spectrometer (GCMSQP2010 Plus model, Shimadzu, Japan) equipped with a split/splitless injector in the splitless mode and at 250 °C during the chromatographic run. VOCs were separated in a capillary column [Rxi-5 MS - (5%-pheny) - methylpolysiloxane; 30 m × 0.25 mm ID × 0.25 µm - Restek, Bellefonte, USA] using helium (99.99%) as carrier gas at a 0.60 mL min^{-1} flow rate. The oven temperature was varied as follows: 40 °C (hold 10 min), then warmed to 50 °C at 0.5 °C min^{-1} (hold 2 min), then 5 °C min^{-1} to 100 °C (hold 2 min), then 8 °C min^{-1} to 175 °C, then 30 °C min^{-1} to 280 °C (hold 2 min), with a total time of 59.88 min. The mass detector conditions were: transfer line temperature of 230 °C, ion source temperature of 230 °C, and ionization mode with electron impact at 70 eV.

VOCs were identified by (1) comparison of the mass spectrum of the compounds with the data system library (NIST 147 Database), and (2) Kovats retention index (KI): values were determined using a homologous series of n-alkanes C_8-C_{40} and the values were compared with values reported in the literature for similar chromatographic columns.

Statistical Analysis

Field Cage Tests. A generalized linear mixed model (Poisson distribution, using log as link function) was used to evaluate the attraction of *C. capitata* (males and females) to the coffee

extract (100%), considering the attractants and sex as fixed factors and block (cohort) as a random factor. A generalized linear model with Poisson distribution (log as link function) was used for experiments with mango essence. The significance of the effect of the fixed factors on the number of captured flies was provided by a Type III Wald Chi-squares or F test (α = 0.05). The pairwise comparisons were made with the Tukey test (α = 0.05). All statistical analyzes were performed with the software R Studio version 1.3.959 with the packages *lme4, car, multcompview,* and *lsmeans* (Bates *et al*. 2015; Fox *et al*. 2012; Graves *et al*. 2019; Lenth 2018).

Effect of attractant age on pH. The presumptions of normality and homoscedasticity were checked with the Bartlett test and Shapiro-Wilk. The pH of the attractants at zero and seven days was compared with a two-way Analysis of Variance (ANOVA) (α = 0.05). The pairwise comparisons were performed using the Tukey test (α = 0.05).

RESULTS

Raw data from field cage and pH tests are available at the link: https://doi.org/10.17632/dxk3246zvh.1

Field Cage Tests

Glycolic Extract of Coffee. The statistical analysis of the tests between water (control) and the coffee extract found a significant effect for the attractive factor (χ^2 = 266.1287; *P*<0.001) and sex (χ^2 = 5.3953, *P*=0.02019) but not for the interaction (χ^2= 0.2397, *P*=0.6244). For all comparisons, the degrees of freedom were 1. In this experiment, the traps filled with the coffee extract captured more males (Tukey Test, *P*<0.05) and females (Tukey Test, *P*<0.05) than the McPhail traps filled with water (Figure 1A).

In the experiments comparing the attractiveness between the coffee extract and hydrolyzed protein, we found significant difference for the attractive factor (χ^2 = 35.0565; *P*<0.001), sex (χ^2 = 3.6901; *P*=0.05) and the interaction between them (χ^2 = 35.5411; *P*<0.001). For all comparisons, the degrees of freedom were 1. The analysis showed that the coffee extract captured more males and less females than the hydrolyzed protein (Tukey Test, P<0.05) (Figure 1B).

Mango Essence. The generalized linear mixed model showed a significant effect of the attractive factor (*F* = 24.545; *P*<0.001), interaction (*F* = 72.8583; *P*<0.001), but not for the sex factor (*F* = 2.8074; *P*=0.104). For all comparisons, the degrees of freedom were 2. Mango essence captured more males than water and hydrolyzed protein (Tukey Test, p<0.05), but less females than water and hydrolyzed protein (Tukey Test, p<0.05) (Figure 2).

Effect of time on the pH of the attractants. The pH was significantly affected by time (F = 7.932; df = 1; *P* = 0.009). The pH was also different among the attractants (F = 509.150; df = 2; *P*<0.001). Moreover, ANOVA showed that the interaction between time and attractant was significant (F = 17.327; df = 2; *P*<0.001).

The pH of the hydrolyzed protein decreased after seven days (Tukey test, *P*<0.05), while in the mango essence and coffee extract, the pH did not change after seven days (Tukey test, *P*>0.05) (Figure 3).

Characterization of Volatile Organic Compounds (VOCs). The VOCs in the samples were determined by the HS-SPME/GC-MS technique, which allowed the identification of 16 compounds (Table 1).

DISCUSSION

The data indicate that the coffee extract and mango essence attract *C. capitata* males compared to water and the hydrolyzed protein. The coffee extract had a higher capacity to attract females than the water, unlike the mango essence, which attracted fewer females than the controls.

The efficacy of the coffee extract to attract male and female *C. capitata*, as observed in this study, could be due to the attractiveness of this species to the coffee odor because it is one of the primary hosts of this species (Liquido *et al*. 1990; Flores *et al*. 2016). Prokopy & Vargas (1996) evidenced that *C. capitata* males and females were more attracted by the odor of intact and macerated ripped fruits of coffee than the other five host fruits. Other parts of the coffee plant (Prokopy *et al*. 1997) and several volatile compounds extracted from coffee grains also attracted *C. capitata* males and females (Warthen *et al*. 1997; Prokopy *et al*. 1998).

The coffee extract was compared with the hydrolyzed protein, the commercial product commonly used for traps in orchards. The most significant number of females captured by the

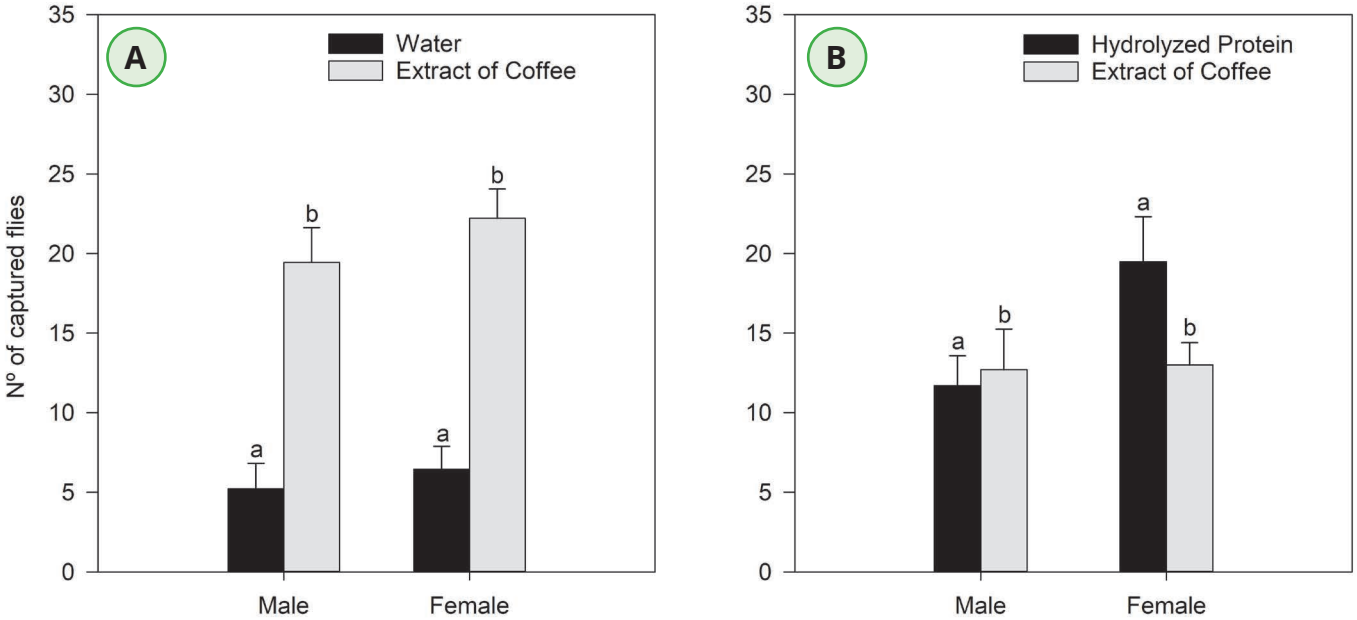

Figure 1. Mean (±SE) of males and females of *C. capitata* captured by each treatment. Different letters above bars from the same-sex indicate statistical differences (Tukey test, p<0.05). (A) Glycolic Extract of Coffee 100% vs. Water; (B) Glycolic Extract of Coffee 100% vs. Hydrolyzed Protein 5%.

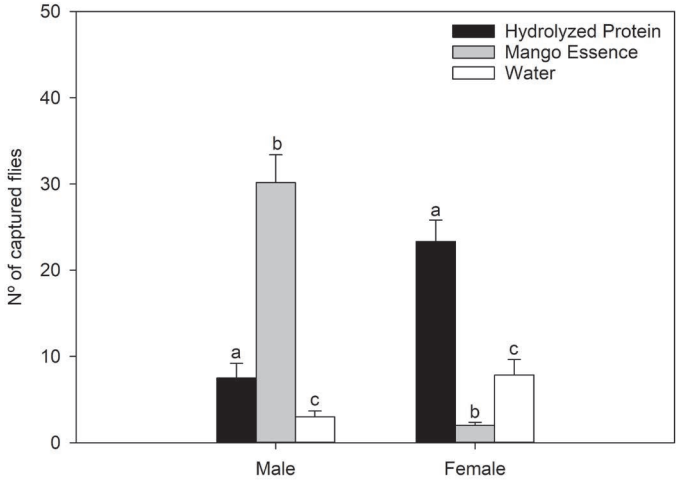

Figure 2. Mean (±SE) of males and females of *C. capitata* captured by McPhail traps filled with Hydrolyzed Protein 5%, Water, and Mango Essence 100%. Different letters above bars from the same-sex indicate statistical difference (Tukey test, p<0.05).

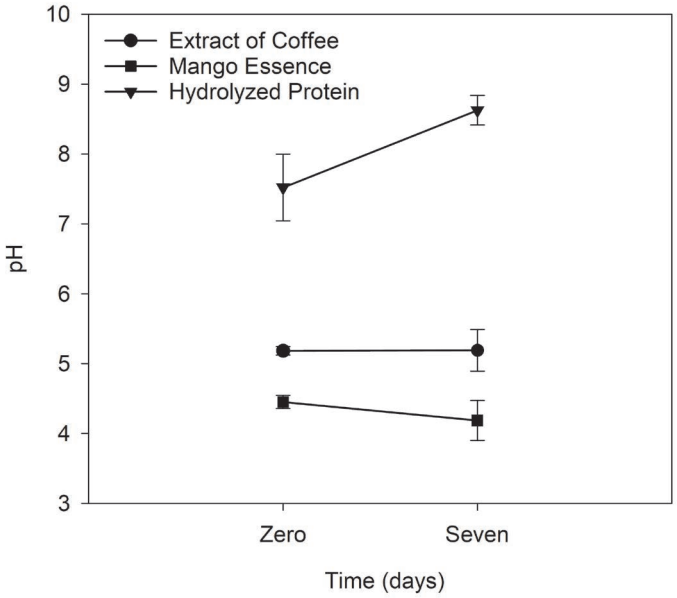

Figure 3. Mean (±SE) of the potential of hydrogen (pH) of the Mango Essence, Glycolic Extract of Coffee, and Hydrolyzed Protein at zero and seven days.

Table 1. Volatile compounds from mango essence, commercial coffee extract, and hydrolyzed protein by HS-SPME/GC-MS.

Compound	IK_{exp}	IK_{lit}	Mango essence	Coffee extract	Hydrolyzed Protein
2,3 butanediol	804	803	-	-	+
(Z) 3-hexen-1-ol	846	846	+	-	-
(E) 3-hexen-1-ol	856	856	+	-	-
2-furanmethanol	858	858	-	+	+
styrene	885	886	+	-	-
α-pinene	924	924	+	-	-
benzaldehyde	956	956	-	+	+
2-furancarboxaldehyde 5 methyl	963	963	-	+	-
β-pinene	972	972	+	-	-
β-myrcene	988	988	+	-	-
benzene 1,2 - dimethoxy	1148	1149	-	+	-
p-ethylguaiacol	1281	1280	-	+	-
bornyl acetate	1287	1287	-	+	-
eugenol	1361	1361	-	+	-
β-ionone	1491	1491	+	-	-
γ-undecalactone	1585	1583	+	-	-

protein hydrolysate traps corroborates the data obtained by Parra *et al*. (1982). Protein-based chemical attractants attract females because these insects need to consume protein to develop the ovary and thus achieve sexual maturation (Heath *et al*. 1994; Cornelius *et al*. 2000a).

Although the mango essence attracted males, it was not attractive to females, capturing a smaller amount than the water, which can be explained by the absence of proteins, sugars, and lipids (Lund & Bryan 1977). Those molecules are used by insects for multiple physiological activities (Silva-Neto *et al*. 2012). Although this essence did not attract females, two VOCs from mango (p-cymene and limonene) efficiently attract *C. capitata* males and females (Hernández-Sánchez *et al*. 2001).

Their searching behavior can explain the male attraction to the coffee extract and the mango essence. The odors from host indicate the location of host plants, where males can exhibit lek behavior for mating (Prokopy & Vargas 1996). In other species of fruit flies, the fruit juice odor was more effective in attracting males than females (Herrera *et al*. 2016). In addition, to aspects of signaling to host plants, evidence exists of positive effects of host plant-derived compounds on the successful mating of male fruit flies (Papadopoulos *et al*. 2001).

Sixteen different VOCs were identified (Table 1). The compounds 2-furamethanol and benzaldehyde were found in food attractant (hydrolyzed protein) and the coffee extract. Both compounds have already been reported in protein hydrolysates, and benzaldehyde is also found in coffee fruits (Buttery *et al.* 1983; Warthen *et al*. 1997; Mesquita *et al*. 2018). Among the compounds identified in mango essence (Table 1), α-pinene, β-pinene, and β-myrcene have already been found in different mango varieties (Mesquita *et al.* 2020). Furthermore, other studies showed that (Z) 3-hexen-1-ol and (E) 3-hexen-1-ol, also present in mango essence, caused electrophysiological responses in *C. capitata* (Light *et al*. 1988; Cossé *et al*. 1995).

We observed many males captured by coffee extract and mango essence. This result demonstrates that these compounds can be used in monitoring *C. capitata* resealed for genetic control (*i.e.*, Sterile Insect Technique), making it possible to monitor their dispersion and longevity (McInnis *et al*. 1994).

Male-specific attractants can be used as part of the mass capture technique of these insects in the field. These compounds, usually synthetic, need to reach a high number of captured insects to be used in this control technique (El-Sayed *et al*. 2006). Among these control technologies, the male annihilation technique uses the principle of mass capture to eradicate these pests in orchards by reducing the male population (Christenson 1963). In this context, methyl eugenol is an example of specific attractants used for males, which works efficiently for the technique of annihilating males of several species of *Bactrocera* Macquart and *Ceratitis* (Singh 2019). In a study conducted in southeastern Ethiopia, the mass capture techniques significantly reduced the natural population of fruit flies. A few months after the mass capture of insects, a reduction to less than half of the fruit infestation rates was observed (Ballo *et al.* 2020). Field experiments must be conducted to determine if commercial coffee extracts and mango essence are efficient in natural conditions.

In addition, the coffee extract was also efficient in capturing *C. capitata* females. This result makes it a comprehensive option for agricultural management of the Mediterranean fruit fly in orchards affected by this agricultural pest. However, more studies must investigate if the coffee extract is efficient after diluting since it's not economically viable to use it in 100% concentration.

Our results showed that mango essence has a high potential for attracting *C. capitata* males, and the extract of coffee captures both sexes. Therefore, these attractants products can be helpful in monitoring and control populations of *C. capitata* in the field. However, tests to evaluate the effect of dilution in the attraction of male in orchards may further elucidate the applicability of this attractant to monitoring and controlling *C. capitata*, as well as the cost-benefit relation of these attractants.

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