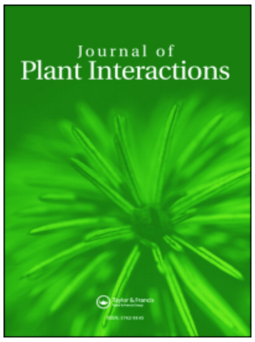

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RESEARCH ARTICLE

# Identification of two monoterpenes and their coding genes in *Coffea arabica* L. associated with the attraction of the coffee berry borer (*Hypothenemus hampei*)

Paula A. Figueroa-Varela[a,b], Claudia P. Martínez[c], Johanna Tapias[c], Aristofeles Ortiz[d], Beatriz E. Mira-Rada[e], Diego Villanueva-Mejía[b] and Carmenza E. Góngora[a]

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**ABSTRACT**

The coffee berry borer (CBB) is the most significant pest affecting coffee in Colombia and, globally, attracted primarily to volatiles from ripe fruits. To identify volatiles involved in host recognition, we conducted olfactometry assays using coffee fruits at different ripening stages and synthetic mixtures. A blend of limonene (25 ppm) and pinene (200 ppm) was significantly more attractive to CBB than ripe fruits or a methanol-ethanol (3:1) lure. GC–MS confirmed both compounds in the coffee fruits, with limonene being the most abundant, especially in the ripe fruits. We identified and analyzed the terpene synthase genes responsible for limonene and pinene biosynthesis in *Coffea arabica*. qPCR showed these genes are exclusively expressed in fruits, with limonene synthase expression 100% higher in ripe fruits, which is correlated with peak CBB attraction. Pinene synthase also varied across ripening stages. These findings demonstrate the developmental regulation of volatile production linked to CBB behavior, revealing potential targets for pest control strategies.

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**KEYWORDS**
*Coffea arabica*; coffee berry borer; volatiles; olfactometryt; erpene synthases

## 1. Introduction

The coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) (CBB) pest greatly affects the economy of Colombian coffee growers, and its management is complex. To control insects, an integrated pest management (IPM) strategy is required that implies the use of intensive labor, requires timely harvesting, the removal of unharvested coffee berries, and the use of chemical insecticides or biological control with *Beauveria bassiana* fungus, among other strategies (Góngora et al. 2023).

Plants can mediate their communication with organisms in their environments, such as pollinators, natural enemies, herbivores, other plants, and microorganisms, through volatile organic compounds (VOCs) (Vivaldo et al. 2017). In particular, insects use visual and chemical cues to locate their plant hosts (Bernays and Chapman 1994). The coffee berry borer has been reported to use both visual and olfactory stimuli for host location (Rejesus and Baldos 1982; Mendoza-Mora 1991; Giordanengo et al. 1993; Mathieu et al. 2001).

Several authors have suggested that CBB uses a complex mixture of volatiles to recognize coffee fruits (Njihia 2014; Castro et al. 2017), and numerous works have focused on identifying the volatiles emitted by coffee fruits. Among the main compounds emitted by fruits are limonene, ocimene, conephtorin, linalool, verbenone, 4,8-dimethyl-1,3,7-nonatriene (DMNT), methyl salicylate, geranyl acetone, acoradiene, farnesene, 4,8,12-Trimethyl-1,3,7,11-tridecatetraene (TMTT), copaene, and pinene (Mathieu et al. 1996; Mendesil et al. 2009; Cruz-López et al. 2016; Blassioli-Moraes 2019). Importantly, different studies have shown that the colonization of coffee plants by

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*H. hampei* females is related to the state of maturation of the fruits, regardless of the coffee variety evaluated (Giordanengo et al. 1993). The ripe coffee fruits elicit the most attraction from the insect (Mathieu et al. 1998; Góngora et al. 2024), being more attractive than green, underripe, and overripe fruits and even more attractive than the mixture of methanol:ethanol used for trapping the insect (Góngora et al. 2024). In experiments on insect preference, by offering the volatiles emitted by ripe vs. green fruits to females in olfactometry tests, they consistently chose the volatiles of ripe fruits (Giordanengo et al. 1993; Mathieu et al. 1996).

To further support this fruit ripeness preference, Mathieu et al. (1998) reported the volatiles emitted by coffee fruits in three different stages of development (green fruits, ripe fruits, and nuts) in two species, *Coffea arabica* and *Coffea canephora*, which are equally susceptible to the insect, and observed that the volatile composition differed at different stages of maturity. While unripe fruits produce few compounds, ripe fruits produce many terpenes in *C. arabica*, and both terpenes and sesquiterpenes in *C. canephora*. As the fruits become overripe and dry, the production of compounds decreases. Concordantly, Mendesil et al. (2009) showed that *H. hampei* females prefer ripe fruits, and through their electroantennography response studies identified electrophysiologically active compounds.

One promising alternative for controlling CBB involves studying the volatile compounds emitted by coffee plants or surrounding vegetation to determine whether these compounds act as attractants or repellents for the insect. Studies conducted by Castro et al. (2017) using olfactometry reported the preference of the CBB for coffee fruits together with different plant species. In this study, it was found that the species *Nicotiana tabacum, Lantana camara*, and *Calendula officinalis* acted as repellent plants; however, the *Emilia sonchifolia* species showed an attraction behavior for CBB. The plants evaluated showed a high emission of volatile compounds, with limonene and pinene being particularly prominent.

Literature reports on volatile compounds emitted by coffee fruits (Mathieu et al. 1996; Mathieu et al. 1998; Mendesil et al. 2009; Cruz-López et al. 2016; Blassioli-Moraes 2019), along with information about volatiles from attractant and repellent plants, have identified limonene and pinene as potential attractants for the CBB to coffee plants, as both compounds are produced by coffee fruits.

Limonene has been consistently identified as a significant volatile component of coffee fruits. Mathieu et al. (1998) reported high levels of limonene production, followed by pinene, in both ripe and green fruits of *C. arabica* and *C. canephora* var. Robusta. Similarly, a study characterizing the volatile profiles of fresh coffee fruits across different cultivated varieties demonstrated that limonene and pinene are the most abundant volatiles in ripe beans of both *C. arabica* and *C. canephora* (Mathieu et al. 1996). Beyond its presence in coffee, limonene is also found in various other plant species, including citrus fruits (*Citrus limon*, *Citrus sinensis*, *Citrus reticulata*) (Bora et al. 2020; Caputo 2020), mint (*Mentha* spp.) (Hudz 2023), sage (*Salvia officinalis*), and pine trees (Alicandri 2022), where it is associated with functions such as defense, pollinator attraction, and plant-plant communication (Ibrahim et al. 2004; Mann 2012; Morehouse et al. 2017).

Prior research has specifically investigated the CBB's response to limonene. Góngora (2020) tested the preference of CBBs for ripe coffee fruits versus coffee fruits augmented with limonene volatiles at different concentrations. Their findings indicated that the treatment with limonene at 25 ppm attracted insects more effectively than ripe coffee fruits alone (Góngora 2020), suggesting a potential role for limonene in mediating CBB attraction to coffee plants.

Pinene, along with limonene, is one of the main volatiles emitted by coffee fruits (Mathieu et al. 1996; Mathieu et al. 1998). It was identified as the second most abundant compound after limonene in both ripe and green fruits of *C. arabica* and *C. canephora*. Similar results have been reported in other analyses of coffee fruit volatiles, confirming the consistent presence of pinene across species. This finding is supported by research on the volatile profiles of fresh coffee fruits, which also indicates a substantial presence of pinene in ripe beans of both coffee species (Mathieu et al. 1996). In other plant species, such as *Pinus sylvestris*, *Pinus pinaster*, *Pinus ponderosa*, *Abies balsamea*, and *Juniperus communis* (Phillips et al. 2003; Alicandri 2022), pinene is associated with defense mechanisms, insect repellent properties, and antimicrobial activity. Furthermore, pinene has been implicated in plant–plant communication, acting as a volatile signal that can induce defensive responses in neighboring plants (Faiola et al. 2015; Riedlmeier 2017; Rosenkranz et al. 2021).

In this work, we aimed to investigate the role of the volatile compounds limonene and pinene in mediating the preference of CBB for coffee fruits. These compounds have been identified as the dominant volatiles emitted by coffee plants. In plants, the diversity of terpene structures is directly linked to the diversity of terpene synthase (TPS) genes. Therefore, identifying and characterizing these genes in coffee plants is critical for understanding the molecular basis of volatile production and for exploring potential strategies to manipulate plant–insect interactions. We characterized the metabolic pathways and genes responsible for their synthesis in the coffee plants. We also established the expression levels of the coding sequences of these genes and correlated them with the CBB attraction response to the coffee fruits.

Our research elucidates the fruit-insect communication mediated by these two principal volatiles and the molecular pathways involved in the production of the terpene synthases responsible for their biosynthesis. We hypothesize that the volatile compounds limonene and pinene, as well as their associated biosynthetic pathways, specifically the limonene and pinene synthase genes, play a key role in the recognition of coffee plants by CBB. In the future, silencing or modifying these genes responsible for the emission of these volatiles could potentially reduce the attractiveness of the plant to the insect.

## 2. Materials and methods

### 2.1. Preference of CBBs for coffee fruits vs. pinene and coffee fruits vs. limonene–pinene mixture

#### 2.1.1. Coffee fruit collection in the field

For the CBB preference studies adapted from Góngora et al. (2024), fruits of *C. arabica* var. Cenicafé 1 were selected in different stages of development during the months of October 2020 and August 2021 from the Naranjal experimental station located in the municipality of Chinchiná Caldas, Colombia (GPS coordinates: 4.991873, −75.597159). The site has an altitude of 1381 m, with an average temperature of 21.4 °C and an average relative humidity of 68%. The fruits were collected between 8 and 10 am to ensure consistent volatile emission profiles and avoid variation caused by temperature or sunlight exposure later in the day. After collection, the fruits were immediately transported to the laboratory initially, the flowering was marked to have fruits of the same physiological age. However, since fruit maturation depends on climatic conditions, we found that fruits of the same developmental age could be in different stages of ripening. Therefore, fruits were chosen according to their color in five stages of maturation: green fruit with approximately 120–150 days of development, underripe 2 (180 days), underripe 3 (200 days), ripe 4–5 (220 days) and overripe 6–7 (240 days) fruits, according to Peñuela-Martínez et al. (2022) scale development and maturation.

#### 2.1.2. Olfactometer

The tests were performed at Cenicafé in the olfactometry laboratory, Entomology Department (Manizales, Caldas, Colombia) under controlled conditions, including a relative humidity of 75 ± 5% and a temperature of 25 ± 2 °C. A uniform fluorescent light (58 W) and an additional 13 W lamp were used.

The response of the CBB to the different treatments was evaluated in the Y-tube olfactometer following the methodology described by Castro et al. (2017). Two 20-cm-long Teflon® hoses were connected to the olfactometer, and the ends of each were attached to polypropylene bag compartments (30 × 18 cm) containing the treatments (compartment A or B). In contrast, each compartment was attached to a 0.1 M long × 0.00635 M Y-shaped glass tube, and the entire system was fitted with a vacuum pump and an air supply system with six charcoal filters (ARS, Gainesville, FL, USA). The airspeed was controlled by pressure regulators at a constant 100 ml/min, according to the recommendations of Sengonca and Kranz (2001), and the air flowed from the pump through the two compartments to the Y-tube.

When the treatments consisted of coffee fruits, 25 freshly collected fruits were placed in each of the olfactometer polypropylene bags.

The olfactometer assays were run 150× for each treatment. Three replicates were performed in different days with 50 insects per day, using one individual insect each time, for a total of 150 insects per treatment. The evaluations were conducted with adult female CBB newly emerged from coffee berries between 13:00 and 16:00 h, which are the hours of highest insect activity.

I. Bioassays

Two types of tests were carried out in which the preference of *H. hampei* females was evaluated.

- Ripe fruits + pinene volatile

The treatments consisted of evaluating ripe fruits against ripe fruits with four different concentrations of pinene. In addition to the fruits, one compartment contains a 25-mm diameter Whatman No. 1 filter paper (Whatman International, Maidstone, UK) treated with 25, 50, 100, and 200 ppm of pinene. The pinene was added to the filter papers by a 50-µl Hamilton microsyringe (Hamilton Company, Reno, Nevada, USA), and 10 µl of each concentration was applied. Owing to the polarity of the volatiles, 0.13% v/v aqueous acetone was used to prepare or dilute each concentration of pinene.

- Fruits in different ripening stages vs. limonene and pinene mixture

The treatments consisted of evaluating green, underripe 2–3, ripe, and overripe fruits among the mixtures of the volatiles limonene (25 ppm) (Góngora 2020) and pinene in their optimal concentration according to the bioassays previously described (ripe fruits vs. limonene + pinene). One of the compartments contained fruits, and the other contained a piece of Whatman No. 1 filter paper with a diameter of 25 mm was placed (Whatman International, Maidstone, Reino Unido). It was treated with a mixture of 25 ppm limonene and 200 ppm pinene, which were added to the filter papers using a 50 µl Hamilton microsyringe (Hamilton Company, Reno, Nevada, USA), and 10 µl of each compound was applied. The different volatiles remained in the compartment for up to 25 min after being applied to the Whatman filter paper, as evidenced by the insect's response to the treatment's attraction during that time when tested.

- Fruits in different ripening stages vs. methanol:ethanol mixture

Each development fruit stage, including green, ripe, and overripe fruits, was evaluated against the mixture of alcohols. A piece of Whatman No. 1 filter paper (Whatman International, Maidstone, United Kingdom) with a diameter of 25 mm treated with 10 µl of the solution in a 3:1 ratio was placed in one of the compartments, and the other contained the fruits. The alcohol mixture was added to the filter paper using a 50 µl Hamilton microsyringe (Hamilton Company, Reno, Nevada, USA).

#### 2.1.3. Statistical analysis for olfactometry assays

Since the distribution of the number of CBBs was binomial, before the treatments were evaluated and their ability to attract or repel insects was identified, the percentage of insects (proportions) that reached the compartments at the end of the Y-tube was first determined. This proportion was 50% when coffee fruits of the same stage of development were placed at both extremes (controls). According to PROC FREQ (SAS® software), which allows for determining Binomial Proportion and the Exact Confident Limits, the null hypothesis that the population proportion equals 50% was accepted.

The data from the olfactometer bioassay were analyzed by calculating the percentage ± standard error of estimate (SEE) of insects that respond in each of the treatments. Based on these results, the binomial proportion and the exact confidence limits were calculated to test the null hypothesis that the population proportion was equal to 50%. If the null hypothesis was rejected, it was determined in favor of which treatment the preference of the insect was greater.

Statistical analyses were performed using the procedure PROC FREQ from SAS® software. The proportions of the response variables were obtained. The outputs show the exact confidence limits and the binomial proportion test with a test of $H_0$: proportion = 0.5. $\Pr > |Z|$, $a = 0.05$.

### 2.2. Identification of volatiles of interest in green, underripe, and ripe fruits using gas chromatography–mass spectrophotometry (GC–MS)

Identification was performed via solid-phase microextraction (SPME). For this purpose, approximately 200 coffee fruits obtained from the field were taken at different stages of development.

The fruits were collected between 7 and 8 am and then taken to the laboratory. They were chosen according to their color in five stages of maturation: green fruit with approximately 120 to 150 days of development, underripe 2 (180 days), underripe 3 (200 days), ripe or mature 4–5 (220 days) and overripe 6–7 (240 days) fruits, following the color scale reported by Peñuela-Martínez et al. (2022).

The 200 fruits were placed in a 500 ml glass container and equilibrated for 24 h at room temperature. The samples were run three times. The compounds were trapped with carboxen/polydimethylxyloxane extraction fiber (Supelco, USA) for 24 h at room temperature. The compounds were detected by gas chromatography coupled to mass spectrophotometry (GC–MS) with a 50 m × 320 µm × 1 µm DBWAX column. The NIST 98 and Wiley 275 libraries were used to identify volatile compounds. The similarity concerning the libraries was expected to be >90% for all the compounds.

For the identification of volatiles, we initially used D-limonene and α-pinene as reference standards. After confirming the identity of these compounds based on their retention times in the chromatographic profiles, we performed a comparative analysis of peak areas across the different fruit ripening stages. This semiquantitative approach allowed us to observe the relative abundance derived from the comparison of peak areas under the same GC-MS operating conditions.

The retention time was identified using the standards D-limonene and alpha-pinene, and identification was not performed using Kovats indices. The chromatographic method was as follows: DB-WAX column, 50 m length, 320 µm diameter, and 1 µm film thickness. The oven program started at 40 °C for 3 min, followed by a temperature ramp of 3 °C/min to 65 °C for 1 min, then 7 °C/min to 130 °C for 1 min, and finally 9 °C/min to 200 °C for 1 min, for a total run time of 31.4 min. The mass spectrometer detector was set in SIM mode, with the ions (68, 77, 93, 105, and 136) monitored.

## *2.3. Identification of metabolic pathway for synthesizing terpene synthases and the genes encoding limonene and pinene synthase in C. arabica*

### *2.3.1. Search for homologous limonene synthase and pinene synthase genes*

A search in the KEEG database (Kanehisa and Goto 2000; Kanehisa 2019; Kanehisa et al. 2021) was carried out to have a metabolic pathway reported in plants for the biosynthesis of monoterpenes and, in this way, to identify the genetic sequences that produce the volatiles limonene and pinene.

Next, we searched GeneBank using the Blast tool (Altschul et al. 1990) to identify possible sequences corresponding to limonene synthase and pinene synthase in *Coffea arabica*. In parallel, the 5′ and 3′ untranslated regions (UTRs) of each gene were analyzed using the Magic-BLAST program (Boratyn et al. 2019). This program was also used to determine the number of gene copies at the *in silico* level, confirming the presence of a single copy for each gene in the *C. arabica* genome.

Based on this information, two specific *in silico* sequences were identified, and gene-specific primers were designed to amplify the corresponding coding regions. Primer design was carried out using Primer-BLAST and Primer3 (Untergasser 2012) and Primer 3 (Untergasser 2012). Two primer sets were evaluated for each gene, taking into account the 5′ and 3′ UTRs to ensure complete sequence coverage (Table S1, Supplementary material).

### *2.3.2. Isolation and sequencing of gDNA and cDNA of limonene synthase and Pinene synthase in C. arabica*

DNA from *C. arabica* was extracted from leaves using the DNeasy Plant Mini Kit extraction kit (Qiagen), and the DNA concentration ranged from 100–400 ng/µl. RNA was extracted using Spectrum plant total RNA kit (Sigma–Aldrich), from a piece of the pericarp and mesocarp from ripe fruits, RNA concentrations ranged from 20–40 ng/µl. cDNA was synthesized with GoScriptTM Reverse Transcription System Kit (Promega) from 300 ng of RNA from each sample. The primers used were oligo (dT) and random primers. The amplification conditions were 25 °C for 5 min, 42 °C for 1 h, and 70 °C for 15 min. The cDNA was used in the shortest time possible. Amplifications were performed using 100 ng of coffee genomic DNA or cDNA in each reaction. The PCR cycle consisted of the following conditions: 94 °C for 5 min, followed by cycles of 94 °C for 1 min; 55 °C for 1 min; 72 °C for 1 min, 72 °C for 5 min; and 32 cycles. The products were purified by taking 50 µl of the PCR product, and the QIAquick PCR purification kit from Qiagen was used. The concentration of each purified product was quantified with Qubit.

The samples were sent to the sequencing service at the University of the Andes, Gencore, in Bogota, Colombia. Oxford Nanopore Technologies (ONT) sequencing technology was used. The ONT PromethION sequencing platform and an R10.4.1 flow cell were used according to the technical and research requirements.

For sample processing and amplicon sequencing, libraries were prepared using the Native Barcoding Kit 24 V14 (EXP-NBD114.24). The construction of the protein model was based on the sequences of gDNA for each gene of interest. FGENESH program (Solovyev et al. 2006) (SoftBerry) was used to predict protein-coding ORFs and gene models for specific gene-finding parameters *C. canephora* genome.

For the prediction of the protein of each gene, protein family, domain, and important motif, we translated the cDNA sequences, and we used the program InterPro (https://www.ebi.ac.uk/interpro/) with the Pfam database (Mistry 2021). For each protein, we searched for the characteristic domains of the terpene synthase family via the program Geneious Prime 2024.0 (https://www.geneious.com/). With the real DNA terpene synthase sequences, along with the validation of RNA-cDNA and expression levels of both genes in coffee fruits and the demonstration of the presence of volatiles, we partially demonstrated these gene areas.

## 2.4. Tissue expression analysis of limonene synthase and pinene synthase in Coffee arabica

Coffee fruits from the Cenicafé 1 variety in different ripening stages, namely, green, underripe, ripe, and overripe, followed the color scale reported by Peñuela-Martínez et al. (2022), and leaves were collected at the Naranjal experimental station. A piece of the pericarp and mesocarp from each coffee fruit was cut and stored in liquid nitrogen until processing. Total RNA was extracted by grinding 100 mg of frozen tissues to a fine powder in liquid nitrogen using the Aldrich Spectrum plant total RNA kit (Sigma–Aldrich). The RNA integrity was verified via agarose gel and quantification using a NanoDrop 2000. The samples were stored in a freezer at −80 °C until later use. Sample collection and expression analysis were performed over 4 years (2021, 2022, 2023, and 2024) in the morning between 9:30–10:30 and in the afternoon between 2:00–3:00 p.m. corresponding to periods of stable environmental and physiological conditions to minimize circadian variation in transcript levels. RNA extraction was performed in individual replicates.

To evaluate the gene expression of the limonene synthase and pinene synthase genes, cDNA was synthesized with GoScriptTM Reverse Transcription System Kit (Promega) from 300 ng of RNA from each of the samples.

The expression analysis was performed using 4 µl of cDNA diluted 1:10 template with the GoTaq® 2-step RT-PCR System Kit (Promega), with 20 µl final volume reactions. The cyclophilin gene was used as a reference gene, as it was previously reported as a housekeeping gene in coffee fruits (Idárraga 2011). The primers that were used to amplify each gene are shown in Table S2 (Supplementary material).

PCR was carried out using BIO-RAD CFX96 Touch system equipment following the thermal profile of 95 °C for 30 s, 42 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. The fluorescence signal was captured at the end of each cycle, and the melting curve was generated from 65 to 95 °C, with increments of 0.5 °C every 5 s, to verify the presence of a single amplicon. For each of the tissues, 3 biological and 3 technical replicates were executed.

Quantitative real-time PCR (qRT-PCR) data were analyzed using the CFX Maestro Software (Bio-Rad), which employs the comparative Cq (ΔΔCq) method to calculate relative normalized expression levels. For each tissue type of *Coffea arabica* fruit, gene expression was evaluated using three biological replicates, each consisting of three technical replicates. The Cq values of the target genes were normalized against a reference gene by calculating ΔCq values (Cq_target—Cq_reference). A cyclophylin gene (calibrator) was selected to determine the ΔΔCq values (ΔCq_sample—ΔCq_calibrator), and the relative expression was then calculated using the $2^{-\Delta\Delta Cq}$ formula. The CFX Maestro software automatically averaged technical replicates, accounted for inter-run variability, and generated standard deviations used to present expression values with error bars.

### 2.4.1. Statistical analysis for gene expression

For the statistical analysis, the probability distribution of the data was estimated using the bootstrap method. For each gene (limonene synthase and pinene synthase) and at each developmental stage (leaf, green, underripe, ripe, and overripe fruits), the bootstrap method was applied to the corresponding datasets, generating 1000 samples based on the measurements obtained during the evaluations. With these samples, the empirical probability distribution function of the data was estimated. Each of the obtained probability functions was subsequently analyzed using the graphical method of Cullen and Frey to identify the parametric function that best fit the empirical distribution of the data. The goodness of fit of the distributions suggested by Cullen and Frey was evaluated using the Kolmogorov test with a significance level of $\alpha = 0.05$. Finally, the distribution with the best fit was selected, and the probability of gene expression was estimated for each developmental stage.

## 3. Results

### 3.1. Preference of CBBs for coffee fruits vs. pinene and coffee fruits vs. limonene–pinene mixture

To evaluate the behavioral response of *H. hampei* to different volatile compounds, a series of dual-choice assays were conducted using an olfactometer. These experiments compared the attractiveness of coffee fruits at various ripening stages with that of individual and mixed synthetic volatiles known to be emitted by coffee plants or associated with insect attraction.

### 3.1.1. Preference of CBBs for coffee ripe fruits vs. coffee ripe fruits + pinene volatile

The CBB preference for ripe coffee fruits vs. coffee fruits accompanied by pinene volatile at different concentrations is shown (Figure 1). Higher CBB attraction was observed at 200 ppm of pinene. Significant differences between treatments were observed ($Pr > |Z|$, $a = 0.05$.) The other concentrations were similar to those of ripe fruits.

### 3.1.2. Preference of the CBB for coffee fruits in different ripening stages vs. limonene–pinene mixture

Compounds of limonene at 25 ppm (Góngora 2020) mixed with pinene at 200 ppm attracted the CBB, showing a more significant attraction effect than the coffee fruits in the different ripening stages. As shown

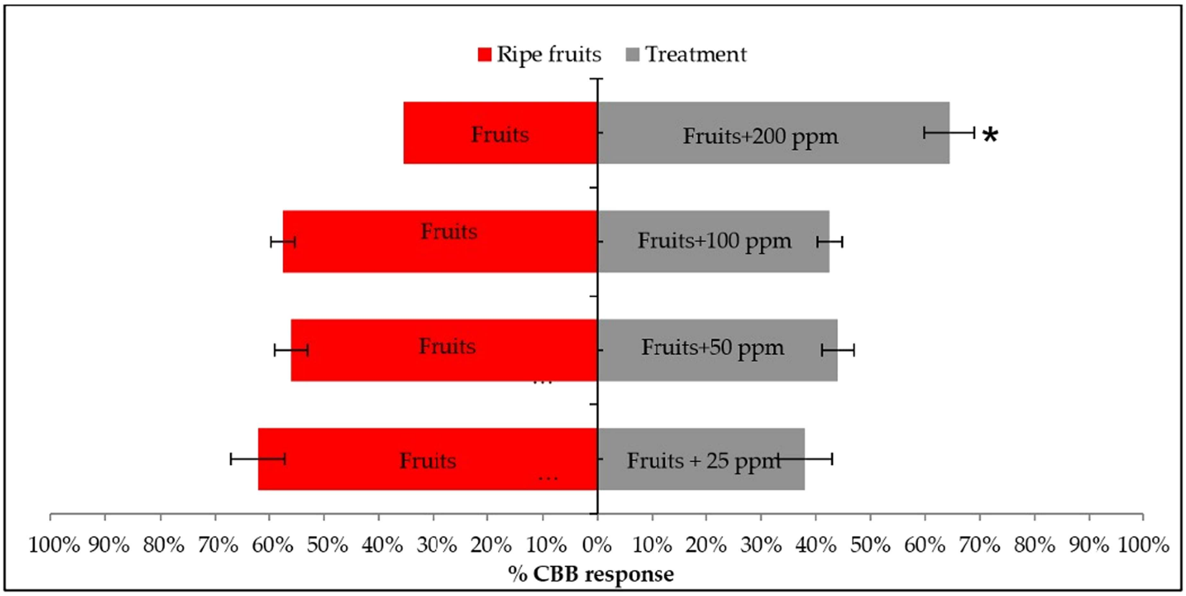

**Figure 1.** Effect of pinene on the CBB preference in a Y-tube olfactometer (percentage + SE, $n = 150$). The asterisk indicates significant differences from a 50% response ($Pr > |Z|$, $a = 0.05$).

in Figure 2, the limonene–pinene mixture attracted between 64% and 77% more than the coffee fruits, independent of the ripening stage. Significant differences between treatments were observed.

### 3.1.3. Preference of the CBB for coffee fruits in different ripening stages and the mixture of methanol:ethanol (3:1) or mixture of limonene–pinene

A mixture of limonene at 25 ppm with pinene at 200 ppm and the ripe coffee fruits showed a higher CBB attraction than the methanol:ethanol mixture 3:1. When the comparison was performed with the other fruit ripening stages, the methanol:ethanol mixture attracted the most. Significant differences between treatments were observed Figure 3.

## 3.2. Identification of volatiles in green, underripe, and ripe fruits using gas chromatography–mass spectrometry (GC–MS)

After identifying the effects of volatiles on insects using olfactometry, it was necessary to determine whether these volatiles were produced by coffee fruits.

To characterize the VOCs associated with coffee fruits across ripening stages, samples were collected and analyzed from green, underripe, and ripe fruits, as shown in Figure 4, volatile collection was carried out using the solid-phase microextraction (SPME) technique, which allows for the efficient capture of headspace volatiles without solvent interference. This capture was made for three stages over one hour, and it was injected directly into the chromatograph for identification.

To identify the retention times and the separation of the volatiles, in this study, two reference standards: D-limonene and α-pinene were used for compound identification, were injected, which were identified at the retention times of 7.46 min and 14.6 min, respectively, as illustrated in Figure 5a. With respect to retention time, we identified each volatile presence in the coffee fruits in the three ripening stages. The limonene was the most abundant component in comparison with pinene; for both volatiles, ripe fruits presented the highest concentration, followed by underripe and green fruits (Figure 5b).

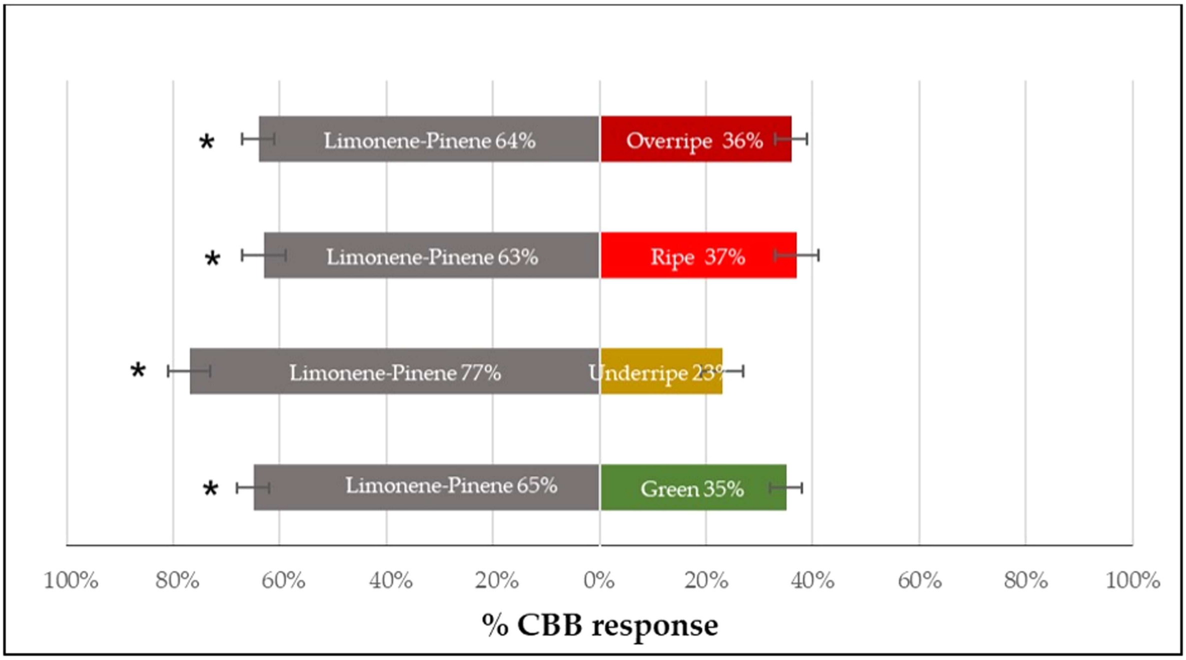

**Figure 2.** Preference of *Hypothenemus hampei* for coffee fruits at different ripening stages vs. limonene + pinene mixture in a Y-tube olfactometer (percentage + SE, $n = 150$). The asterisks indicate significant differences from a 50% response ($\Pr > |Z|$, $a = 0.05$).

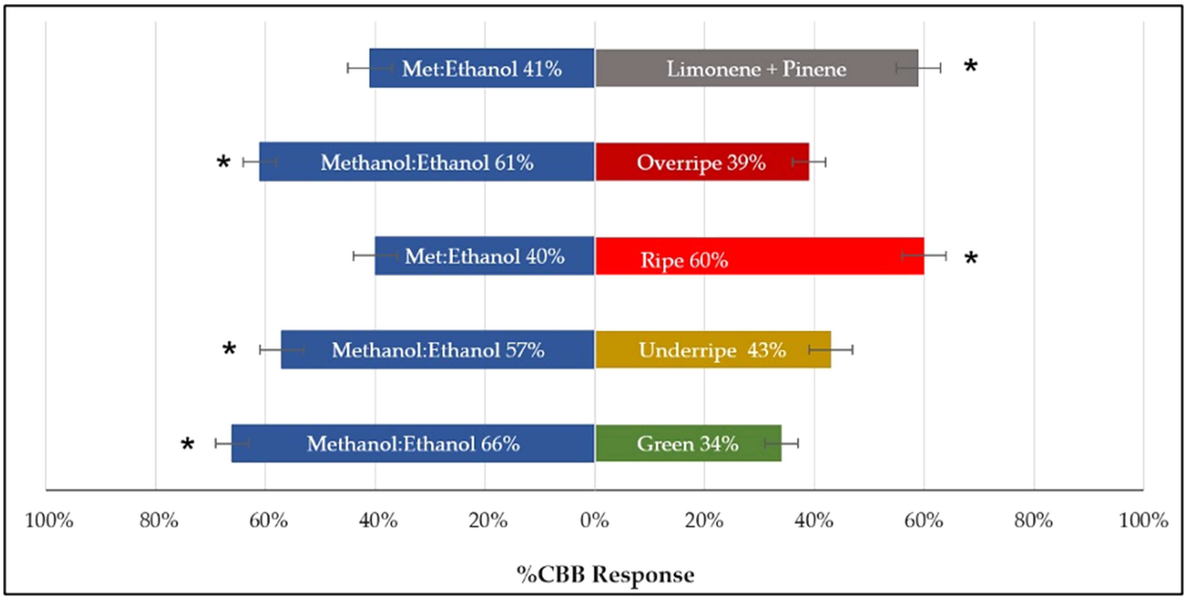

**Figure 3.** Preference of *Hypothenemus hampei* for coffee fruits at different ripening stages and the mixture of limonene + pinene vs. methanol:ethanol in a Y-tube olfactometer (percentage + SE, $n = 150$). The asterisks indicate significant differences from a 50% response ($Pr > |Z|$, $a = 0.05$).

**Figure 4.** Ripening stages of coffee fruits used for identification of volatile compounds. From left to right, ripe, underripe, and green fruits are shown.

### 3.3. Identification of metabolic pathway for synthesizing terpene synthases and the genes encoding limonene and pinene synthase in *C. arabica*

To validate that the volatile compounds limonene and pinene detected in *Coffea arabica* fruits are biosynthesized by the plant itself, it was essential to identify and confirm the presence of the genes encoding their respective synthases. This was achieved through homology-based sequence searches, amplification, and sequencing.

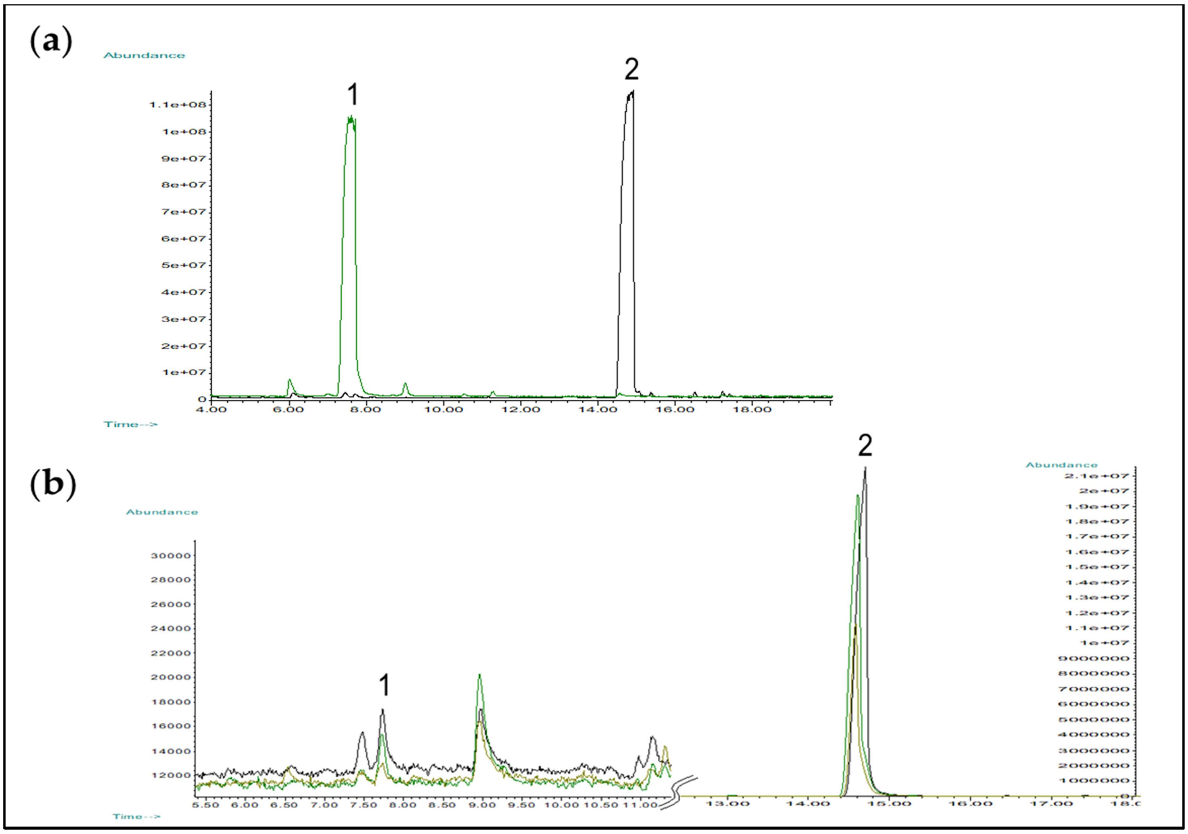

**Figure 5.** GC–MS analysis of coffee fruits at different ripening stages. (a) Chromatogram with standard retention times for pinene (1) and limonene (2) volatiles. (b) Chromatogram of the coffee fruit samples. 1 = pinene (7.6 min); 2 = limonene (14.6 min). The black curve represents ripe fruits, the green curve represents underripe fruits, and the yellow curve represents green fruits.

The search for the limonene synthase gene was guided by prior findings from Del Del Terra (2013), who reported a cDNA sequence encoding a 566 amino acid protein in *C. arabica*. This gene has a total length of 2908 bp, with a 2021 bp coding region (GenBank accession: HE985292.1). Based on the homologue sequence, primers were designed, and the genes were amplified in *C. arabica* genome, and the complete real sequence was obtained, and sequence data for limonene synthase were deposited in GenBank under the accession number PV256510. The limonene synthase has 2904 bp and contains an 1821 bp open reading frame that encodes a 606 amino acid protein (see supplementary material). Sequence alignment revealed a single nucleotide variation (A > G at position 1229) compared to the reference. Our sequence shows >98% identity but presents a few single-nucleotide polymorphisms (SNPs) and small indels that slightly modify the CDS length and predicted protein size. These differences are consistent with allelic or cultivar-specific variants within the *C. arabica* genome, which is allotetraploid and known to harbor polymorphisms between the *C. eugenioides* and *C. canephora* subgenomes. Phylogenetic analysis showed high identity with limonene synthase from *Coffea eugenioides* and *Olea europaea* var. *sylvestris* (98.95% and 99.01%, respectively), and at the protein level, the sequence shared 98% identity with *C. eugenioides* and 96% with *C. canephora*. It also exhibited moderate similarity (57%–59%) with alpha-terpineol synthases from *Vitis* species. This gene was located on chromosome 2e.

For the volatile pinene, the monoterpene synthase proteins of *Coffea arabica* have a high percentage of similarity with other species, such as *Vitis vinifera*, whose monoterpene biosynthesis genes show high similarity to those of coffee (Martin 2010). A candidate gene was identified through amino acid-level homology, showing 57.04% identity with *V. vinifera*. This gene has a size of 4593 bp and consists of a coding region of 1827 bp with 608 amino acids (GenBank: accession number XM_027247536.1). Pinene synthase has been previously reported both in *Abies grandis* and in various conifers (Steele et al. 1998). Based on the homologue sequence, primers were designed, the genes were amplified in coffee genome, the complete real sequences were obtained, and sequence data for pinene synthase were deposited in GenBank under the accession number PV256511. Pinene synthase has 4748 bp, and the coding sequences are 1482 bp and 482 aa (supplementary material). The gene comprises six exons and was mapped to chromosome 2 c. At the nucleotide level, it shares 98.20% identity with *C. eugenioides* and exhibits high similarity with pinene synthases from *Quercus suber*, *Q. lobata*, and *Q. ilex* (up to 92%). The protein sequence shares over 97% identity with terpene synthase 10-like proteins from *C. eugenioides* and *C. canephora*. The CDS and deduced protein sequences for both genes are provided in the Supplementary Material (Appendices A and B) for reference and reproducibility.

At the amino acid level, both proteins belong to the family of Terpene Cyclases, Class 1. This family converts the acyclic isoprenoid diphosphates geranyl diphosphate (GPP), farnesyl diphosphate (FPP), or geranylgeranyl diphosphate (GGPP) into monoterpenes, diterpenes or sesquiterpenes (Bohlmann et al. 1998; Dewick 2002).

Conserved protein domains were identified in both enzymes: the terpene cyclase-like 1 C-terminal domain spans residues 262–587 in limonene synthase and residues 280–604 in pinene synthase. Additionally, both proteins contain motifs typical of terpene synthases, including DDXXD (aspartate-rich motif), $RR(X)_8W$, and the NSE/DTE motif, which are essential for catalytic activity and metal ion coordination.

According to Gene Ontology (GO) annotations, both enzymes are involved in the diterpenoid biosynthetic process (GO:0016102), with associated molecular functions, including terpene synthase activity (GO:0010333), lyase activity (GO:0016829), and magnesium ion binding (GO:0000287). However, considering their clustering within the TPS-b subfamily, which is characteristic of monoterpene synthases in angiosperms, these genes are functionally involved in the monoterpenoid biosynthetic process (GO:0016099), which is consistent with the volatiles detected in coffee fruits. These findings support the functional role of these genes in the biosynthesis of behaviorally relevant volatiles detected in coffee fruits.

### 3.4. Tissue expression analysis of limonene synthase and pinene synthase in Coffea arabica

To investigate the expression patterns of the limonene synthase and pinene synthase genes in *Coffea arabica*, transcript levels were analyzed in various tissues: green, underripe, ripe, and overripe fruits, as well as leaves, across multiple years (2021–2024).

The expression levels observed for the limonene synthase gene in the years 2021, 2023 (am and pm), and 2024 (am and pm) in most of the years evaluated were similar according to the type of tissue (Figure 6). In the ripe fruit, the expression levels were always the highest, followed by the underripe fruit and overripe, then those in the green fruit; finally, the expression was the lowest in the leaves. However, data for the year 2022 showed a large variability among biological and technical replicates. The expression levels were not homogeneous and did not represent the same expression pattern as the abovementioned years. The limonene synthase expression of limonene synthase in ripe fruits is approximately 127.5% higher than the average expression in other fruits.

The expression levels of the pinene synthase gene exhibit distinct behavior compared to the expression of the limonene synthase gene across various fruit ripening stages, with significant variation between years and among different tissues. In 2022, substantial variability in gene expression was also observed among biological and technical replicates (Figure 7). Table 1 summarizes the average gene expression levels for each tissue over the four years of the study.

### 3.5. Tissue statistical expression analysis of limonene synthase and pinene synthase in Coffee arabica

To calculate the probability of expression of both genes, the probability distribution that best fits the data was determined. Using a bootstrap resampling procedure, over 1000 samples were generated based on the original data, and various probability distributions were tested to identify the best fit. The probability of expression in the tissues of green fruits, underripe fruits, and ripe fruits can be modeled using a gamma

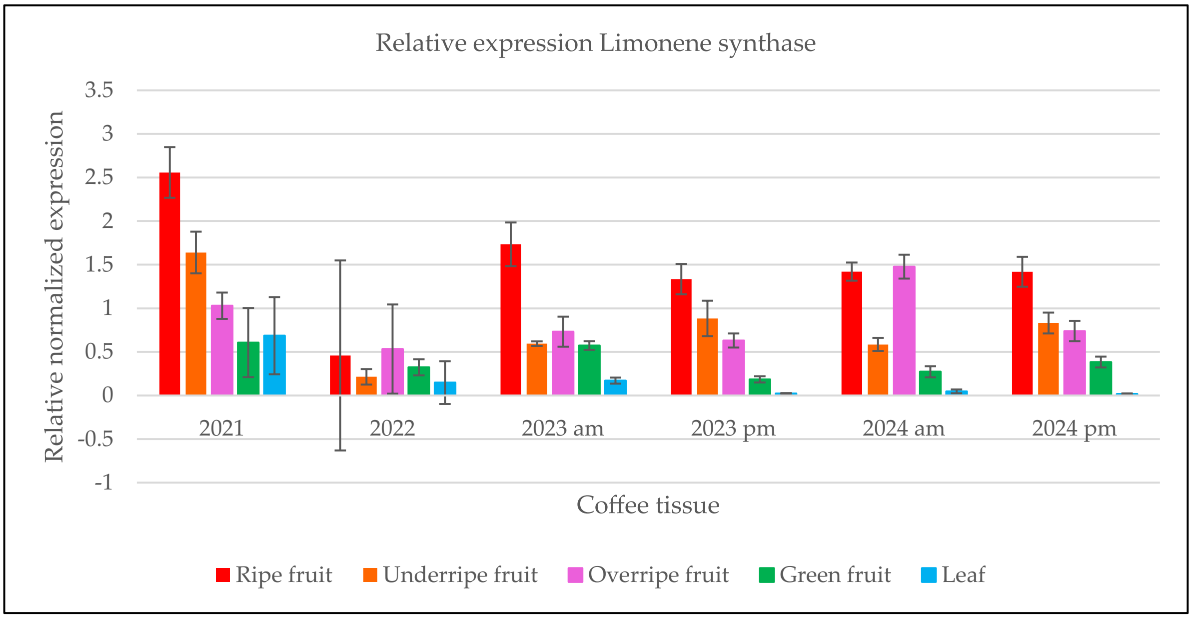

**Figure 6.** Relative expression of the limonene synthase gene in ripe, underripe, overripe, and green fruits and leaves for four years.

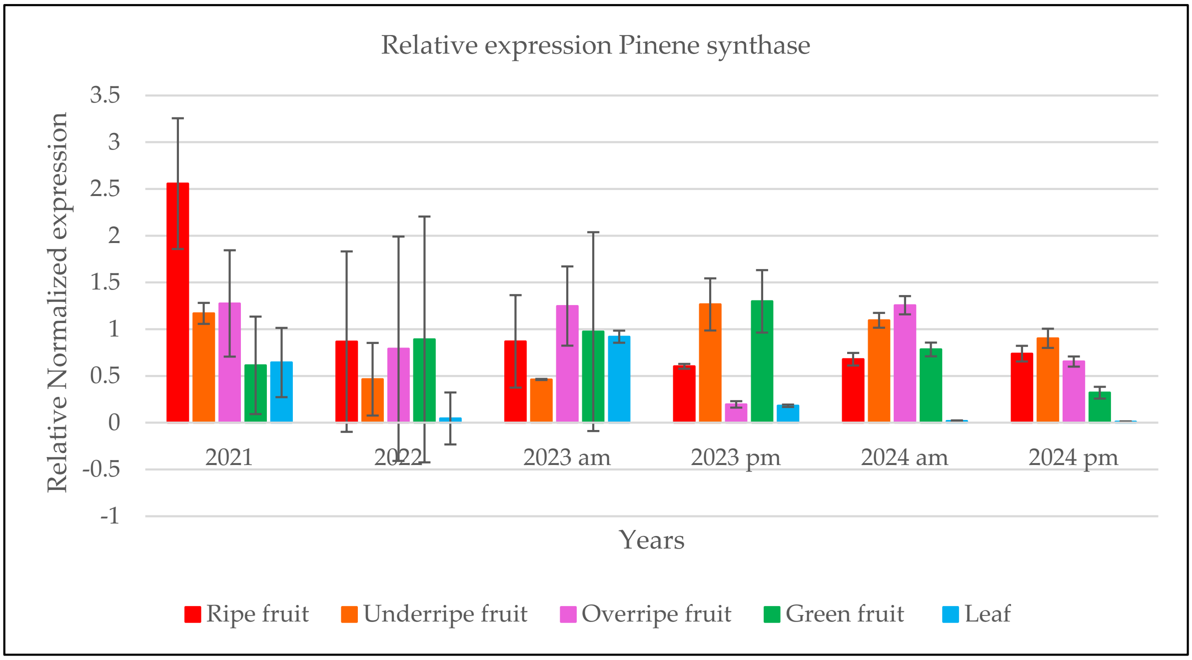

**Figure 7.** Relative expression of the pinene synthase gene in ripe, underripe, overripe, and green fruits and leaves for four years.

distribution, while for leaf tissues and overripe fruits, an empirical distribution was selected. Table 2 presents the probability of expression of the limonene synthase and pinene synthase genes in each tissue and the probability parameters for each model.

The expression levels observed for the limonene synthase gene in the years evaluated were similar for each of the tissues (Figure 8). In the ripe fruit, the probability of expression levels was always the highest, followed by the underripe fruit, and overripe, then green and finally, the leaf. The leaf's probability of limonene synthase is near 0%; for this gene, the ripe fruit had the highest likelihood of expression, with 66.4% of the relative expression being greater than 1.

**Table 1.** Average relative normalized expression for each gene in different coffee tissues.

| Gene | Coffee tissue | Average relative normalized expression |
|---|---|---|
| Limonene synthase | Ripe fruit | 1,6928 |
| | Underripe fruit | 0,9066 |
| | Overripe fruit | 0,9213 |
| | Green fruit | 0,4039 |
| | Leaf | 0,1886 |
| Pinene synthase | Ripe fruit | 1,0892 |
| | Underripe fruit | 0,9787 |
| | Overripe fruit | 0,9253 |
| | Green fruit | 0,7980 |
| | Leaf | 0,3548 |

**Table 2.** Probability of gene expression of limonene synthase and pinene synthase in each tissue and probability parameters for each model.

| Gene | Coffee tissue | Expression probability (greater than 1) | Fitted distribution | Parameters Shape | rate |
|---|---|---|---|---|---|
| Limonene synthase | Leaf | 0.0% | Empirical | – | – |
| | Green fruit | 13.7% | Gamma | 0.8 | 1.7 |
| | Underripe fruit | 33.0% | Gamma | 3.3 | 3.8 |
| | Ripe fruit | 66.4% | Gamma | 2.1 | 1.0 |
| | Overripe fruit | 40.0% | Empirical | – | – |
| Pinene synthase | Leaf | 0.0% | Empirical | – | – |
| | Green fruit | 33.0% | Gamma | 2.0 | 2.3 |
| | Underripe fruit | 37.8% | Gamma | 1.37 | 1.40 |
| | Ripe fruit | 39.5% | Gamma | 1.39 | 1.38 |
| | Overripe fruit | 34% | Empirical | – | – |

The expression levels of the pinene synthase gene show a different behavior concerning the expression of the limonene synthase gene in the various fruit ripening stages. The expression probability levels did not show differences between the different tissues, except for the leaf, where the probability of pinene synthase expression was always the lowest one (Figure 9).

## 4. Discussion

Previous studies have reported the preference results of the CBB to *C. arabica* fruits at different stages of ripening, showing that ripe fruits attract insects the most, drawing approximately 25%–30% more attraction than green or underripe fruits and 20% more than overripe fruits (Góngora et al. 2024). As has been demonstrated before, green fruits were the least attractive, and the volatiles they release are also the least appealing (Giordanengo et al. 1993; Mathieu et al. 1996).

The preference of CBB to different volatiles has been widely studied (Ortiz et al. 2004; Mendesil et al. 2009; Castro et al. 2017; Castro et al. 2018; Blassioli-Moraes 2019; Tobing et al. 2022). However, not many studies have tried to identify mixtures and concentrations of these volatiles that can be involved in the insect coffee attraction process. When comparing CBB preference for coffee fruits and terpene limonene attraction was observed at 25 ppm (Góngora 2020), and this is corroborated by the antennogram studies carried out by Mendesil et al. (2009), where CBB females were electrophysiologically active when responding to volatile limonene.

The information on volatiles produced by attractant plants and the comparison with the volatiles emitted by coffee fruits reported in the literature (Mathieu et al. 1996; Mendesil et al. 2009; Cruz-López et al. 2016; Magalhães 2018; de la Rosa-Cancino et al. 2021), allowed us to find in this study that in addition to limonene, there is another compound that could be an attractant of the insect, pinene. This pinene compound at 200 ppm together with coffee fruits has a greater attraction effect than ripe fruits alone. This effect is comparable with the concentration of 25 ppm of limonene previously reported (Góngora 2020).

The combination of methanol–ethanol alcohols has an attractive effect on CBB, and it is commonly used as an attractant of CBB on traps, it believes that this mixture mimics the metabolic components involved in the ripening process of coffee fruits. According to Ortiz et al. (2004), alcohols, especially

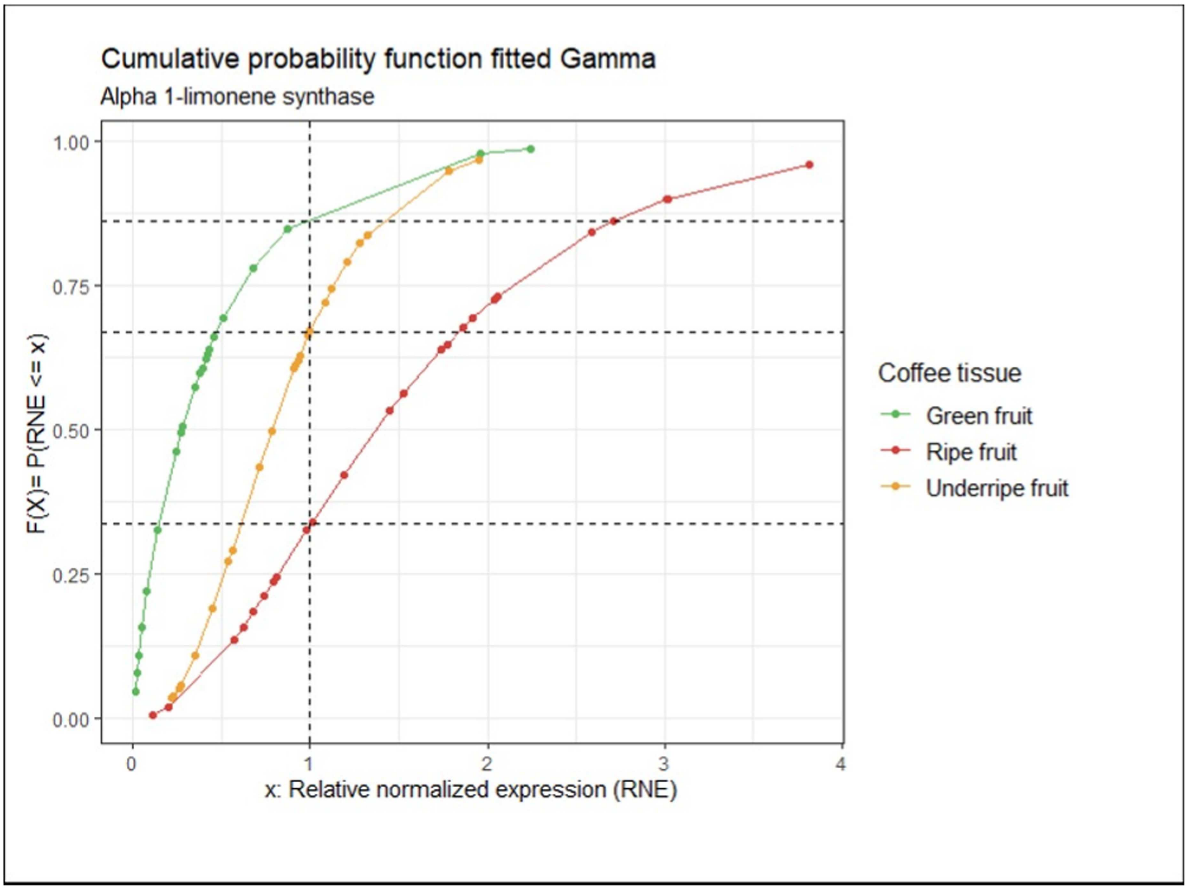

**Figure 8.** Probability expression of the limonene synthase gene in ripe, underripe, overripe, and green fruits and leaves.

ethanol, were among the volatile compounds coffee fruits released at every maturity stage. Specifically, the 3:1 methanol:ethanol mixture mimics the volatile profile associated with fermenting or physiologically stressed coffee berries, which are preferred oviposition and feeding substrates for the CBB (Ortiz et al. 2004;Barrera et al. 2006). Empirical studies have demonstrated that this specific 3:1 ratio result in the highest attraction rates (Barrera et al. 2006; Tobing et al. 2022), and this formulation has since been widely adopted in field traps across coffee-producing regions.

Here, the mixture of pinene and limonene at specific concentrations can have a higher attraction than the mixture of methanol:ethanol and by itself can attract CBB the same as the ripe fruits. Based on these findings, it was possible to assume that limonene and pinene were partly responsible for the attraction of the insects to the coffee fruits. Therefore, other compounds, not only alcohols, must be considered. Ripe and underripe fruits show the highest level of terpenes and monoterpenes (Mathieu et al. 1998; Cruz-López et al. 2016; Góngora 2020), which could also induce strong attraction. Beyond alcohols and terpenes, other volatiles such as kaurene, β-caryophyllene, and hexanal have also been reported in the volatile profiles of coffee berries and have shown varying degrees of influence on insect behavior (Mathieu et al. 1996; Mathieu et al. 2001; Ortiz et al. 2004; Góngora et al. 2024). The presence and proportion of these volatiles may enhance or modulate the behavioral response of the insect, supporting the idea of a synergistic or combinatorial effect among different volatile classes.

The identification of limonene and pinene as volatiles emitted by *Coffea arabica* during different fruit ripening stages using GC–MS analysis provides a strong foundation for understanding the chemical cues involved in host selection by *H. hampei*. Among these volatiles, limonene was found to be the most abundant compound, which is consistent with prior findings. Mathieu et al. (1996) reported that, through headspace analysis, limonene constituted 72.1% of the volatiles emitted by ripe coffee berries across six coffee varieties, while pinene represented only 4.97%. Similarly, in ripe fruits of *C. arabica*, limonene accounted for 93.8%, and pinene for 4.7% of the total volatile profile (Mathieu et al. 1998). This finding is

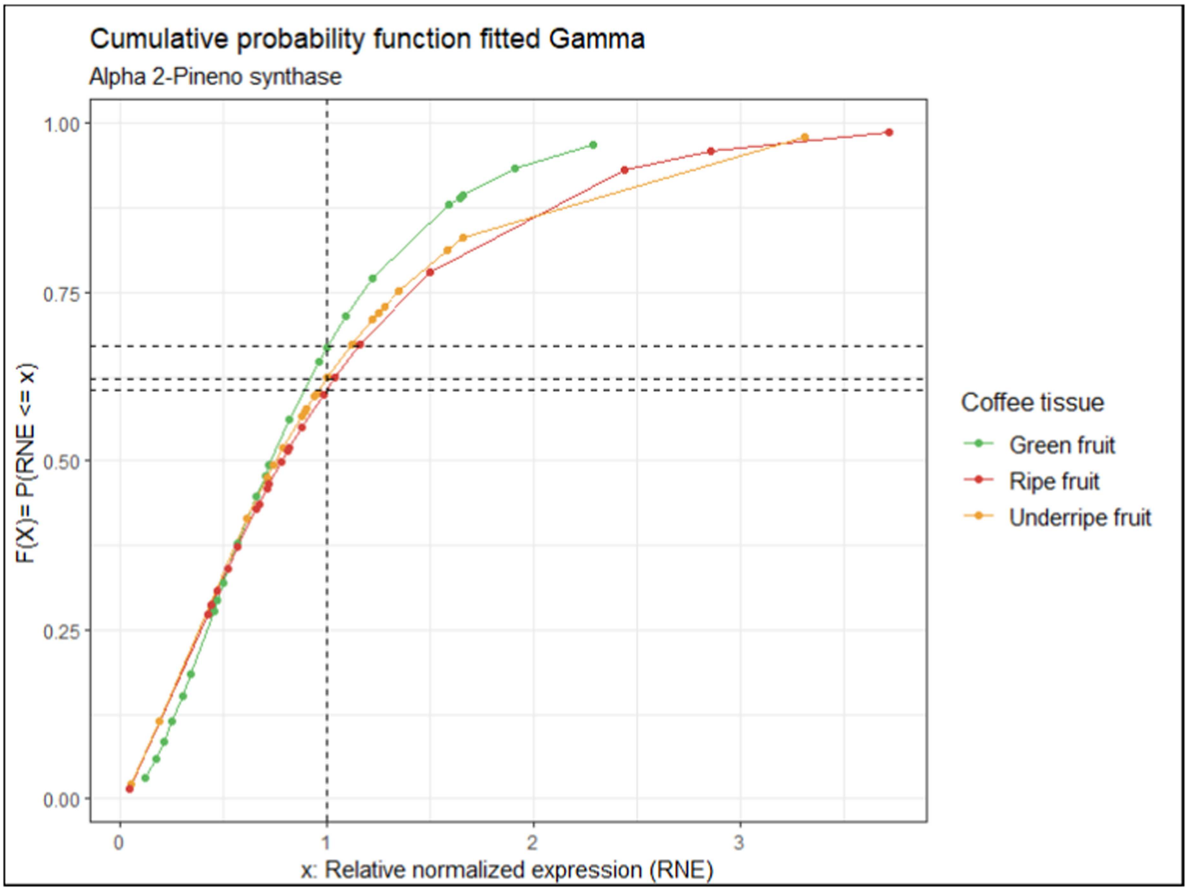

**Figure 9.** Probability expression of the pinene synthase gene in ripe, underripe, overripe, and green fruits and leaves.

also consistent with our findings because as the fruit ripening progresses, the presence of these two volatiles increases, suggesting that those two can be strongly involved in CBB attraction, explaining why green and underripe fruits are less attractive than the ripe fruits (Góngora et al. 2024).

To explore the ecological function and biosynthesis of these volatiles, we further examined the molecular basis for their production by identifying and characterizing the genes encoding limonene synthase and pinene synthase. Identifying these allowed us to test the hypothesis that these two compounds are involved in the recognition of the plant by the insect. Gene amplification and sequencing confirmed that both enzymes belong to the TPS gene family. For limonene synthase, homologous sequences were predominantly found in *Citrus sinensis*, followed by *Cannabis sativa* subsp. *sativus*. Limonene synthase plays key ecological roles, acting not only as an insect attractant but also as a deterrent for oviposition, an antifungal agent, and a signal to pollinators (Langenheim 1994).

Similarly, the pinene synthase genes showed high homology to sequences from *Cryptomeria japonica*, *Rosa rugosa*, and *Malus sylvestris*. Pinene, the product of this enzyme, is widely recognized for its role in plant defense. For instance, in *Dendroctonus valens* (red turpentine beetle), higher concentrations of α-pinene were associated with reduced boring activity and shorter gallery lengths, suggesting a repellent or feeding-inhibitory effect (Xu et al. 2014). Moreover, pinene is involved in plant–plant signaling, facilitating communication between neighboring plants under biotic stress (Riedlmeier 2017).

Structurally, both proteins have the DDXXD motif (aspartic acid-rich motif), which is involved in the coordination of divalent metals such as $Mg^{2+}$ or $Mn^{2+}$ (Tholl 2006; Chen et al. 2011); additionally, they have the RRX8W motif, which is conserved in monoterpene synthases (Williams et al. 1998). Additionally, the NSE/DTE motif is also present in both proteins, which is an extra metal ion-binding motif (Schnee et al. 2006). The presence of these motifs plays a key role in the catalytic functions of these TPS proteins (Zhang 2022).

Regarding the expression of limonene and pinene synthases in *C. arabica* fruit tissues and their potential involvement in interactions with the coffee berry borer, our results show that both enzymes are predominantly expressed in fruit tissues, with very low expression detected in leaves. These findings suggest a fruit-specific expression pattern for these terpene synthases. Del Terra (2013) evaluated the expression of several terpene synthase enzymes, including limonene synthase, in different tissues: flowers and fruits at four different ripening stages (10, 15, 25, and 32 weeks after pollination). RT-PCR analysis was performed to verify the presence of the enzyme transcripts. They identified for the first time that *limonene synthase* was expressed in fruits 25 weeks after pollination, corresponding to a mature fruit of approximately 224 days. In contrast, *limonene synthase* transcripts were not detected in the analyzed samples of flowers, leaves, or seeds, and no amplicons were obtained from fruits at other ripening stages. Our results complement this research and show by qPCR that also the limonene synthase expression changes according to the stage of fruit ripening, with the highest probability of relative expression (greater than 1) in ripe fruits, almost 70% more than that in the other stages, followed by underriping with 30% and overriping with 50%. The green fruits showed the lowest expression probability (10%) compared with the other stages.

Marie (2024) also reported on limonene synthase expression using RNA-Seq in underripe (210 DAF) and ripe (240 DAF) fruits. When evaluating the level of expression (normalized RNA-seq counts) of limonene synthase, they reported that the expression levels are higher in underripe fruits than in ripe fruits, and its expression decreased in the final stages of ripening. This conclusion differs from our findings; our study included a more comprehensive analysis across all ripening stages (green, underripe, ripe, and overripe) while Marie (2024) focused only on two time points within the late stages of fruit development. By omitting early ripening stages, such as green fruits, their study may not capture the full expression dynamics of limonene synthase. In contrast, our inclusion of intermediate stages allows for a clearer observation of the expression trajectory throughout maturation.

Currently, there are no reports on the expression levels of pinene synthase in coffee species. However, functional studies have been conducted in other plants, such as $\alpha$-pinene in *Pinus taeda* (Phillips et al. 2003), *Cannabis sativa* (Page et al. 2007) and *Picea sitchensis* (McKay 2003). The expression patterns of this enzyme in *C. arabica* do not show differences between the probabilities of relative expression greater than 1 in the fruit tissues because, for all fruits, the probability was 33%–39.5%, except for the leaves, probably because its expression is not conditioned to a particular ripening stage, representing an almost constant expression throughout the ripening stages.

It is important to consider that *Coffea arabica* is an allotetraploid ($2n = 4x = 44$), with an allelic load from both *C. eugenioides* and *C. canephora*. The terpene synthase gene families are found in both subgenomes. Although there is no reported global dominance of one subgenome over the other (Salojärvi 2024), for the TPS family, there are genes that are more expressed in one of the two subgenomes (Combes et al. 2023; Salojärvi 2024).

In this study, the expression profile of pinene synthase in *Coffea arabica* displayed considerable variability; however, on average, expression levels were relatively similar across all fruit ripening stages. In contrast, limonene synthase expression varied significantly with ripening, showing notably higher levels in ripe fruits. This finding suggests that while pinene synthase may be constitutively expressed throughout fruit development, limonene synthase is more dynamically regulated, likely in response to fruit maturation processes.

Both volatiles, limonene and pinene, appear to serve as olfactory cues for host recognition by the coffee berry borer. Nevertheless, the higher abundance of limonene in ripe fruits likely explains the insect's stronger attraction to this ripening stage, as supported by previous behavioral data. Pinene, despite its relatively stable expression, seems to play a complementary role in this chemical signaling. Our findings suggest that it is not the presence of individual volatiles alone but rather the ratio of their emission that is critical for host selection by the insect, reinforcing the ecological importance of this volatile blend in CBB behavior.

At the transcriptional level, higher differential expression of genes has been found in green and ripe fruits (Cheng et al. 2018). Considering the nutritional requirements of insects, ripe fruits with a main composition of galactomannans (Cheng et al. 2018) can better supply the nutritional needs of CBB larvae than green or overripe fruits (Góngora et al. 2024). Green coffee fruits contain only about 10% galactomannans, but as they mature, this polysaccharide content rises by 50% (Redgwell et al. 2003). Additionally, insects possess mannanase as the main enzyme responsible for breaking down the galactomannan in coffee berries (Cheng et al. 2018). During the maturation process, there is an increased expression of βglucosidase, which leads to a higher rate of cellulose conversion into glucose (Redgwell et al. 2003). The endosperm of the mature fruit stores nutrient reserves, including polysaccharides, proteins, and fatty acids, which are utilized by the seed embryo and CBB larvae. This explains, at an evolutionary level, the preference of the insects for the ripe fruits (Johnson et al. 2020).

For the expression of the genes in the different years of evaluation, a large variation was observed in the year 2022 with respect to the other years, with the lowest levels for both volatiles. The La Niña event, characterized by increased rainfall and lower temperatures, could partly explain these results. It is known that the gene expression pattern can be related to environmental factors such as relative humidity, light, atmospheric CO2, and temperature, which can influence the emission of VOCs (Staudt and Bertin 1998; Gouinguené and Turlings 2002; Dudareva et al. 2013). In the context of monoterpenes such as limonene and pinene, temperature has been identified as one of the most critical abiotic drivers. Numerous studies have demonstrated a direct, often exponential, relationship between temperature and the rate of terpene emission, largely due to its effect on enzyme activity and volatilization kinetics (Mochizuki et al. 2020; Malik 2023). Low temperatures, as observed during La Niña years, can suppress both the transcriptional activity of terpene synthase genes and the physical volatilization of terpenes from plant tissues. This could explain the lower expression levels observed in our dataset for 2022, which coincided with strong La Niña conditions. Additionally, in La Niña years, the CBB infestation level in Colombia is lower compared to other climatic years (Constantino et al. 2021).

In coffee plants, monoterpene biosynthesis appears to be transcriptionally regulated. Two pathways may explain the fact that there is differential expression depending on the ripening stage. First, the biosynthesis of these monoterpenes may be controlled by posttranscriptional and/or posttranslational mechanisms, or the products may accumulate in ripe fruit after the end of active monoterpene synthesis (Del Terra 2013). The latter idea seems more likely because in other plants, it has been demonstrated that monoterpenes are stored during fruit ripening in nonvolatile glycosylated forms (Mateo and Jiménez 2000; Weckerle et al. 2003; Maicas and Mateo 2005).

Our chromatography studies observed a higher concentration of limonene and -pinene in ripe fruits. However, the RNA expression levels of pinene synthase did not appear to be higher in ripe fruit tissues, the levels, although higher, did not show statistical differences, because of the large variability. One possible explanation is that these monoterpenes expression and biosynthesis levels are not proportional to their volatilization rate. For instance, it has been observed in some plants, terpenes are stored in separate compartments, one for synthesis and one for emission (Loughrin et al. 1994; Gershenzon et al. 2000). This finding indicates that the emission of monoterpenes comes from a different pool in the cell, and probably there is a different source with continuous expression of monoterpene genes; additionally, this volatilization rate is mediated by the ripening stage, glycosylation state, and postharvest handling factors such as temperature, storage, atmosphere, and mechanical damage (Gershenzon et al. 2000; p. 20; El Hadi et al. 2013; Huang et al. 2021). However, more information is needed to understand the physiological drivers and physicochemical parameters behind volatilization.

The compounds identified in this study are common among plants and are not exclusive to any taxonomic group, indicating that limonene and pinene are present in many different plant species. For that reason, CBB needs to localize the host based on an olfactory contrast hypothesis, where the proportion of compounds released by the plant is important (qualitative and quantitative patterns), with respect to the background odor (Hilker and Mcneil 2008; Magalhães 2018), in contrast to the hypothesis where the location is based on species-specific olfactory recognition. Volatiles emitted by some plants change the proportion of the blend over time, indicating a physiological change in the host plant. This reflects the suitability or growth state of the host and requires CBB to have enough plasticity to detect and identify these variations in the volatile pattern (Dudareva et al. 2004; Bruce and Pickett 2011). This can be supported by the fact that coffee fruits terpene volatiles vary according to the physiological state of the fruit. The expression of limonene synthase changes; however, pinene synthase seems to remain constant throughout ripening. This suggests that what determines CBB's identification or preference for a ripe fruit over others is the change in expression of limonene synthase; however, the pinene has to be present.

Studies involving the analysis of volatiles in *C. arabica* are usually linked to quality and the cup profile since the volatile limonene appears to be related to the quality and cup profile (Del Terra 2013; Marie 2024), specifically the aroma characteristics. However, this study shows novelty because it is the first to report an association of the volatile compounds limonene and pinene synthesized and the terpene limonene and $\alpha$-pinene synthase, respectively, and a correlation with the attraction of CBB to coffee fruits. Through an integrated analysis of gene expression, volatile profiling, and olfactometry assays, we demonstrated that the preference of CBB for ripe fruits is associated with higher concentrations of limonene and pinene, particularly limonene, whose expression peaks in the ripe stage. This correlation between gene-level expression and insect attraction represents a novel contribution to understanding the molecular and ecological basis of host selection in the CBB.

Based on these findings, we propose that the specific blend and ratio of volatiles emitted by the coffee plant cause CBB olfactory stimuli that allow host localization. We propose that a change in the expression levels of the terpene synthase proteins identified in this study would alter the production pattern and, consequently, the mixture of volatiles released by the plant, affecting the ability of the insect to find the coffee plant in such a way that the insect would not identify the host plant. Understanding these processes could help develop plants with reduced volatile emissions and better agroecological insect control strategies, thereby diminishing coffee plant attraction to the coffee berry borer and potentially reducing economic losses in coffee production worldwide.

## 5. Conclusions

In the present study, it was reported that the CBB preferences are largely toward ripe coffee fruits; in this way, two volatile compounds have been identified: limonene and pinene at concentrations of 25 ppm and 200 ppm, respectively, which together attract the insect. The GC/MS analyses recorded the presence of limonene and pinene in all stages of coffee fruits, and both volatiles were captured in higher concentration in the ripe fruits, followed by the underripe fruits and finally the green fruits.

The genes associated with the production of those two volatile compounds in the genome of *C. arabica* were identified as limonene synthase and pinene synthase. Additionally, the RNA expression levels of both genes exhibit a differential expression profile with respect to the fruit ripening stage.

According to the results obtained, the hypothesis that these two compounds are involved in the recognition of the plant by the insect could be correct because both volatile compounds exert a synergistic effect on the attraction of the insect to the coffee fruits, specifically the ripe fruits. We identified these volatile compounds by GC-MS in coffee fruits, with a higher concentration in ripe fruits. At the same time, this pair of volatiles are found in the biosynthetic route of coffee monoterpenes, and the genetic sequences of each of them were obtained. Additionally, it was confirmed that there is a differential expression depending on the ripening stage, maybe indicating that the expression of these terpene synthases modulates the plant-insect interaction by the host-selection.

Studies at the antennogram level still need to be carried out to determine how CBB females respond electrophysiologically to the volatile pinene and the mixture of limonene and pinene. More studies should also be carried out on how the two volatiles are involved in the monoterpene biosynthesis pathway, specifically in coffee, because this pathway is based on other plant species.

With the findings of this study, it can be proposed that integrated pest management strategies are based on changing the volatile profile of the coffee plant to avoid identification by the CBB.

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## Author contributions

Conceptualization and supervision CEG, DV-M. Methodology PAF-V, AO, CEG, DV-M. Formal analysis PAF-V, JT, CEG, DV-M. Writing—original draft preparation PAF-V, CEG; writing—review and editing, CEG, JT, DV-M; conducted laboratory experiments JT, CPM, PAF-V, AO. Statistical analysis BEM-R. All authors have read and agreed to the published version of the manuscript.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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## Data availability statement

Sequence data for limonene synthase and pinene synthase have been deposited in GenBank under accession numbers PV256510 for limonene synthase and PV256511 for pinene synthase.

Additional information, including raw data, code, and supplementary materials, is available upon reasonable request from the corresponding author.

## References

Alicandri E et al. 2024. Monoterpene synthase genes and monoterpene profiles in *Pinus nigra* subsp. laricio. Plants. 11(3):2022. https://doi.org/10.3390/PLANTS11030449

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol. 215(3):403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Barrera JF, Herrera J, Villacorta A, GH CL. 2006. Trampas de metanol-etanol para detección, monitoreo y control de la broca del café Hypothenemus hampei. In: Simposio sobre Trampas y atrayentes en detección, monitoreo y control de plagas de importancia económica. Sociedad Mexicana de Entomología y El Colegio de la Frontera Sur. p 71–83.

Bernays EA, Chapman RE. 1994. Host-plant selection by phytophagous insects. In: Host-plant selection by phytophagous insects. 1. Springer. https://doi.org/10.1007/B102508

Blassioli-Moraes MC et al. 2019. Influence of constitutive and induced volatiles from mature green coffee berries on the foraging behaviour of female coffee berry borers, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae). Arthropod Plant Interact. 13(3):349–358. https://doi.org/10.1007/s11829-018-9631-z

Bohlmann J, Meyer-Gauen G, Croteau R. 1998. Plant terpenoid synthases: molecular biology and phylogenetic analysis. Proc Natl Acad Sci U S A. 95(8):4126–4133. https://doi.org/10.1073/PNAS.95.8.4126/ASSET/61F15E06-ADDC-4AE8-99CE-976F4D2251DD/ASSETS/GRAPHIC/PQ0980694006.JPEG

Bora H, Kamle M, Mahato DK, Tiwari P, Kumar P. 2020. Citrus essential oils (CEOs) and their applications in food: an overview. Plants [Internet]. 9(3):357. https://doi.org/10.3390/PLANTS9030357

Boratyn GM, Thierry-Mieg J, Thierry-Mieg D, Busby B, Madden TL. 2019. Magic-BLAST, an accurate RNA-seq aligner for long and short reads. BMC Bioinform. 20(1):1–19. https://doi.org/10.1186/s12859-019-2996-x

Bruce TJA, Pickett JA. 2011. Perception of plant volatile blends by herbivorous insects – finding the right mix. Phytochemistry. 72(13):1605–1611. https://doi.org/10.1016/J.PHYTOCHEM.2011.04.011

Caputo L et al. 2020. Chemical composition and biological activities of essential oils from peels of three citrus species. Molecules. 25(8):1890. https://doi.org/10.3390/MOLECULES25081890

Castro AM, Benavides P, Góngora CE. 2018. Push-pull strategy for handling the coffee berry borer. Trends Entomol. 14:51–61.

Castro AM, Tapias J, Ortiz A, Benavides P, Góngora CE. 2017. Identification of attractant and repellent plants to coffee berry borer, *Hypothenemus hampei*. Entomol Exp Appl. 164(2):120–130. https://doi.org/10.1111/EEA.12604

Chen F, Tholl D, Bohlmann J, Pichersky E. 2011. The family of terpene synthases in plants: a mid-size family of genes for specialized metabolism that is highly diversified throughout the kingdom. Plant J. 66(1):212–229. https://doi.org/10.1111/J.1365-313X.2011.04520.X

Cheng B, Furtado A, Henry RJ. 2018. The coffee bean transcriptome explains the accumulation of the major bean components through ripening. Sci Rep. 8(1):1–11. https://doi.org/10.1038/s41598-018-29842-4

Combes MC, Joët T, Stavrinides AK, Lashermes P. 2023. New cup out of old coffee: contribution of parental gene expression legacy to phenotypic novelty in coffee beans of the allopolyploid *Coffea arabica* L. Ann Bot. 131(1):157–170. https://doi.org/10.1093/AOB/MCAC041

Constantino LM, Gil ZN, Montoya EC, Benavides P. 2021. Coffee Berry Borer (*Hypothenemus hampei*) emergence from ground fruits across varying altitudes and climate cycles, and the effect on coffee tree infestation. Neotrop Entomol. 50(3):374–387. https://doi.org/10.1007/S13744-021-00863-5

Cruz-López L, Díaz-Díaz B, Rojas JC. 2016. Coffee volatiles induced after mechanical injury and beetle herbivory attract the coffee berry borer and two of its parasitoids. Arthropod Plant Interact. 10(2):151–159. https://doi.org/10.1007/s11829-016-9417-0

Del Terra L et al. 2013. Functional characterization of three *Coffea arabica* L. monoterpene synthases: Insights into the enzymatic machinery of coffee aroma. Phytochemistry. 89:6–14. https://doi.org/10.1016/j.phytochem.2013.01.005

Dewick PM. 2002. The biosynthesis of C5-C25 terpenoid compounds. Nat Prod Rep. 19(2):181–222. https://doi.org/10.1039/B002685I

Dudareva N, Klempien A, Muhlemann JK, Kaplan I. 2013. Biosynthesis, function and metabolic engineering of plant volatile organic compounds. New Phytol. 198(1):16–32. https://doi.org/10.1111/nph.12145

Dudareva N, Pichersky E, Gershenzon J. 2004. Biochemistry of plant volatiles. Plant Physiol. 135(4):1893–1902. https://doi.org/10.1104/pp.104.049981

El Hadi MAM, Zhang FJ, Wu FF, Zhou CH, Tao J. 2013. Advances in fruit aroma volatile research. Molecules. 18(7):8200–8229. https://doi.org/10.3390/MOLECULES18078200

Faiola CL, Jobson BT, VanReken TM. 2015. Impacts of simulated herbivory on volatile organic compound emission profiles from coniferous plants. Biogeosciences. 12(2):527–547. https://doi.org/10.5194/BG-12-527-2015

Gershenzon J, McConkey ME, Croteau RB. 2000. Regulation of monoterpene accumulation in leaves of peppermint. Plant Physiol. 122(1):205–214. https://doi.org/10.1104/PP.122.1.205

Giordanengo P, Brun LO, Frerot B. 1993. Evidence for allelochemical attraction of the coffee berry borer, *Hypothenemus hampei*, by coffee berries. J Chem Ecol. 19(4):763–769. https://doi.org/10.1007/BF00985007

Góngora CE et al. 2020. Evaluation of terpene-volatile compounds repellent to the Coffee Berry Borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae). J Chem Ecol. 46(9):881–890. https://doi.org/10.1007/s10886-020-01202-5

Góngora CE, Gil ZN, Constantino LM, Benavides P. 2023. Sustainable strategies for the control of pests in coffee crops. Agronomy 2023. 13(12):2940. https://doi.org/10.3390/AGRONOMY13122940

Góngora CE, Martinez CP, Figueroa-Varela PA, Medina-Rivera R. 2024. Preference of *Hypothenemus hampei* for coffee fruits according to state of maturation and infestation. Int J Pest Manag. 1–10. https://doi.org/10.1080/09670874.2024.2349897

Gouinguené SP, Turlings TCJ. 2002. The effects of abiotic factors on induced volatile emissions in corn plants. Plant Physiol. 129(3):1296–1307. https://doi.org/10.1104/PP.001941

Günnewich N, Page JE, Köllner TG, Degenhardt J, Kutchan TM. 2007. Functional expression and characterization of trichome-specific (-)-limonene synthase and (+)-α-pinene synthase from *Cannabis sativa*. Nat Prod Commun. 2(3):223–232. https://doi.org/10.1177/1934578X0700200301

Hilker M, Mcneil J. 2008. Chemical and behavioral ecology in insect parasitoids: how to behave optimally in a complex odorous environment. In: Behavioral ecology of insect parasitoids: from theoretical approaches to field applications. Wiley-Blackwell. p 92–112. https://doi.org/10.1002/9780470696200.CH5

Huang Y, Xie FJ, Cao X, Li MY. 2021. Research progress in biosynthesis and regulation of plant terpenoids. Biotechnol Biotechnol Equip. 35(1):1800–1809. https://doi.org/10.1080/13102818.2021.2020162

Hudz N et al. 2023. *Mentha piperita*: essential oil and extracts, their biological activities, and perspectives on the development of new medicinal and cosmetic products. Molecules. 28(21):7444. https://doi.org/10.3390/MOLECULES28217444

Ibrahim MA, Oksanen EJ, Holopainen JK. 2004. Effects of limonene on the growth and physiology of cabbage (*Brassica oleracea* L.) and carrot (*Daucus carota* L.) plants. J Sci Food Agric. 84(11):1319–1326. https://doi.org/10.1002/JSFA.1819

Idárraga SM et al. 2011. Sequences and transcriptional analysis of *Coffea arabica* var. Caturra and *Coffea liberica* plant responses to coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae: Scolytinae) attack. J Plant Interact. 7(1):56–70. https://doi.org/10.1080/17429145.2011.573151

Johnson MA, Ruiz-Diaz CP, Manoukis NC, Rodrigues JCV. 2020. Coffee berry borer (*Hypothenemus hampei*), a global pest of coffee: perspectives from historical and recent invasions, and future priorities. Insects. 11(12):1–35. https://doi.org/10.3390/insects11120882

Kanehisa M. 2019. Toward understanding the origin and evolution of cellular organisms. Protein Sci. 28(11):1947–1951. https://doi.org/10.1002/PRO.3715

Kanehisa M, Goto S. 2000. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 28(1):27–30. https://doi.org/10.1093/NAR/28.1.27

Kanehisa M, Furumichi M, Sato Y, Ishiguro-Watanabe M, Tanabe M. 2021. KEGG: integrating viruses and cellular organisms. Nucleic Acids Res. 49(D1):D545–D551. https://doi.org/10.1093/NAR/GKAA970

Langenheim JH. 1994. Higher plant terpenoids: a phytocentric overview of their ecological roles. J Chem Ecol. 20(6):1223–1280. https://doi.org/10.1007/BF02059809/METRICS

Loughrin JH, Manukian A, Heath RR, Turlings TCJ, Tumlinson JH. 1994. Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plant. Proc Natl Acad Sci U S A. 91(25):11836–11840. https://doi.org/10.1073/PNAS.91.25.11836

Magalhães DM et al. 2018. Identification of volatile compounds involved in host location by anthonomus grandis (Coleoptera: Curculionidae). Front Ecol Evol. 6:1–12. https://doi.org/10.3389/fevo.2018.00098

Maicas S, Mateo JJ. 2005. Hydrolysis of terpenyl glycosides in grape juice and other fruit juices: a review. Appl Microbiol Biotechnol. 67(3):322–335. https://doi.org/10.1007/S00253-004-1806-0/METRICS

Malik TG et al. 2023. Environmental factors affecting monoterpene emissions from terrestrial vegetation. Plants. Vol 12(17):3146. https://doi.org/10.3390/PLANTS12173146

Mann RS et al. 2012. Induced release of a plant-defense volatile 'deceptively' attracts insect vectors to plants infected with a bacterial pathogen. PLoS Pathog. 8(3):e1002610. https://doi.org/10.1371/JOURNAL.PPAT.1002610

Marie L et al. 2024. Combined sensory, volatilome and transcriptome analyses identify a limonene terpene synthase as a major contributor to the characteristic aroma of a *Coffea arabica* L. specialty coffee. BMC Plant Biol. 24(1):1–19. https://doi.org/10.1186/s12870-024-04890-3

Martin DM et al. 2010. Functional annotation, genome organization and phylogeny of the grapevine (vitis vinifera) terpene synthase gene family based on genome assembly, FLcDNA cloning, and enzyme assays. BMC Plant Biol. 10(1):226. https://doi.org/10.1186/1471-2229-10-226

Mateo JJ, Jiménez M. 2000. Monoterpenes in grape juice and wines. J Chromatogr A. 881(1–2):557–567. https://doi.org/10.1016/S0021-9673(99)01342-4

Mathieu F, Malosse C, Cain A, Frérot B. 1996. Comparative headspace analysis of fresh red coffee berries from different cultivated varieties of coffee trees. J High Resolut Chromatogr. 19(5):298–300. https://doi.org/10.1002/jhrc.1240190513

Mathieu F, Gaudichon V, Brun LO, Frérot B. 2001. Effect of physiological status on olfactory and visual responses of female *Hypothenemus hampei* during host plant colonization. Physiol Entomol. 26(3):189–193. https://doi.org/10.1046/J.1365-3032.2001.00232.X

Mathieu F, Malosse C, Frérot B. 1998. Identification of the volatile components released by fresh coffee berries at different stages of ripeness. J Agric Food Chem. 46(3):1106–1110. https://doi.org/10.1021/jf970851z

McKay SAB et al. 2003. Insect attack and wounding induce traumatic resin duct development and gene expression of (—)-pinene synthase in sitka spruce. Plant Physiol. 133(1):368–378. https://doi.org/10.1104/PP.103.022723

Mendesil E et al. 2009. Semiochemicals used in host location by the Coffee berry Borer, *Hypothenemus hampei*. J Chem Ecol. 35(8):944–950. https://doi.org/10.1007/S10886-009-9685-6

Mendoza-Mora JR. 1991. Reposta da broca-do-café, *Hypothenemus hampei*, a estimulos visuais e semioquimicos. Universidade Federal de Vicosa.

Mistry J et al. 2021. Pfam: the protein families database in 2021. Nucleic Acids Res. 49(D1):D412–D419. https://doi.org/10.1093/NAR/GKAA913

Mochizuki T, Ikeda F, Tani A. 2020. Effect of growth temperature on monoterpene emission rates of *Acer palmatum*. Sci Total Environ. 745:140886. https://doi.org/10.1016/J.SCITOTENV.2020.140886

Morehouse BR et al. 2017. Functional and structural characterization of a (+)-Limonene synthase from *Citrus sinensis*. Biochemistry. 56(12):1706–1715. https://doi.org/10.1021/ACS.BIOCHEM.7B00143/SUPPL_FILE/BI7B00143_SI_001.PDF

Njihia TN et al. 2014. Spiroacetals in the colonization behaviour of the coffee berry borer: a "push-pull" system. PLoS One. 9(11):e111316. https://doi.org/10.1371/journal.pone.0111316

Ortiz Américo, Ortiz Aristófeles, Vega FE, Posada F. 2004. Volatile composition of coffee berries at different stages of ripeness and their possible attraction to the coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae). J Agric Food Chem. 52(19):5914–5918. https://doi.org/10.1021/jf049537c

Peñuela-Martínez AE, Guerrero A, Sanz-Uribe JR. 2022. Cromacafé® Herramienta para identificar los estados de madurez de las variedades de café de fruto rojo. AVT. 535:1–8. https://doi.org/10.38141/10779/0535

Phillips MA, Wildung MR, Williams DC, Hyatt DC, Croteau R. 2003. cDNA isolation, functional expression, and characterization of (+)-α-pinene synthase and (−)-α-pinene synthase from loblolly pine (*Pinus taeda*): stereocontrol in pinene biosynthesis. Arch Biochem Biophys. 411(2):267–276. https://doi.org/10.1016/S0003-9861(02)00746-4

Redgwell RJ, Curti D, Rogers J, Nicolas P, Fischer M. 2003. Changes to the galactose/mannose ratio in galactomannans during coffee bean (*Coffea arabica* L.) development: implications for in vivo modification of galactomannan synthesis. Planta. 217(2):316–326. https://doi.org/10.1007/s00425-003-1003-x

Rejesus BM, Baldos EP. 1982. The biology of coffee berry borer *Hypothenemus hampei* (Ferr.) (Scolytidae, Coloeptera) and its incidence in the Southern Tagalog Provinces [Philippines]. Philipp Entomol. 4:303–316.

Riedlmeier M et al. 2017. Monoterpenes support systemic acquired resistance within and between plants. Plant Cell. 29(6):1440–1459. https://doi.org/10.1105/TPC.16.00898

de la Rosa-Cancino W, Alavez-Rosas D, Rojas JC. 2021. Host conspecific infestation level guides the preference of *Hypothenemus hampei* for robusta coffee berry volatiles. Arthropod Plant Interact. 15(4):573–582. https://doi.org/10.1007/s11829-021-09845-1

Rosenkranz M, Chen Y, Zhu P, Vlot AC. 2021. Volatile terpenes – mediators of plant-to-plant communication. Plant J. 108(3):617–631. https://doi.org/10.1111/TPJ.15453

Salojärvi J et al. 2024. The genome and population genomics of allopolyploid *Coffea arabica* reveal the diversification history of modern coffee cultivars. Nature Genetics 2024. 56(4):721–731. https://doi.org/10.1038/s41588-024-01695-w

Schnee C et al. 2006. The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores. Proc Natl Acad Sci U S A. 103(4):1129–1134. https://doi.org/10.1073/PNAS.0508027103

Sengonca C, Kranz J. 2001. A modified, four-amed olfactometer for determining olfactory reactions of beneficial arthropods. Anz Schadlingskd. 74(5):127–132. https://doi.org/10.1046/J.1439-0280.2001.01022.X

Solovyev V, Kosarev P, Seledsov I, Vorobyev D. 2006. Automatic annotation of eukaryotic genes, pseudogenes and promoters. Genome Biol. 7 Suppl 1(1):1–12. https://doi.org/10.1186/GB-2006-7-S1-S10/TABLES/8

Staudt M, Bertin N. 1998. Light and temperature dependence of the emission of cyclic and acyclic monoterpenes from holm oak (*Quercus ilex* L.) leaves. Plant Cell Environ. 21(4):385–395. https://doi.org/10.1046/J.1365-3040.1998.00288.X

Steele CL, Crock J, Bohlmann J, Croteau R. 1998. Sesquiterpene synthases from grand fir (*Abies grandis*). J Biol Chem. 273(4):2078–2089. https://doi.org/10.1074/jbc.273.4.2078

Tholl D. 2006. Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. Curr Opin Plant Biol. 9(3):297–304. https://doi.org/10.1016/J.PBI.2006.03.014

Tobing MC, Sinaga SCT, Pramayudi N. 2022. The used of attractants from coffee at various heights traps to control Coffee Berry Borer and quality test of coffee berry. IOP Conf Ser Earth Environ Sci. 974:012024. https://doi.org/10.1088/1755-1315/974/1/012024

Untergasser A et al. 2012. Primer3-new capabilities and interfaces. Nucleic Acids Res. 40(15):e115–e115. https://doi.org/10.1093/nar/gks596

Vivaldo G, Masi E, Taiti C, Caldarelli G, Mancuso S. 2017. The network of plants volatile organic compounds. Sci Rep. 7(1):1–18. https://doi.org/10.1038/s41598-017-10975-x

Weckerle B, Tóth G, Schreier P. 2003. Linalool disaccharides as flavour precursors from green coffee beans (*Coffea arabica*). Eur Food Res Technol. 216(1):6–10. https://doi.org/10.1007/S00217-002-0614-Y

Williams DC, McGarvey DJ, Katahira EJ, Croteau R. 1998. Truncation of limonene synthase preprotein provides a fully active 'pseudomature' form of this monoterpene cyclase and reveals the function of the amino-terminal arginine pair. Biochemistry. 37(35):12213–12220. https://doi.org/10.1021/BI980854K

Xu BB, Liu ZD, Sun JH. 2014. The effects of α-pinene on the feeding performance and pheromone production of *Dendroctonus valens*. Entomol Exp Appl. 150(3):269–278. https://doi.org/10.1111/EEA.12161

Zhang CP et al. 2022. Genome-wide identification and characterization of terpene synthase genes in *Gossypium hirsutum*. Gene. 828:146462. https://doi.org/10.1016/J.GENE.2022.146462