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Volatile Composition of Coffee Berries at Different Stages of Ripeness and Their Possible Attraction to the Coffee Berry Borer *Hypothenemus hampei* (Coleoptera: Curculionidae)

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The analysis of volatile emissions of coffee berries in different physiological states of ripeness was performed using dynamic headspace and gas chromatography/mass spectrometry analysis for *Coffea arabica*, var. Colombia. The composition of the volatiles emitted by coffee berries is dominated by very high levels of alcohols, mainly ethanol, in all stages of ripeness in comparison with other compounds. Overripe coffee berries have high volatile emissions and show a composition dominated mainly by esters followed by alcohols, ketones, and aldehydes. The lowest level compounds were monoterpenes. 2-Methyl furan was detected in various ripening stages; this compound has not been previously reported as a coffee berry volatile. The presence of ethanol and other alcohols in the volatile composition might explain the effectiveness of using traps with mixed alcohols for detection and capture of coffee berry borers.

KEYWORDS: ***Coffea*; volatiles; attraction; coffee berry borer; *Hypothenemus*; broca**

INTRODUCTION

The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae), is widely considered to be the most serious pest of coffee throughout the world (*1*). Research on this insect increased dramatically after its introduction to the major coffee producing countries in South and Central America and, in particular, Colombia. Control of the coffee berry borer initially relied on the use of chemical insecticides such as endosulfan, which in some countries has been banned for its negative impact on human health and the environment; furthermore, cases of coffee berry borer resistance to endosulfan have been reported (*2*). Nowadays, there is a great deal of information on biocontrol approaches based on parasitoids and entomopathogenic fungi that can be used against the coffee berry borer (*1*). Cultural practices, including a thorough and complete harvest of all berries, also contribute to reducing coffee berry borer population levels. However, coffee berries that are not picked during harvest or that fall on the ground are a source of coffee berry borer inoculum from which to start new infestations (*1*).

When an adult female coffee berry borer emerges from a berry in which it has mated with a sibling male, it has a short window of time in which to attack another berry. The ripeness of berries is known to influence colonization by the beetles. In choice tests, adult females preferred red berries to green berries and were more attracted to volatiles emitted by red berries (*3*). Similarly, Mendoza et al. (*4*) reported an increased attraction to red or black (overripe) berries over green or yellow berries and to red berries that were either healthy or attacked by the insect over green berries.

Knowledge of the specific composition of volatiles emitted by coffee berries at different ripening stages could lead to a better understanding of how individual components or combinations of these components affect coffee berry borer behavior. With this information, it might be possible to infer plant resistance mechanisms or to develop attractants or repellents that can be used in integrated pest management programs and in the design of insect traps for detection, population estimation, and mass capture. The purpose of this report is to elucidate the composition of volatile emissions of coffee berries in different physiological states of ripeness of *Coffea arabica* var. Colombia.

MATERIALS AND METHODS

Berries of *C. arabica* var. Colombia were collected in a 3 year old plantation growing at 1400 m above sea level at the National Coffee Research Center (Cenicafé) in Chinchiná, Caldas, Colombia. Each berry was cut from the tree with scissors leaving the peduncle attached to the berry. The berry collections were always made for one defined ripeness stage at each specific collection time, near midday (10.00 a.m.−12.00 noon) and repeated on different days for three replicate analyses in each specific ripeness stage (data presented in the Results section are the averages for three replicates). This procedure was carried out

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from May to August 2003. The berries (240 g for each ripeness stage replicate) were classified according to ripeness: (i) green berries were a uniform green color, weighing ca. 1.70 g each; (ii) half-ripe berries were mixed light green to yellow and pale red colors, weighing ca. 2.1 g each; (iii) ripe berries were a glossy red color, weighing ca. 2.3 g each; and (iv) overripe berries were a very dark red color like blackberries, weighing ca. 2.2 g each. The time elapsed from collection to analysis of each sample was less than 1 h, considering the short distance (<2 km) between the coffee plantation and the laboratory.

Isolation of volatile emissions of coffee berries and analysis by dynamic headspace, trap concentration, and thermal desorption were performed on coffee berries detached from the plant using the ex situ dry technique of Jakobsen (*5*). Coffee berry samples were introduced into a glass chamber; the 240 g sample size was chosen in order to fill the chamber completely. The glass chamber was adapted to a Tekmar 3000 (Tekmar-Dorman, Mason, OH) purge and trap system coupled through a fused silica transfer line set at 150 °C to a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, Avondale, PA) and a Trio 2000 mass spectrophotometer (Micromass Limited, Wythenshawe, United Kingdom) operated with MassLynx NT version 3.2 (Micromass Limited).

The purge and trap system was operated in an automatic sequence, beginning by purging the glass sample chamber for a defined period of time at ambient temperature and collecting the volatiles swept from the sample of coffee berries in a concentrating trap of Tenax (Tekmar-Dorman, Trap 1), glass-lined, 12 in. long × 1/8 in. outer diameter and maintained at 40 °C. The moisture was then driven from the Tenax trap by purging with dry gas for 1 min, bypassing the sample chamber. The Tenax trap was then heated to 225 °C, the operating temperature specified for desorption, and coupled by a six port valve set at 150 °C through the transfer line to the gas chromatograph in a direct mode introduction at the injection port of the chromatograph. The desorption time was 2 min, selected as optimal after experimenting with 1−4 min. Finally, the Tenax trap was uncoupled from the gas chromatograph and set in bake out mode for 10 min to clean the trap of any highly concentrated compounds or nonvolatile compounds.

After various times for purging and concentrating volatile emissions from the different coffee berries samples were evaluated, it was determined that 1 h was a sufficient period; a 2 h period resulted in no differences in the individual relative concentrations of components in chromatograms of samples of the same ripeness stage. Helium was chosen as the purging gas, with a flow of 40 mL per minute, after observing no significant differences in chromatograms using helium, nitrogen, or air. Some consider that anaerobic conditions result in increased alcohol dehydrogenase activity in plant material (*6*) although Buterry et al. (*7*) found no significant difference among the constituents isolated when using air or nitrogen as the sweeping gas. The purging gas and carrier gas for the chromatograph, also helium, were supplied through a common hydrocarbon trap. The gas chromatography separation of volatiles was carried out using a DB-Wax capillary column (50 m × 0.32 mm i.d., 1 μm film thickness, J&W Scientific, Folsom, CA) with a split−splitless injection port temperature at 250 °C and an oven temperature programmed at 35 °C for 5 min ramped to 220 °C at 7 °C per minute and held at 220 °C for 4 min. The carrier gas was operated in the constant flow mode at 1.2 mL/min. The transfer line between the gas chromatograph and the mass spectrometer was held at 250 °C. The mass spectrometer was operated in the electron impact ionization mode at 70 eV, scanning from 30 to 330 amu at a rate of 2 scans per second.

RESULTS

Typical total ion current chromatograms of headspace samples of coffee berries in different stages of ripeness are shown in **Figure 1**. The chromatograms have been stripped by subtracting a single spectrum of the representative background to remove unwanted background and noise included by the low limit of the mass scan scale (30 amu). Because of the large concentrations of some individual compounds, it was necessary to amplify 100 times the vertical scale of abundances of the chromatograms of the green, half-ripe, and ripe berries, while leaving the chromatogram of the overripe berries unamplified in order to appreciate the differences in the profiles of composition of volatiles in all stages of ripeness (see **Figures 2** and **3**). All chromatograms are presented with link vertical axes, showing the same vertical scale for peak heights, where the maximum height is presented on the chromatogram of overripe berries (8.8 min), thus allowing an appreciation of the characteristic evolution of various common compounds. All of the compounds represented in the green stage berries chromatogram are common to the other stages of ripeness, and there are large differences in their abundances as the stage of ripeness of the berries advances. The overripe berries show the largest number of compounds detected, with some of them at the highest level of abundance. The ripening process, from the last stage of green ripeness to ripe glossy red berries, is characterized by the development of a complex aroma dominated by large quantities of ethanol.

Table 1 presents the headspace analysis of coffee berries in different stages of ripeness with chemical identification of the detected compounds obtained by comparison of each spectrum with NIST 98 Libraries and Structures and the WILEY AccessPak Library. **Table 1** shows the name of the compound with its retention time (t_R) identified according to the best quality value reported by the process of comparison with the used libraries (above 90%). The total number of compounds detected in each ripeness stage and the total abundance are presented in area counts per sample of 240 g for each ripeness stage and its area count distribution in each individual compound.

A large increase in volatile emissions (**Table 1**) was detected from the last stage of green maturation (51494 total area counts) to the half-ripe (304560 total area counts) and ripe stages (332035 total area counts). The high volatile concentration in the ripe stage is normally due to physiological phenomena of degradation products, texture change, drying, and loss of weight of the berries (*6*). Meanwhile, the number of compounds in each stage of ripeness goes from 27 in the green to 68 in the overripe stage (**Table 1**). Almost all volatile components are common in all berry stages tested but at different intensities, except for a few: 2-Methyl-propanal, methyl acetate, 3-methyl-furan, 3-methyl-butanal, 3-methyl-2-butenal, and β-ocimene begin to be detected at the half-ripe stage and have a low increasing emission in the ripe stage. The volatile composition in all stages of ripeness is dominated by high levels of ethanol, increasing steeply from the green to the half-ripe stage of maturation and then increasing more slowly in the ripe to overripe stages. However, in this latter stage, ethanol is not the major component, occurring at a lower concentration than the acetic acid ethyl ester. Alcohols may have been magnified by the polar column used (DB-Wax), but the same profile was obtained with the almost nonpolar (5% phenyl−95% dimethylpolisiloxane) column DB-5.

2-Methyl-furan has not been previously reported as a coffee berry volatile (*8*); this compound exhibited a decreasing intensity from green to half-ripe to ripe berries (**Table 1**), which is in marked contrast to the normally increasing intensity from the green to the overripe stage of most aldehydes, ketones, and alcohols.

Dimethyl disulfide is present in all stages of ripeness at very low levels, and dimethyl sulfide was detected in some instances at trace levels in green berries. Monoterpenes have been reported in coffee berries (*8*), and some such as β-myrcene, 1-phellandrene, α-terpinene, β-ocimene, and (+)-2-carene were found at very low and trace levels in the headspace analysis. To rule

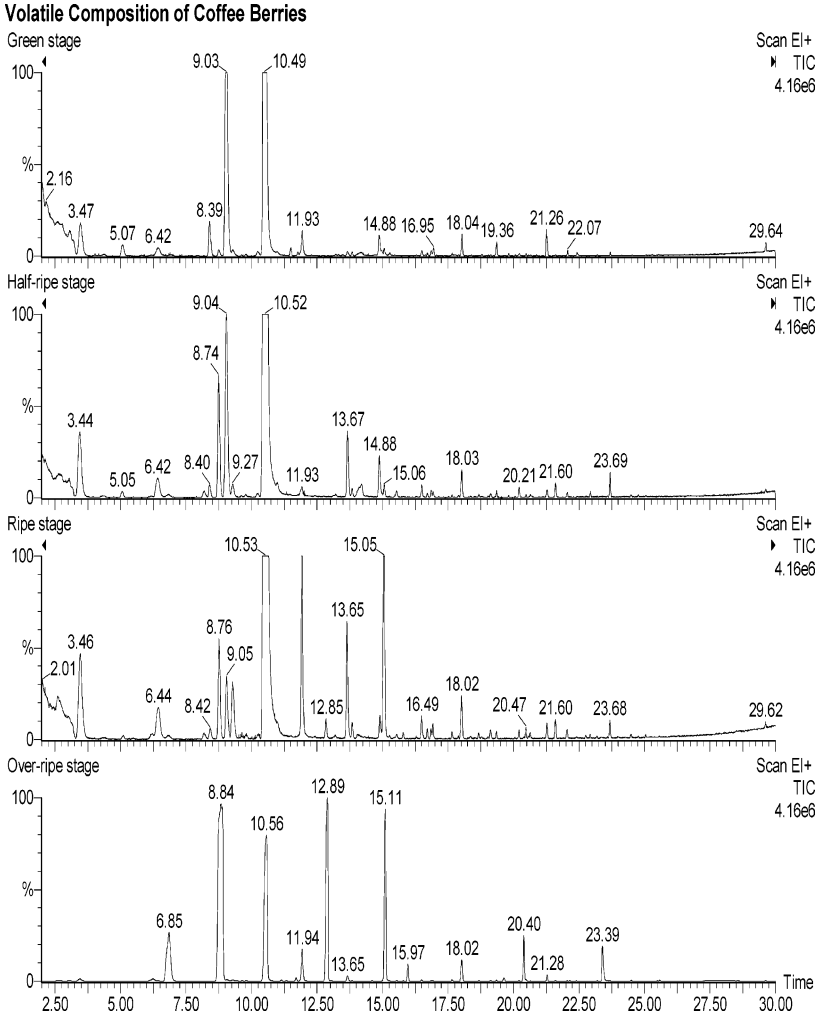

Figure 1. Typical total ion current chromatograms of headspace samples of coffee berries in different stages of ripeness.

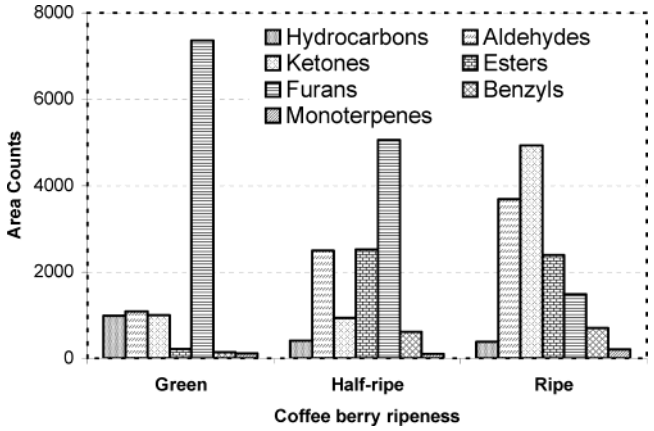

Figure 2. Volatile composition by functional group for green, half-ripe, and ripe berries using data presented in **Table 1**. Each bar contains the sum of area counts for compounds in each functional group.

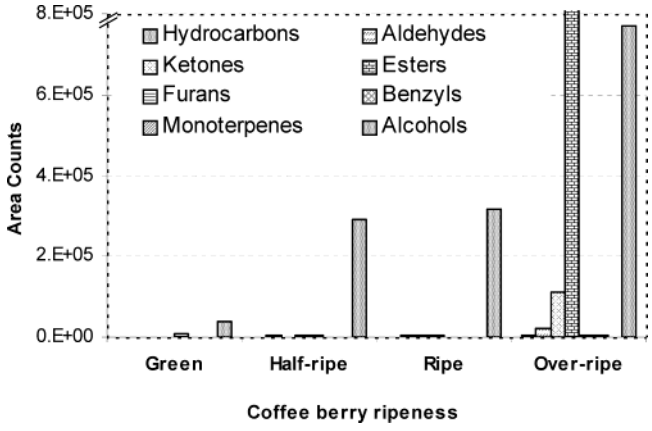

Figure 3. Volatile composition by functional group including the overripe berries to reflect the large intensity of esters and alcohols in this stage of ripeness.

out the possibility of oxidative decomposition in Tenax traps, small samples of a few pine tree needles—known to be a good source of monoterpene compounds—were analyzed following the headspace methodology used for coffee berries and resulted in good separation and intensities of most known compounds of this type.

Other compounds detected in this study include acetaldehyde, acetic acid ethyl ester, ketones (e.g., 2-propanone and 3-methyl-2-butanone), and alcohols (e.g., 1-propanol and 2-methyl-1-propanol). There was an overall increasing compound concentration from the green to the ripe stage of development with a pronounced expression in the overripe stage, where the volatile composition is dominated by esters and alcohols (**Figures 2** and **3**).

DISCUSSION

The presence of ethanol and other alcohols in the volatile emissions of coffee berries may explain why traps using mixed alcohols are effective for the detection and capture of coffee

Table 1. Headspace Analysis of Coffee Berries in Different Stages of Ripeness with Chemical Identification of the Detected Compounds Obtained by Comparison of Each Spectra with NIST 98 Libraries and Structures and the WILEY AccessPak Library

volatile composition of coffee berries		area counts/ 240 g sample				volatile composition of coffee berries		area counts/ 240 g sample			
compound	t_R	green	half-ripe	ripe	overripe	compound	t_R	green	half-ripe	ripe	overripe
1,3-butadiene, 2-methyl-	2.68				1839	β-myrcene	16.92	112	73	154	593
unknown	3.04	160	97		1529	1-phellandrene	17.16				196
acetaldehyde	3.45	1027	2201	3017	8731	acid acetic, pentyl ester	17.28				91
octane	5.06	282	138	70	396	α-terpinene	17.53				252
propanal, 2-methyl-	6.23			170	9831	2-heptanone	17.66			79	1703
2-propanone	6.42	353	711	1289	789	isoamyl alcohol	18.02	258	371	582	31265
methyl acetate	6.85		89	125	193375	3-pentanol, 2-methyl-	18.39				211
furan, 3-methyl-	8.23		155	160	207	β-ocimene	18.68		32	58	614
nonane	8.39	703	231	207	541	1-pentanol	19.03				153
acetic acid, ethyl ester	8.84	115	2372	1846	853170	1,3,7-octatriene, 3,7-dimethyl	19.12		46	107	867
furan, 2-methyl-	9.07	7361	4573	1331	3987	3-octanone	19.35	155	76	77	1194
unknown	9.28	159	210	1245	3040	styrene	19.64				4270
unknown	9.45				589	(+)-2-carene	20.06				168
propanal, 2,2-dimethyl-	9.64				488	unknown	20.21	29	120	100	892
butanal, 3-methyl	9.80		34	75	738	3-hydroxy-2-butanone	20.40				54001
propanoic acid, 2-methyl-, methyl ester	9.95				313	heptanol	20.48	16	27	98	2052
ethanol	10.56	39055	289641	308099	437377	cyclopentanol, 2-methyl, *trans*	20.64			49	200
unknown	11.00		331		252	2-buten-1-ol, 2-methyl-	20.71				135
propanoic acid, ethyl ester	11.14			44	1658	3-ethyl, 2-pentanol	21.14				156
propanoic acid, 2-methyl ethyl ester	11.34				856	hexanol	21.28	301	81	175	6530
acetic acid, propyl ester	11.70	46			5050	anisole	21.60		151	199	470
2-butanone, 3-methyl-	11.94	487	149	3487	53753	3-hexen-1-ol, formate	22.05	59	59	120	385
acetic acid, 2-methylpropyl ester	12.89			258	364014	butanol, 3-methyl-, acetate	22.15				462
1-propanol	13.65	86	1121	1896	9495	3-ethyl, 4-methylpentan-1-ol	22.40				535
toluene	13.85	51	102	195	376	pentanol, 3,4-dimethyl-	22.77			34	181
2-butenal	14.03			72	132	1-octen-3-ol	23.19				441
acetic acid, butyl ester	14.61				1071	acetic acid	23.39				53452
disulfide, dimethyl-	14.88	390	593	256	138	benzene, 1-methoxy-3-methyl	23.68	32	243	168	859
1-propanol, 2-methyl-	15.11	106	138	5507	281861	formic acid, octyl ester	24.49			35	908
2-butenal, 2-methyl-	15.54		102	43	513	unknown	25.08				157
2-pentanol	15.80			77	535	unknown	25.50				376
1-butanol, 3-methyl-, acetate	15.97				23299	propanedioic acid, dimethyl-	25.58				1109
1-butanol	16.49	64	162	316	1800	total area counts		51494	304560	332035	2427296
O-xylene	16.71	30	46	106	98	no. of compounds		27	34	41	68
1-hexene-3-ol	16.86	56	83	112	578						

berry borers. Mendoza-Mora (*9*) was the first to report ethanol: methanol as a coffee berry borer attractant, and subsequent work has shown this mixture to be an effective and inexpensive attractant (*10*−*14*), although further increases in attraction can be achieved with organic extracts of coffee berries. Velasco Pascual et al. (*15*, *16*) compared crude ethanol and methanol extracts from ripe berries of three different coffee varieties and found significant differences in attraction levels, although these differences among varieties were not consistent over time. Velasco Pascual et al. (*15*, *16*) showed that the berry extract has some components that increase attraction over ethanol or methanol alone or ethanol:methanol mixtures. Similarly, Gutiérrez-Martínez and Ondarza (*17*) reported increased coffee berry borer attraction toward ripe coffee berry extracts obtained with methylene chloride; solvents alone attracted significantly fewer insects. In addition, they report caffeine dissolved in ethanol as a strong attractant. Cárdenas (*12*) used caffeine in his trapping devices with no significant increases in the number of coffee berry borers captured.

It is important to differentiate between attraction toward the berry and colonization of the berry; the latter is known to depend on the dry matter content, which has to be above 20% (*1*). Thus, volatiles might be the initial factor in attracting the coffee berry borer to the berry, and colonization would then depend on internal factors within the berry. Our results show various chemical group volatiles present in all stages of ripeness, including green berries, in contrast to Mathieu et al. (*8*) who only identified a single compound, limonene, in green berries. They reported that *C. arabica* produced high terpene levels while *Coffea canephora* produced high terpenes and sesquiterpenes and concluded by stating that further studies would be conducted to determine the role of terpenes in attracting the coffee berry borer. We detected trace amounts of five monoterpenoids (β-myrcene, 1-phellandrene, α-terpinene, β-ocimene, and (+)-2-carene), and all but two (β-myrcene and β-ocimene) occurred only in overripe berries. Monoterpenes are known to serve as volatile cues that attract insects (*18*, *19*); thus, β-myrcene and β-ocimene are good candidates for future coffee berry borer attraction tests. In an earlier study, Mathieu et al. (*20*) reported a total of 28 volatiles in *C. arabica* and *C. canephora* red berries although not all were detected across all samples. In the present study, we detected 27 chemicals in green berries, 34 in half-ripe berries, 41 in ripe berries, and 68 in overripe berries. We detected high levels of furans in green berries, with levels decreasing as the ripeness increased. Furans are common volatiles in green and roasted coffee, which have been implicated in aroma (*21*); the high levels of furans in green beans warrant further tests to determine whether they attract the coffee berry borer.

In this study, we have identified the major volatiles emitted by *C. arabica* berries at various stages of development. This information may serve as a framework for the formulation of improved coffee berry borer attractants with defined compositions. Testing some of the chemicals detected in this study under

controlled conditions in the laboratory, e.g., using electroantennograms and choice olfactometer bioassays (*22*), should pinpoint what chemicals elicit responses in the coffee berry borer, resulting in a scientific-based selection of coffee berry borer attractants and, perhaps, repellents. The current use of ethanol:methanol mixtures, which is based on empirical tests, has room for improvement.

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