First Characterisation of Volatile Organic Compounds Emitted by Banana Plants

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Banana (Musa sp.) ranks fourth in term of worldwide fruit production, and has economical and nutritional key values. The Cavendish cultivars correspond to more than 90% of the production of dessert banana while cooking cultivars are widely consumed locally around the banana belt production area. Many plants, if not all, produce Volatile Organic Compounds (VOCs) as a means of communication with their environment. Although flower and fruit VOCs have been studied for banana, the VOCs produced by the plant have never been identified despite their importance in plant health and development. A volatile collection methodology was optimized to improve the sensitivity and reproducibility of VOCs analysis from banana plants. We have identified 11 VOCs for the Cavendish, mainly (E,E)-α-farnesene (87.90 ± 11.28 ng/μl), methyl salicylate (33.82 ± 14.29) and 6-methyl-5-hepten-2-one (29.60 ± 11.66), and 14 VOCs for the Pacific Plantain cultivar, mainly (Z,E)-α-farnesene (799.64 ± 503.15), (E,E)-α-farnesene (571.24 ± 381.70) and (E)β-ocimene (241.76 ± 158.49). This exploratory study paves the way for an in-depth characterisation of VOCs emitted by Musa plants.

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We have determined that eight VOCs were emitted by both Cavendish and Pacific Plantain varieties, representing a relative proportion of 73 and 57% of the total emitted VOCs for Cavendish and Pacific Plantain respectively. Among these, three VOCs ((Z) β-ocimene, 6-methyl-5-hepten-2-one, and β-ionone) were produced in similar proportions between the two varieties. The relative proportions among the remaining common VOCs (myrcene, Z and E β-farnesene, linalool and alloocimene. The main compounds emitted were ketones, esters and aldehydes.

Eight compounds were common between the two varieties (myrcene, Z and E β-ocimene, 6-methyl-5-hepten-2-one, 6-methyl-3,5-heptadien-2-one, α-farnesene, methyl salicylate and β-ionone). The main compounds emitted by Cavendish and Pacific Plantain cultivars are (E,E)-β-ocimene, 6-methyl-5-hepten-2-one and β-ionone). The only exception was (Z) β-ocimene, 6-methyl-5-hepten-2-one and β-ionone, which were 1.5 times higher in Cavendish than in Pacific Plantain. Limonene, α- and β-pinene were only detected with Cavendish while Pacific Plantain emitted specifically (E)-hex-2-enal, DMNT, (E)-β-farnesene, linalool and alloocimene.

### Table 1. Volatile organic compounds for banana plants.

| Compounds* | CAS | IUPAC names | RI Cal | RI Std | Ref # | Identification* | Class | Ref RI |
|------------|-----|-------------|--------|--------|-------|-----------------|-------|--------|
| α-pinene   | 80-56-8 | 4,6,6-trimethylcyclo[3.1.1]hept-3-one | 1031 | 1018 | 1032 | MS RI STD | Ae | 3.30 ± 1.24 | — | A |
| β-pinene   | 127-91-3 | 6,6-dimethyl-4-methylidenecyclo[3.1.1]heptane | 1109 | 1102 | 1124 | MS RI STD | Ae | 1.23 ± 0.47 | — | A |
| Myrcene    | 123-35-3 | 7-methyl-3-methylidenedeca-1,6-diene | 1163 | 1158 | 1156 | MS RI STD | Ae | 3.84 ± 1.95 | 16.89 ± 12.85 | A |
| Linalool   | 138-86-3 | 1-methyl-4-prop-1-en-2-ylcyclohexene | 1191 | 1186 | 1178 | MS RI STD | Ae | 15.19 ± 5.54 | — | B |
| (E)-hex-2-enal | 6728-26-3 | (E)-hex-2-enal | 1218 | 1213 | 1207 | MS RI STD | Ad | — | 60.23 ± 34.64 | A |
| (Z)-β-ocimene | 3338-55-4 | (3Z)-3,7-dimethylocta-1,3,6-triene | 1228 | 1222 | 1245 | MS RI STD | Ae | 8.92 ± 4.04 | 10.36 ± 7.19 | B |
| (E)-β-ocimene | 13877-91-3 | (3E)-3,7-dimethylocta-1,3,6-triene | 1244 | 1240 | 1242 | MS RI STD | Ae | 8.86 ± 4.45 | 241.76 ± 158.49 | B |
| 4,8-dimethyl-1,3,7-nonatriene | 19945-61-0 | (3E)-4,8-dimethylhexa-1,3,7-triene | 1302 | — | — | MS | Ae | — | 226.20 ± 62.01 |
| 6-methyl-5-hepten-2-one | 110-93-0 | 6-methylhept-5-en-2-one | 1328 | 1329 | 1340 | MS RI STD | K | 29.60 ± 11.66 | 18.56 ± 20.89 | D |
| Alloocimene | 673-84-7 | (4E,6E)-2,6-dimethylocta-2,4,6-triene | 1361 | 1364 | 1367 | MS RI STD | Ae | — | 3.82 ± 2.65 | D |
| Linalool | 78-70-6 | 3,7-dimethylocta-1,6-dien-3-ol | 1535 | 1539 | 1537 | MS RI STD | Ae | — | 6.96 ± 2.89 | B |
| 6-methyl-3,5-heptadien-2-one | 1604-28-0 | (3E)-6-methylhepta-3,5-dien-2-one | 1576 | 1583 | 1590 | MS RI STD | K | 14.83 ± 10.84 | 18.78 ± 26.86 | E |
| (E)-β-farnesene | 18794-84-8 | (6E)-7,11-dimethylidenodeca-1,6,10-triene | 1654 | 1661 | 1658 | MS RI STD | Ae | — | 126.02 ± 87.79 | B |
| (Z,E)-α-farnesene | 26560-14-5 | (3Z,6E)-3,7,11-trimethylenodeca-1,3,6,10-tetraene | 1716 | 1715 | 1727 | MS RI STD | Ae | — | 799.64 ± 503.15 | C |
| (E,E)-α-farnesene | 502-61-4 | (3E,6E,7,11)-trimethylenodeca-1,3,6,10-tetraene | 1740 | 1742 | 1756 | MS RI STD | E | 87.90 ± 11.28 | 571.24 ± 381.70 | C |
| Methyl salicylate | 119-36-8 | methyl 2-hydroxybenzoate | 1755 | 1765 | 1745 | MS RI STD | E | 33.82 ± 14.29 | 145.42 ± 163.03 | B |
| (Z)-β-ionone | 79-77-6 | (E)-4-(2,6,6-dimethylcyclohexen-1-yl)but-3-en-2-one | 1924 | 1934 | 1918 | MS RI STD | Ae | 3.76 ± 0.85 | 3.47 ± 1.76 | A |

### Results

#### Adaptation of VOCs collection and analysis protocol.

The development of a reliable and sensitive protocol was a challenge due to the low level of VOCs emission by the banana plant. Several steps of the extraction protocol (sampling method, sampling time) were optimized compared to existing protocols and the improved protocol was described in the material and methods section.

We have robustly identified 11 compounds emitted by Cavendish plants and 14 emitted by Pacific Plantain (Table 1). The selection criteria and validation of the VOCs extracted for each representative variety were: their presence in at least 3 of 4 repetitions, and the difference in emissions or total absence in the control sample. The identification of the compounds was through their Mass Spectrum (MS) and their Retention Index (RI). Most of these compounds belong to the terpenes group (8 for Cavendish, 10 for Pacific Plantain).

The VOCs quantities were obtained by cavity (ng/l), respectively.
An extensive bibliographical analysis was carried out on the 17 VOCs identified in this study. A first comparison has been made with the VOCs known to be produced by other banana organs (see Table 2). Among the VOCs identified in this study, four were already detected in *Musa* sp. flowers (α and β-pinene, limonene and myrcene)\(^{11}\), three in the whole fruit of *M. acuminata* cv. Nanicão (α-pinene, limonene, and ocimene), and two in the pulp of the aforementioned cultivar (limonene, (E)-hex-2-enal)\(^{10}\). Limonene is therefore the only VOC detected in the four studies related to *Musa* genus. It was however only found on Cavendish plants in our study.

In addition, VOCs were compared to the identified compounds of other plants in the same Order (Zingiberales), and in particular to the ones emitted by the flowers of *Hedychium coronarium*, commonly named “Ginger lily”\(^{13}\). Fourteen VOCs (Table 2) were identified in these studies. We observed that three of them (limonene and α and β-pinene) were specifically emitted by Cavendish, four were specific to Pacific Plantain ((E)-hex-2-enal, DMNT, alloocimene and linalool) and seven were common between the two varieties. Furthermore, all the 17 VOCs detected in our work have already been identified in other plants\(^{14}\) (Table 2), but to our knowledge, the blend of VOCs emitted by Pacific Plantain and Cavendish varieties might be unique, in confirmation of the general likelihood of a taxa-specific VOCs emission\(^{15,16}\). This hypothesis would nevertheless need the additional testing of many other varieties and closely related species.

According to Pherobase and a bibliographical survey\(^{14}\), all 17 identified VOCs are known to play a role in insects control, and 11 identified VOCs (7 for Cavendish (64% of the total emission) and 8 for Pacific Plantain (57%)) have a role in controlling plant diseases. Among all identified VOCs, and regarding their functions in insect management, 14 showed an attraction function (91% for Cavendish and 79% for Pacific Plantain), 13 are allomones (82% for Cavendish and 71% for Pacific Plantain), 16 are kairomones (91% for Cavendish and 93% for Pacific Plantain), and 15 are pheromones (100% for Cavendish and 86% for Pacific Plantain). It is worth mentioned that methyl salicylate (second most abundant compound emitted by Cavendish and fifth by Pacific Plantain) is also known to be an herbivore induced compound, that the plant used as an insect protection agent\(^{17}\). In addition, 11 VOCs have also shown antimicrobial effects. Alpha and β-pinene have antimicrobial activities\(^{18,19}\). Myrcene is known for its antifungal\(^{20}\) and antibacterial properties\(^{21}\). Limonene presents a strong antifungal activity\(^{20,22–24}\) and it has been proven that antifungal activity of citrus essential oils on *Aspergillus flavus*, *Penicillium chrysogenum* and *Penicillium verrucosum* was explained by its prevalence\(^{25}\).

The (E)-hex-2-enal, belonging to the Green Leaf Volatiles (GLVs) group, is well known to exhibit an antimicrobial activity\(^{26}\) and a strong antifungal activity *in-vitro*\(^{27}\), and in fields of soybean, conference pears, stone fruits, and common bean\(^{28}\). This VOC is also implicated as a defence response mechanism in the Lima bean\(^{29}\), similarly to *Nicotiana tabacum*\(^{30}\) when infected. Another defence signalling compound is the (E)-β-farnesene, an alarm pheromone\(^{31,32}\).

Herman *et al.*\(^{33}\) found that linalool significantly increased the antimicrobial activity of essential oils when it was combined to them, and suggest that the synergism could be through enhancing the uptake of other antimicrobials by the cell wall of the pathogens\(^{33}\). In addition, methyl salicylate, which is the main compound of the essential oils of *Laportea aesteans*, showed inhibitory potential against *E. coli* and *S. aureus*\(^{34}\). At the opposite, 6-methyl-5-hepten-2-one (third most abundant in Cavendish and ninth in Pacific Plantain) was found to be an endogenous germination stimulators of uredospores of *Puccinia graminis* var. *tritici*\(^{35}\).

All the compounds emitted specifically by Pacific Plantain show protection properties. (E)-hex-2-enal, DMNT\(^{36}\) and linalool\(^{37}\) have insect and disease control properties. (E)-β-farnesene, is an alarm pheromone
emitted by aphids in case of predator or parasitoid attack, that can be used by plants as repulsive agent and disease control properties. In addition, the GLV (E)-hex-2-enal, and methyl salicylate, are reported to be involved in the plant-plant communication, as an alarm signal for the neighbouring plants, when they are exposed to injured plants VOCs.

This study opens therefore the field on new research axes to further investigate if several Cavendish and/or Pacific Plantain VOCs identified, are involved, individually or in a blend with specific concentration ratios, in plant-pathogen and plant-plant interaction.

Conclusion

This study reports for the first time the VOCs emitted by aerial parts of Cavendish and Pacific Plantain banana plants. Despite low levels of emission, the VOCs collection revealed that Cavendish emitted 11 compounds VOCs, mainly (E,E)-α-farnesene (87.90 ± 11.28 ng/µL), methyl salicylate (33.82 ± 14.29) and 6-methyl-5-hepten-2-one (29.60 ± 11.66), while Pacific Plantain emitted 14 VOCs, mainly (Z,E)-α-farnesene (799.64 ± 503.15), (E,E)-α-farnesene (571.24 ± 381.70) and (E) β ocimene (241.76 ± 158.49). Even if the majority of the detected compounds (11 of all the VOCs combined) have known disease and insect control properties, they are produced at low levels and their potential effect in Musa should be further confirmed. In conclusion, this exploratory study paves the way for an in-depth characterisation of VOCs emitted by the Cavendish and Pacific Plantain, focusing on their roles and emissions in biotic and abiotic stress management.

Materials and Methods

Banana plants. The plant material used was the banana cultivar Williams, of the Cavendish cultivar group (AAA genome), and the banana cultivar Pacific Plantain, of the Maia Maoli/Popoulu cultivar group (AAB genome), grown from in vitro meristems, kindly provided by the International Transit Center (ITC) for banana germplasm (Bioversity, Leuven, Belgium). Growing conditions in greenhouse were 25 °C at 16 h/8 h (Day/Night) photoperiod. The plants were grown in pots of 20 cm diameter, containing a peat moss substrate, and irrigated every 1 or 2 days when needed, until they reach 40–60 days of age, stage of VOCs extraction, having 5–6 leaves. The plants submitted to VOCs sampling were virus-free, they had neither visible disease symptoms nor observable pests, and did not present any wound.

Collection and analysis of VOCs. The volatile collection protocol was a dynamic system, and a thermal desorption was performed for the analysis method, due to its efficiency compared to a solvent desorption. Four entire banana plants (50 cm high, 5 to 6 leaves) of each variety were placed at each extraction in 4 identical glass chambers (40,000 ml), previously cleaned with RBS detergent (Chemical Products R. Borghgraef S.A., Belgium) and rinsed with distilled water, to collect the volatile compounds, using a "Push-Pull" system (Fig. 2). The air was pushed through the system by an air pump (Rocker 430 Vacuum/Pressure Pump, NSE, GA, USA) that provided a constant airflow set at 1000 ml/min. The air entering the chamber was previously cleaned (Clean Air Supply System CASS 6, Volatile assays, Rensselaer, NY, USA). A filter, made of a glass cartridge filled with 60 mg of absorbent material (TENAX TA wax trap, Gerstel, Mülheim an der Ruhr, Deutschland), was placed at the exit of the glass chamber and was used to catch the volatile compounds carried by the air being pulled from the chamber using a second air pump (Rocker 400 Vacuum Pump, NSE, GA, USA). The filters were previously cleaned by a thermal conditioner (TC2, Gerstel, Mülheim an der Ruhr, Deutschland), for a period of 11 hours at 300 °C.

Table 2. Occurrence of VOCs in plants. aBestmann et al. 11, bFacundo et al. 10, cPherobase.com 14.
prior to each extraction. Four replicates per variety were conducted simultaneously, along with a fifth chamber containing a pot filled with the same substrate, but without the plant. The VOCs collection was performed at 24 ± 2 °C, with a relative humidity of 47 ± 6% and an artificial photoperiod of 16 h/8 h (Day/Night) under LED lamps (77 lmol/sqm/s).

The collected VOCs were analyzed by a Gas Chromatography-Mass Spectrometry (GC-MS) thermal desorption method (7890A, Agilent Technologies, Santa Clara, CA, USA). In this system, the content of the TENAX TA trap was thermally desorbed (Thermal Desorption Unit, Gerstel, Mülheim an der Ruhr, Deutschland) at 280 °C for 10 minutes prior to the injection. In each sample, 1 μl of an Internal Standard (I.S.) (butylbenzen, 100 ng/ml), diluted from a pure solution (Sigma-Aldrich, Saint-Louis, MO, USA) in a methanol solvent (VWR, West Chester, PA, USA) was automatically added for the quantification control purpose (MultiPurpose Sampler, Gerstel, Mülheim an der Ruhr, Deutschland).

The entire sample was injected in the column (polar column VF-WAXms, 30 m, internal diameter: 0.25 mm, thickness: 0.25 μm, Agilent Technologies, Santa Clara, CA, USA) of the GC machine. Conducted by a Helium gas (1.5 ml/min), the VOCs were prone to the following temperature program: 35 °C for 2 minutes, followed by an increase of 5 °C/min up to 155 °C, and then 20 °C/min until reaching 250 °C, which was maintained for 10 minutes.

The identification that followed was insured by the Mass-Spectrometer (5973, Agilent Technologies, Santa Clara, CA, USA) via the electronic impact ionisation (70 eV), and a separation through the quadripolar filter at 150 °C.

MSD ChemStation Software (Agilent Technologies, Santa Clara, CA, USA) was used to characterize the chromatograms received from all previous analyses, by the mean of manual integration, comparing the retention times with the WILEY275 spectral database, only taking into consideration the compounds found at least in 3 of the 4 repetitions. The compounds were rejected if similar quantities are collected from both the control and the plants. Further identification was carried out by comparing the theoretical Kovats retention indices (RI) with calculated ones for each molecule. RI were calculated by injecting a saturated n-alkane standard solution (C7–C30) at 1,000 μg/mL in hexane (Supelco, Belgium), following the definition of van Den Dool and Kratz43. In addition, identifications were confirmed by injecting commercial standards provided by Sigma-Aldrich (Sigma-Aldrich, Saint-Louis, MO, USA).

Quantifications were determined by constructing calibration curves, for each volatile chemical group, injecting authentic standards in the same previews analysis conditions.

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Author Contributions
B.C., D.C.C. and L.C. performed the experiments, B.C. performed the quantifications, B.C., D.C.C., F.M.-L., B.A., K.A., V.F. and M.S. commented and discussed the manuscript, L.C. provided the figure, D.C.C., F.M.-L., B.A., K.A., V.F. and M.S. commented and discussed the manuscript.
Additional Information

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