http://dx.doi.org/10.5935/0103-5053.20160194

J. Braz. Chem. Soc., Vol. 27, No. 8, 1484-1492, 2016.
Printed in Brazil - ©2016 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Diurnal Pattern of Leaf, Flower and Fruit Specific Ambient Volatiles above an Oil Palm Plantation in Pará State, Brazil

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Oil palm plantations are rapidly expanding in the tropics because of insatiable global demand for fruit oil to be used in food, biofuels and cosmetics. Here we show that three tissue-specific volatiles can be quantified in ambient air above an African-American hybrid oil palm plantation in Brazil and linked photosynthesis (isoprene), floral scent (estragole), and for the first time, fruit oil processing (6-methyl-5-hepten-2-one, MHO). Plant enclosure techniques verified their tissue specific emission sources with ambient concentrations displaying distinct diurnal patterns above the canopy. Isoprene concentrations were near zero at night, but dramatically increased during the day while estragole showed elevated concentrations at night suggesting a light-independent, temperature-driven emission pattern from flowers. MHO also showed elevated concentrations at night and both estragole and MHO increased during the day. Our observations demonstrate that the African-American oil palm hybrid is strong isoprene emitter and suggest that MHO is a specific oxidation product of lycopene released during the industrial processing of palm oil. This study highlights the potential value of quantifying volatile oil palm signals in the atmosphere as a novel, non-invasive method to better understand biological functioning and its interactions with the environment including carbon assimilation, floral-insect interactions, and fruit oil production/processing.

Keywords: bioactive compounds, biomarkers, chemical ecology, essential oils, environmental analysis/quality, mass spectrometry

Introduction

Oil palm is cultivated in the tropics as a major global source of healthful and low-cost vegetable oil for use in food additives and as a cooking oil with its widespread use in a multitude of food products increasingly consumed around the world.[1] It is highly desirable due to its low cost, high stability to oxidation (e.g., during transport and processing), and healthy aspects including a composition of trans-free fatty acids (predominantly oleic acid in the *cis* confirmation) as well as cholesterol-free.[2] In addition to food, palm oil has many diverse uses including as biofuels, industrial lubricants, and cosmetics.[3,4] The high global demand for palm oil has resulted in a concerning trend of rapid placement of rainforest with oil palm plantations, particularly in Southeast Asia.[4] However, the replacement of forest is not a requirement as the replacement of anthropogenic grasslands is also occurring, for example in Brazil. Estimates suggest that global demand for palm oil has not peaked with an estimated demand of 240 Mt by 2050, nearly twice the total in 2009.[5]

Globally, the largest oil palm plantations are concentrated in the Asian countries of Malaysia and Indonesia where an African species (*Elaeis guineensis* Jacq.) is utilized.[6] In 2015, relatively little oil palm was grown outside of Malaysia and Indonesia, which accounted for roughly 85.0% of total global

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production.[4] However, some tropical areas in Latin America and Africa are also being explored as suitable areas for oil palm cultivation. In Brazil, land used for oil palm plantations is dominated by Pará State, where an initial planting of 52,058 hectares in 1995 increased to 255,000 hectares by 2014.[7] The limited success of this expansion is, in part, due to the development of an intra-specific hybrid obtained by crossing the African palm oil (*Elaeis guineensis* Jacq.) with the American palm (*Elaeis oleifera* (Kunth) Cortés), which conferred partial resistance against the fatal yellowing disorder, responsible for the loss of thousands of plants in Brazil.[8] In Brazil, oil palm plantations were initially established in the 1970's, although only a few studies have been published discussing the physiology of the African-American hybrid with the majority of publications focusing on the various problems limiting the successes and expansion of plantations in Brazil including pollination, oil palm bud rot,[8] and fatal yellowing disorder.[9] In Brazil, land-use change associated with this expansion process has acted to convert degraded cattle pasture to oil palm plantations, and therefore enhance water cycling and ecosystem carbon assimilation and storage.[3] This land-use change may also be associated with the emission of oil palm specific volatile organic compounds (VOC) into the atmosphere, which impact air quality and climate as well as participate in key biological processes including photosynthesis, reproduction, and response to abiotic and biotic stress.[10] However, little is known about oil palm-specific VOC emissions from the African-American hybrid in Brazil, as to our knowledge no studies have been reported to-date.

VOC emission studies in Southeast Asia demonstrated high leaf and plantation-scale isoprene (C_5H_8) emissions from the African oil palm (*Elaeis guineensis*) during the daytime.[11] Moreover, nighttime estragole emissions ($C_{10}H_{12}O$), involved in recruitment of natural pollinators, have been reported as large fluxes from flowers and plantations of Asian oil palm.[12] However, neither leaf isoprene nor floral estragole emissions have been verified from the African-American hybrid. Although the exact mechanisms are under investigation, isoprene production in plants has been shown to protect photosynthesis during oxidative stress associated with high light and leaf temperatures typical of tropical climates[13] and derive carbon intermediates directly from photosynthesis.[14]

While isoprene emissions have been extensively characterized in mid-latitude tree species, little is known about its distribution of this reactive alkene among natural and managed tropical ecosystems,[15] which are estimated to dominate global VOC emissions.[16] In the atmosphere, VOCs exert a large effect on atmospheric chemistry, air quality, and climate including the formation of ground-level ozone[17] and the formation/growth of secondary organic aerosols,[18] and by controlling the lifetimes of atmospheric oxidants and greenhouse gases.[19] Therefore, understanding the chemical composition and geographical distribution of VOC emissions in the tropics is very important because of the complex biosphere-atmosphere interactions and feedback processes linking terrestrial carbon and water cycling with atmospheric chemistry and climate. Understanding these interactions is particularly important in the tropics where large-scale land-use change, increasing global temperatures and changing precipitation patterns are dramatically altering terrestrial carbon, water, and energy cycling, with local to global consequences. Therefore, there is an urgent need to better understand these interactions, in particular those associated with palm oil production in Brazil.

Although oil palm plantations provide numerous products of high commercial value, to our knowledge, a specific VOC linked with oil production and processing has not been described. As deployed in South East Asia,[11] online mass spectrometers are widely used in VOC studies in the atmosphere (e.g., proton transfer reaction-mass spectrometry, PTR-MS), which provide high time resolution, but often limited compound identification capabilities. In this study, we first present a new automated method for trace (few ppb to tens of ppt) identification and quantification of VOCs in ambient air above an African-American hybrid oil palm plantation near Belém, Brazil. The method allows the characterization of the diurnal patterns ambient air VOC concentrations, and therefore aids the characterization of different biological and industrial sources of VOCs and their environmental controls (e.g., light, temperature, vertical mixing). The new method is based on automated sequential collection of air samples from a tower platform onto 28 individual thermal desorption tubes using a self-contained waterproof air sample collection system (Less-P®, Signature Science Inc.), followed by VOC analysis with gas chromatography-mass spectrometry (GC-MS). To verify the identity of different VOC sources within the oil palm plantation, we used manual collections of air samples on thermal desorption tubes from plant enclosures on the ground to identify tissue-specific VOC emissions from leaves, flowers, and fruits, including the first report of atmospheric 6-methyl-5-hepten-2-one (MHO) as a volatile biomarker of lycopene oxidation, possibly during fruit oil processing. Diurnal pattern of VOC concentrations in ambient air above the plantation demonstrates the ability to study these biological and industrial processes at the landscape-scale. Our study provides a foundation for future biosphere-atmosphere studies on African-American hybrid oil palm plantations

in Brazil and provides new insights into key biological and industrial processes including photosynthesis and growth, floral scents and reproduction, and palm oil production and processing. We suggest that the atmospheric based methods described here can be used to study these important biological and industrial processes in the future from the scales of individual tissues, plantations, and regions as oil palm plantations expand throughout the pantropics.

Experimental

Site description

The field data collection was conducted in an oil palm plantation situated about 130 km from Belém, capital of Pará State, Brazil. The plantation contains a flux tower situated in its center (1º58'43.22''S; 48º36'52.36''W) for studying trace gas and energy fluxes, as well as a palm oil processing plant on the edge of the plantation (see Figure 1).

The experiment was conducted using two methods for tower-based and ground-based VOC air sampling. The tower-based method utilized a programmable automatic air sampler to collect VOCs from ambient air on the walkup tower above the canopy (once *per* hour) throughout a diurnal period. On the ground, individual thermal desorption tubes were manually collected onto thermal desorption tubes from plant enclosures without (background control) and with biological oil palm tree tissue including leaves, flowers, and fruits, in order to isolate and verify oil palm tree VOC sources.

Ambient air VOCs

A diurnal pattern of VOC concentrations in ambient air was analyzed on April 1, 2015, by installing a thermal desorption tube autosampler (Less-P®, Signature Science Inc.) just above the oil palm canopy (8.1 m height) on the walkup tower in the center of the plantation. Air samples were delivered to the Less-P® system through 1 m of 1/8" O.D. Teflon PFA tubing extending out roughly 0.5 m from the tower using an aluminum boom. The Less-P® internal battery was fully charged before field deployment and was loaded with 28 freshly conditioned thermal desorption tubes purchased commercially (QTC®, Markes International), which were packed with quartz wool, Tenax TA, and Carbograph 5TD adsorbents. No sample was collected from the first tube in the Less-P® (blank), with the remaining tubes sequentially activated for sampling by passing 50 mL min^{-1} of ambient air through the tube for one hour (3.0 L total). Sampling was initiated at 10:40 pm on the previous day and stopped at 5:40 pm on April 1, 2016, due to the onset of a rainstorm. Thus, an

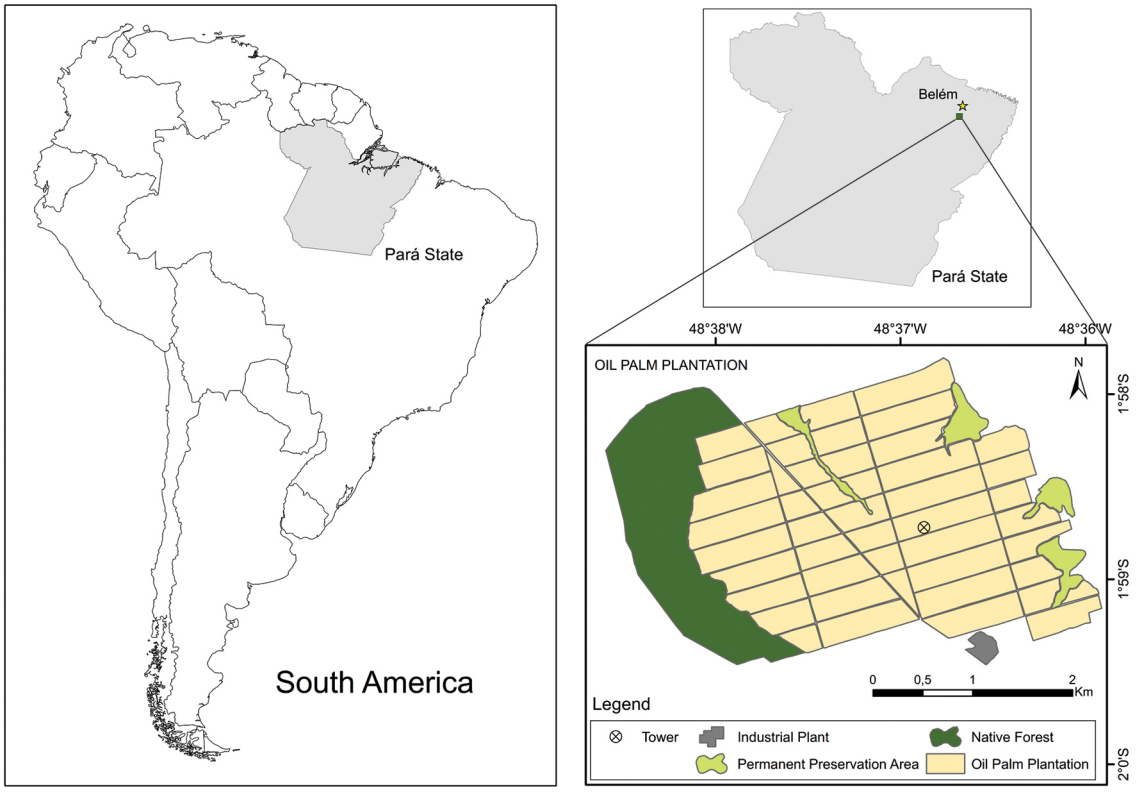

Figure 1. Location of the oil palm plantation and the tower near Belém, Brazil, where the study was conducted.

hourly time series consisting of 21 consecutive samples were collected with VOCs identified and quantified using thermal desorption GC-MS. This study represents the first field deployment of the Less-P® VOC autosampler (serial No. 008) for automated tower-based collection of ambient VOCs throghout a diurnal period.

Tissue-specific VOC emissions from leaves, flowers and fruits

In order to evaluate potential oil palm tree sources of VOCs quantified in the ambient air study, plant enclosure and ambient air proximity measurements were performed for leaves, fruits, and flowers. The same type of thermal desorption tubes used in ambient air sampling (quartz wool, Tenax-TA, carbograph 5TD), were used in plant enclosure/proximity studies. For all samples, a portable battery powered pump was used to draw air samples through the thermal desorption tube at 150 mL min^{-1} for 10 min (1.5 L).

Leaf emissions of VOCs were evaluated in the morning to mid-afternoon on April 1, 2015, by utilizing a 300 mL glass enclosure in the sun with the enclosure inlet exposed to ambient air. For each leaf sample studied, an enclosure background sample was collected consisting of an empty enclosure in the sun. For study, two trees were selected near the tower and two leaves were selected *per* tree with one on the top of the tree and the other closer to the ground. Following the collection of the background sample, thermal desorption samples were collected immediately upon placing the leaf inside the enclosure. The first leaf sample was measured at 9:20 am and the last one at 4:30 pm on April 1, 2015. In total, 16 leaf samples were measured inside the glass chamber.

Emissions of floral VOCs were evaluated by collecting ambient air samples at the very top of the walkup tower as a background sample, and roughly 0.5 m from inflorescences of the oil palm. In total, three samples of different inflorescences from different oil palm individuals were collected. The first flower sample was collected at 8:40 am and the last sample was collected at 11:25 am on April 1, 2015.

VOCs emitted by damaged oil palm fruits and/or their oils were analyzed using two thermal desorption tube samples including with no air sample collection and an enclosure air sample collection containing three partially damaged oil palm fruits placed inside.

VOC's analysis by GC-MS

Thermal desorption tubes were analyzed for VOCs collected on thermal desorption tubes using a thermal desorption system (TD-100®, Markes International) interfaced with a gas chromatograph/electron impact mass spectrometer with a triple-axis detector (5975C series, Agilent Technologies) at the National Institute for Amazon Research (INPA) in Manaus, Brazil, as previously described.[14] After loading a tube in the TD-100 system, the collected samples were dried by purging for 4 min with 50 mL min^{-1} of ultra-high-purity helium (all flow vented out of the split vent) before being transferred (290 °C for 5 min with 50 mL min^{-1} of helium) to the TD-100 cold trap (air toxics) held at 30 °C. During GC injection, the trap was heated to 290 °C for 3 min while backflushing with carrier gas at a flow of 3.5 mL min^{-1}. Simultaneously, 2 mL min^{-1} of this flow was directed to the split, and 1.5 mL min^{-1} was directed to the column (Agilent DB624, 60 m × 0.32 mm × 1.8 µm). The GC oven temperature was programmed with an initial hold of 3 min at 40 °C followed by an increase to 230 °C at 6 °C min^{-1}, followed by a hold at 230 °C for 3 min. The mass spectrometer was configured for trace analysis with a 15-times detector gain factor and operated in scan mode (*m/z* 35-150). To minimize the analyte loss during sample transfer, all tubing downstream of the thermal desorption tube inside the TD100, as well as the sample transfer line to the GC, were continuously maintained at 190 °C.

The GC-MS was calibrated to authentic standards of estragole and 6-methyl-5-hepten-2-one (MHO) (99%, Sigma Aldrich) in methanol using the dynamic solution injection (DSI) technique.[20] The GC-MS was calibrated to isoprene by dynamic dilution with hydrocarbon free air of a 1.0 ppm gas-phase standard in nitrogen (Apel-Riemer, Inc.).

Results and Discussion

Plant enclosures

In order to characterize VOC sources from the African-American hybrid oil palm, tissue-specific VOC emissions from leaves, flowers, and fruits, were characterized using manual collections of air samples on thermal desorption tubes from plant enclosures (leaf and fruit) and using proximity ambient air samples (flowers). From GC-MS chromatograms obtained, the occurrence of three main VOCs were found to be produced/emitted by oil palm tissues: isoprene, estragole, and 6-methyl-5-hepten-2-one (MHO), representative of leaves, flowers and fruits, respectively.

Leaves

For VOCs emissions from oil palm leaves, the glass enclosure method detected the emission of large

amounts of the compound isoprene (C_5H_8) from all leaf samples collected. The occurrence of isoprene emissions from tropical leaves has been previously described as mainly deriving from carbon and energy resources from photosynthesis through a direct utilization of photosynthetic products by the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway; the isoprene formation pathway that occurs within the thylakoid membranes of chloroplasts located inside the mesophyll cells of leaves.[13,14] The GC-MS chromatogram obtained from the glass chamber with a leaf inside shows a strong peak for this compound occurring with a retention time of 7.2 min (Figure 2). In contrast, a relatively small peak could be observed for the empty enclosure, likely due to isoprene present in the ambient air, while thermal desorption tubes without an air sample collection showed a negligible isoprene peak.

Figure 2 also shows the mass spectra of an example isoprene peak from a leaf sample (top mass spectra) plotted together with the mass spectra of isoprene from the National Institute of Standards and Technology (NIST 2011) mass spectral library (bottom mass spectra). The results show a good match between these two mass spectra (a match greater than 90%). Moreover, the isoprene peak from the leaf samples shares the same mass spectra and retention time of the standard (not shown). These results gives us a high degree of certainty that like the African oil palm in Asia,[11] African-American hybrid oil palm trees in Brazil also produce copious amounts of isoprene in their leaves during photosynthesis. While potentially limiting the carbon and energy resources that could be utilized for other processes including oil production, our observations are consistent with a functional role of isoprene as an antioxidant in protecting photosynthesis during abiotic stress including high light and temperature conditions characteristic of tropical regions.

Flowers

For all ambient air samples collected near oil palm flowers, strong peaks of the aromatic VOC estragole were detected. As an example, GC-MS chromatograms collected near a flower showed a large peak for this compound with a retention time of 30.8 min, while the ambient air sample at the top of the tower during the day showed a relatively smaller peak (Figure 3). Thermal desorption tube samples without an air sample collection showed negligible peaks for estragole. Figure 3 also shows the mass spectra of an estragole sample peak from an ambient air sample collected near a flower (top mass spectra) and the mass spectra of estragole from the NIST 2011 mass spectral library (bottom mass spectra), demonstrating a good match between these two peaks

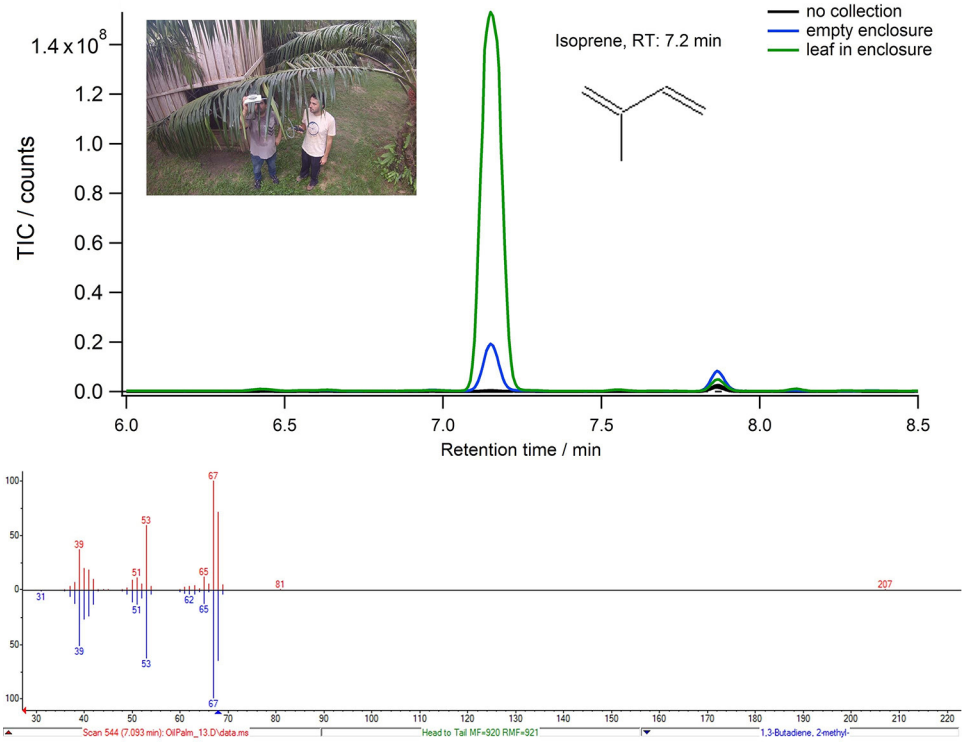

Figure 2. GC-MS chromatogram showing the peak for isoprene (RT: 7.2 min) from three thermal desorption tube samples including no air sample collection, an air sample collection from an empty glass leaf enclosure with ambient air, and the same enclosure with a palm leaf. Also shown is the mass spectra of the isoprene sample peak (top mass spectra) and the mass spectra of isoprene from the NIST 2011 mass spectral library (bottom mass spectra).

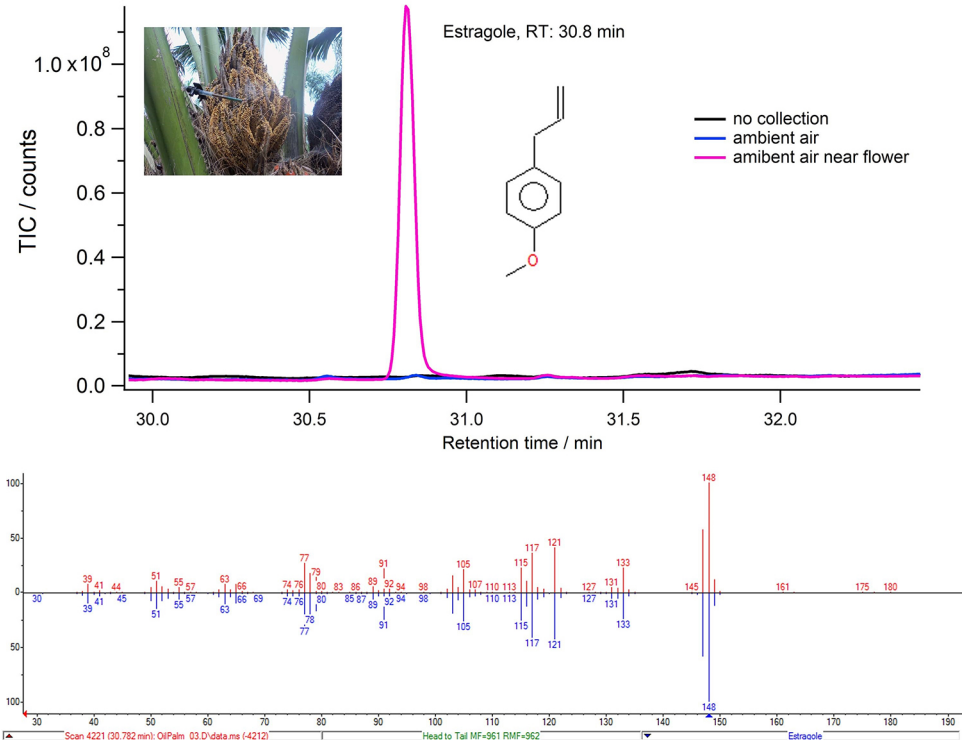

Figure 3. GC-MS total ion chromatogram (TIC) showing the peak for estragole (RT: 30.8 min) from a thermal desorption tube samples including with no air sample collection, with an ambient sample collection at the top of the flux tower, and an ambient air collection less than 0.5 m from an oil palm flower. Also shown is the mass spectra of the estragole sample peak (top mass spectra) and the mass spectra of estragole from the NIST 2011 mass spectral library (bottom mass spectra).

(> 90%). These results give us a high degree of confidence that like the African oil palm in Asia,[12] African-American hybrid oil palm tree flowers in Brazil emit large amounts of estragole.

Estragole emissions have been previously described to act as a powerful attractant for pollinators including the weevil, *Elaeidobius kamerunicus*, which was shown to improve pollination and increase fruit set in African oil palm plantations in Malaysia.[21] However, in Brazil, pollination is carried out by artificial methods, which increases labor and material costs.[22] Thus, quantifying the temporal dynamics of estragole emissions should be an essential part of future biological pollination activities in Brazilian oil palm plantations.

Fruits

When VOC emissions were evaluated from fruits, no significant emissions were detected from intact fruits placed in an enclosure (not shown). However, a small, but clear peak of 6-methyl-5-hepten-2-one (MHO), was detected from damaged fruit placed in the enclosure (Figure 4). This could most clearly be observed in the selected ion chromatogram (*m/z* 43) which shows a peak for MHO with a retention time of 24.8 min in thermal desorption tube samples collected from enclosures with damaged oil palm fruits, but not from blank tubes without samples collected. When compared with the mass spectra of MHO from the NIST 2011 mass spectral library (Figure 4, bottom mass spectra), the MHO sample peak (Figure 4, top mass spectra) showed a good match (> 80%) and presented the same retention time and mass spectra as an MHO standard. These observations strongly suggest that the damaged palm fruit emitted MHO as a volatile, which is widely recognized to be a specific biomarker of temperature-dependent lycopene oxidation in red fruit.[23-25]

Crude palm oil has a rich orange-red color due to its high content of carotenoids which is dominated by α, β-carotene and lycopene.[26] These nutritional components of palm oil, in particular lycopene, have been shown to reduce chronic diseases in humans such as cancer and heart disease through their antioxidant behavior.[27] However, palm oil carotenoids are oxidized during oil processing giving rise to volatile oxidation products which are removed from the oil during a deodorization step.[26] This gives rise to the intriguing possibility that MHO emissions from palm oil processing and refining could be used to study both palm oil stability and quality during processing, and the environmental impacts of palm oil production through tower based measurements or other atmospheric platforms, such as drones. If verified in future studies, MHO emission measurements may, therefore, provide a new tool in the study of industrial processes associated with the loss of

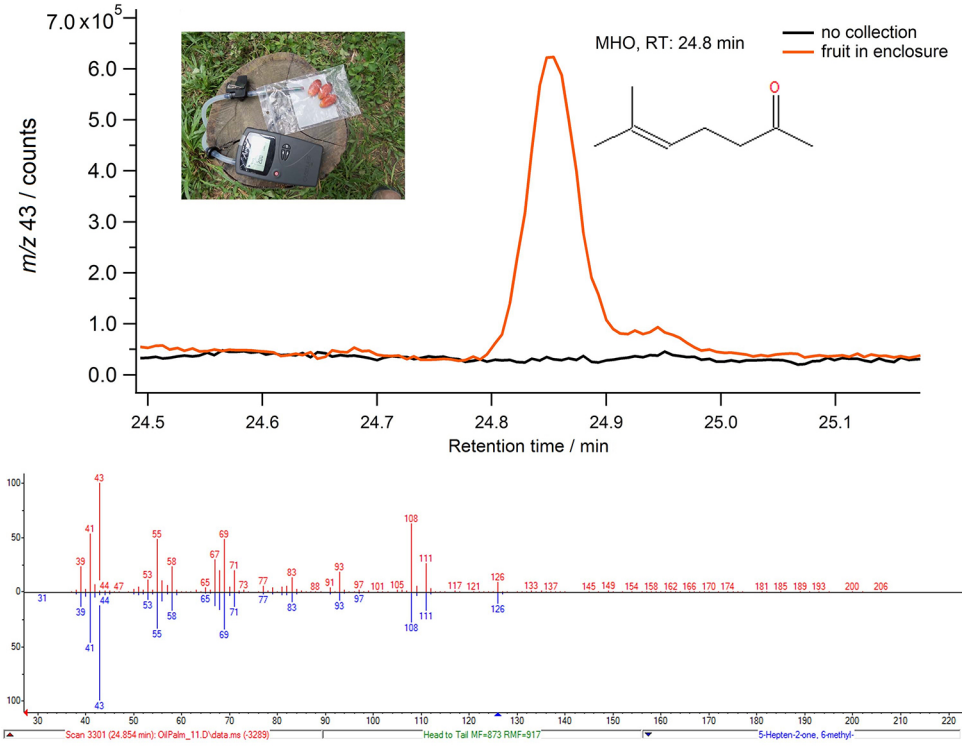

Figure 4. GC-MS selected ion chromatogram (*m/z* 43) showing the peak for MHO (RT: 24.8 min) from two thermal desorption tube samples including with no air sample collection and an enclosure air collection with three partially damaged oil palm fruits placed inside. Also shown is the mass spectra of the MHO sample peak (top mass spectra) and the mass spectra of MHO from the NIST 2011 mass spectral library (bottom mass spectra).

the nutritionally important carotenoids such as lycopene, which reduces palm oil stability through a decrease in antioxidant activity.

Ambient air

Ambient concentrations of the three VOCs found to be emitted from leaf, flower, and fruit tissues of African-American oil palm hybrids were also quantified in the atmosphere just above the canopy of the oil palm plantation on the walkup tower. Diurnal observations throughout the night and day revealed that each volatile had a distinct temporal pattern, probably due to environmental controls over biological emissions, wind direction and transport of industrial emissions, as well as vertical atmospheric dilution (Figure 5).

Consistent with a strict light dependence linked with photosynthesis, ambient isoprene concentrations during the night were very low, but strongly increased after 8:00 am, following sunrise. Isoprene ambient concentrations continued to increase in the morning, peaking near mid-day at over 12 ppb when light and temperature were the highest. In contrast, the volatile floral compound estragole, showed elevated concentrations at night (up to 2 ppb) suggesting a light-independent, temperature-driven emissions from flowers. This is consistent with similar observations of estragole ambient concentrations in an oil palm plantation in Borneo, which also accumulated at night, and whose emission rates correlated with ambient temperature.[12] However, following sunrise, estragole ambient concentrations dramatically decreased, probably due to a dilution effect from the initiation of vertical turbulent mixing of the boundary layer driven by surface heating. Following this initial dilution in the morning, estragole ambient concentrations continued to increase throughout the day, likely due to increased emission rates as the temperature increased.

The diurnal variation of MHO concentrations showed a similar pattern to estragole with elevated concentrations of this compound during the night, possibly as a result of the industrial palm oil processing/refining activities that were occurring 24 hours a day (Figure 5). However, ambient concentrations of MHO gradually decreased throughout the night, possibly due to the shifting of wind directions away from the oil processing plant. Following the early morning minimum, MHO ambient concentrations increased sharply at 8:00 am, possibly due to the shifting of winds and/or the recontamination of the ambient air by the palm oil processing emissions. MHO ambient concentrations remained high throughout the afternoon. In the late afternoon, clouds were building and by 4:00 pm, an intense rainstorm began, driving down the concentrations

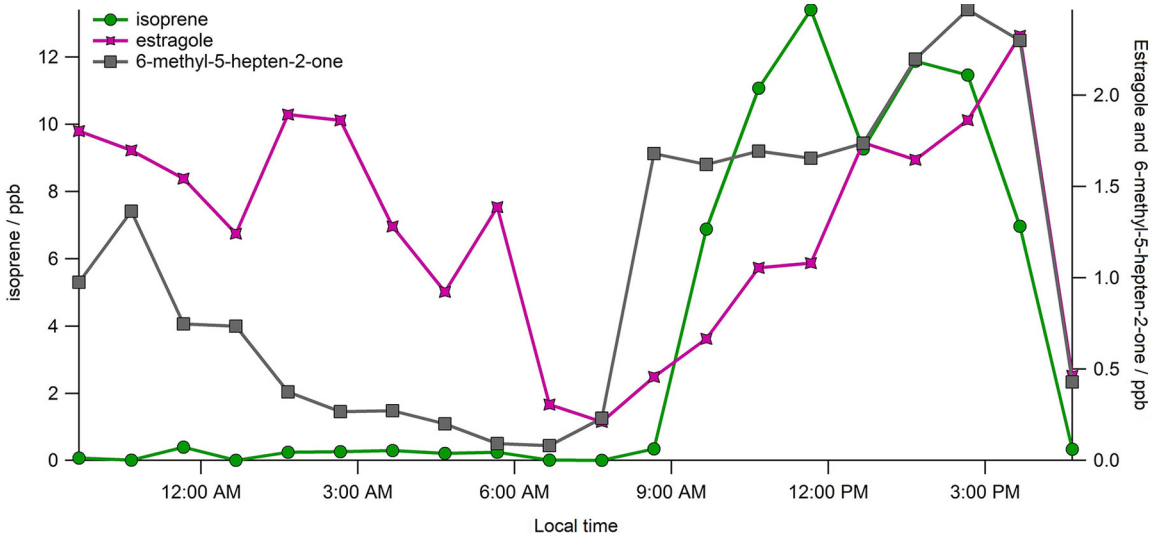

Figure 5. Diurnal variations in ambient concentrations of isoprene, estragole, and 6-methyl-5-hepten-2-one (MHO) at 8.1 m height above an oil palm plantation near Belém, Brazil. Note the last sample was collected during the onset of a rainstorm.

of all ambient VOCs, likely through the both decrease in biological emission rates and increase in dilution from vertical mixing of the boundary layer.

Conclusions

Oil palm plantations in Brazilian Amazon are currently limited in productivity and size due to numerous biological diseases and other biological problems, including lack of a biological pollinators, oil palm bud rot, fatal yellowing disorder, and saturated soils during the rainy season. Nonetheless, the Brazilian Amazon has suitable cleared land for oil palm which, in total, could account for twice that used globally.[22] Demand for food and biofuel products from oil palm are expected to grow in the future with Brazil poised to become the leading global exporter. Therefore, a better biological understanding of oil palm physiological functioning in the tropical environment is needed including new methods which enable a remote monitoring of plantations from towers and other atmospheric platforms. In this study, we provide a new, compound specific method to monitor VOCs emitted from oil palm plantations in Brazil on diurnal time-scales at the whole stand scale. We provide the first observations which verify that the African-American oil palm hybrid is a strong isoprene emitter during leaf photosynthesis (daytime) and a strong estragole emitter during floral emissions (both night and day). These VOCs could, therefore, be used to monitor plant growth/carbon assimilation, including responses to abiotic stress, as well as future biological pollination activities, respectively.

We observed significant ambient concentrations of MHO (both night and day), which has been previously described as a specific oxidation product biomarker of the carotenoid lycopene found in many red fruits. As damaged fruits showed small emissions of MHO, we suggest the possibility that atmospheric emissions of MHO are largely due to industrial palm oil processing rather that emissions from the intact fruits themselves. If future studies verify this possibility, this would open the door to the use of MHO as a specific volatile biomarker of the palm oil industry for both environmental and industrial purposes. Atmospheric based monitoring of oil palm processing could aid studies on the impact the oil palm industry has on the environment, as well as provide the industry with a new tool for evaluating the oxidation of nutritionally important carotene during palm oil processing, and also evaluate its role in oil stability through antioxidant properties.

Acknowledgments

We acknowledge the support from the Central Office of the Large Scale Biosphere Atmosphere Experiment in Amazonia (LBA), the Instituto Nacional de Pesquisas da Amazônia (INPA), and the Brazilian Agricultural Research Corporation (EMPRAPA). This material is based upon work supported as part of the Green Ocean Amazon (GoAmazon 2014/5) project as well as the Next Generation Ecosystem Experiments-Tropics (NGEE-Tropics) funded by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research through contract No. DE-AC02-05CH11231 to LBNL, as part of DOE's Terrestrial Ecosystem Science Program.

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Submitted: March 28, 2016
Published online: June 28, 2016