Article

Wounding-induced VOC emissions in five tropical agricultural species

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Abstract: Leaf mechanical wounding triggers a rapid, within minutes, release of a blend of volatile organic compounds (VOCs). Wounding-induced VOC blend is mainly composed of oxygenated ubiquitous stress volatiles such as methanol and volatile products of lipoxygenase (LOX) pathway (mainly C5 and C6 alcohols and aldehydes and their derivatives), but also includes multiple minor VOCs that collectively act as infochemicals inducing defences in non-damaged plant leaves, neighbouring plants and attracting herbivore enemies. Till present, interspecific variability of the rate of induction and magnitude of wounding-induced emissions, and the extent to which plant structural traits and physiological activity alter these emissions are poorly known. Particularly scarce is the information of the induced emissions in tropical agricultural plant species despite their economic importance and large area of cultivation at regional to global scales. We chose five tropical crops with varying photosynthetic activity and leaf structural characteristics: *Abelmoschus esculentus*, *Amaranthus cruentus*, *Amaranthus hybridus*, *Solanum aethiopicum* and *Telfairia occidentalis* to characterize the kinetics and magnitude of wounding-induced emissions, hypothesizing that the induced emission response is greater and faster in physiologically more active species with greater photosynthetic activity than in less active species. Rapid highly repeatable leaf wounds (12-mm cuts) were generated by a within-leaf-chamber cutting knife. Wounding-induced VOC emissions were measured continuously with a proton-transfer reaction time-of-flight mass spectrometer and gas-chromatography mass spectrometry was used to separate isomers. Twenty-three ion VOCs and twelve terpenoid molecule structures were identified, whereas ubiquitous stress volatiles methanol (on average 40% of total emissions), hexenal (24%), and acetaldehyde (11%) were the main compounds across the species. Emissions of low-weight oxygenated compounds (LOC, 70% of total), and LOX products (29%) were positively correlated across species, but minor VOC components, monoterpenoids and benzenoids were negatively correlated with LOC and LOX, indicating a reverse relationship between signal specificity and strength. There was a large interspecific variability in the rate of induction and emission magnitude, but the hypothesis of a stronger emission response in physiologically more active species was only partly supported. In addition, the overall emission levels were somewhat lower with different emission blend compared to the data reported for wild species, as well as different shares for the VOCs in the blend. The study demonstrates that wounding-dependent emissions from tropical agricultural crops can significantly contribute to atmospheric volatiles, and these emissions cannot be predicted based on current evidence of wild plant model systems.

Keywords: abiotic stress, acetaldehyde, hexenal, LOX products, mass spectrometry, methanol, proton-transfer reaction, tropical crop species,

1. Introduction

Leaf mechanical damage by piercing or chewing herbivores or strong wind or falling plant debris depicts one of the many stressors to which plants are exposed throughout their life span [1-3]. Wounding of the leaf under precise laboratory conditions provides an experimental setup to quantitatively study the sequence of physiological responses elicited in response to or mechanical damage, e.g. damage by herbivory [4,5]. Leaf tissue wounding triggers a rapid release of a blend of volatile organic compounds (VOCs) mainly composed of short- to medium-length alcohols and aldehydes [4,5]. It contains volatile products of lipoxygenase (LOX) pathway (also called green leaf volatiles), which are the C5 and C6 alcohols and aldehydes derived as the result of break-down of poly-unsaturated fatty acids that are released from damaged membranes as the result of constitutive activity of different lipoxygenases [6,7]. A major methanol emission burst from damaged cell walls is also characteristic to leaf wounding [4,5,8,9] and reflects activation of pectin methylesterases [10,11]. In addition, other low-mass oxygenated compounds such as formaldehyde and acetaldehyde, ketones, esters, and volatile isoprenoids [1,4,9,12-17] are released in a sequence characterizing the activation of different pathways in different leaf compartments. As a general consequence of an open wound, temporarily, there is an uncontrolled loss of water from free water surface generated by wounding and enhanced emission of compounds that are dissolved in cell wall water and in cytosol [9]. In addition, major emission bursts can result from damage of VOC-storing specialized cell compartments, or specialized structures in the leaf interior (e.g. resin ducts and oil glands) or on leaf surface (glandular trichomes) [9,18].

The role of the alcohols and aldehydes emitted upon wounding is the immediate protection against herbivory by repelling the feeders, e.g. by eye and oral irritation [15,17]. The release of volatile LOX products and other wounding-associated volatiles to the surrounding air also plays a signalling role, warding off or attracting herbivores or attracting herbivore natural enemies during a leaf feeding event [19]. Moreover, synthesis of some volatiles such as (*E*)-2-hexenal and (*Z*)-3-hexenol contributes to protection against further microbial pathogen attacks through the open wound [20,21]. Synthesis of volatile plant hormones, typically jasmonic acid and its volatile methylated form methyl jasmonate, is also induced [22,23]. Jasmonates act as emergency messengers for conspecifics and trigger the biosynthesis of benzenoids and terpenoids in leaves of the attacked plants and other neighbouring individuals [24], thereby reducing leaf palatability. From an atmospheric perspective, VOCs emitted after major leaf wounding events are released in significant amounts to the atmosphere, thereby contributing to aerosol and ozone formation, directly influencing the air quality at local to regional scales [25-28].

There is a high diversity of plant species growing within a given ecosystem and across different ecosystem types in different climatic conditions. The species differ in the activity of primary metabolism, but also have a large variation in the suites of secondary defence metabolism with important implications for the herbivore feeding behaviour. Research on the VOC emission composition, magnitude and dynamics upon wounding has a potential to provide insight into the rate and strength of herbivory signal in different species, and might further be informative for resolving co-evolution strategies among plants, their feeders and enemies of herbivores. Until present, the research on the rapid VOC emission dynamics upon leaf wounding has been primarily conducted with a few temperate climate model plants: e.g. poplars [8,12,29,30], *Arabidopsis* [31-33], *Dactylis glomerata* [4], and *Trifolium repens* [12]. Wound responses of agricultural plants that are grown over large areas worldwide are currently understudied with the exception of some crops such as *Phaseolus vulgaris* [9]. This is a significant limitation as agricultural species are potentially very vulnerable to herbivore attacks due to their high nutritive value, low secondary metabolite contents and cultivation in monocultures [34,35]. There is particularly limited information of VOC emissions in tropical agricultural plants, in particular, about their wounding responses despite their high contribution to worldwide vegetation cover. Given the high physiological activity of agricultural plants, including

high photosynthetic and growth rates, the rate and degree of induction of defences under different stresses such as wounding stress are expected to be particularly large in agricultural plants [36]. In contrast, long-living plants in natural communities invest more in structural and chemical constitutive defences and are expected to have greater stress tolerance [36]. Nevertheless, there is a wide range of variation in photosynthetic and structural traits in crops [37-39]. It is expected that there is an analogous variation in induced stress responses, but to our knowledge, interspecific variation in crop induced volatile responses to mechanical stress has not yet been studied.

Given the rapid initial wounding response, fast instruments such as proton transfer reaction time-of-flight mass spectrometer (PTR-TOF-MS) are required for continuous monitoring both the emissions of wound-induced VOCs and their temporal dynamics. These recently developed instruments allow real-time measurement of a large spectrum of VOCs (typically from 1 to 512 a.m.u.) with a time resolution of 1 to up to 10 Hz and a limit of detection under 1 ppb. The instrument is suitable for determining plant responses to rapidly occurring stresses such as wounding and measuring the overall magnitude and kinetics of the volatile emissions [4,5,12].

In this paper, we studied the wound-induced emissions in five tropical dicot crop species with contrasting photosynthetic activity. The species studied – okro (*Abelmoschus esculentus* (L.) Moench), red amaranth (*Amaranthus cruentus* L.), green amaranth (*Amaranthus hybridus* L.), African eggplant (*Solanum aethiopicum* L.) and fluted pumpkin (*Telfairia occidentalis* Hook. f.) – are currently of regional importance, but they are considered as promising future crops to diversify the food basket. *Abelmoschus esculentus*, *A. cruentus* and *A. hybridus* are annual herbs, *S. aethiopicum* is a woody perennial, but grown typically as annual herb and *T. occidentalis* is a perennial vine. The two amaranths have C_4 photosynthetic metabolism, and the three other species have C_3 metabolism. Thus, we expected that there is a significant variation in foliage photosynthetic activity and structural characteristics among species. We used a PTR-TOF-MS device to assess the rate of elicitation and magnitude of wounding-induced stress response from leaf damage until cessation of the immediate stress response. We hypothesized that both the rate of elicitation and magnitude of elicitation of the key wounding-related volatiles, methanol, and LOX pathway compounds, are greater in species with greater photosynthetic activity. We also compared the induced responses with the non-crop model species studied previously, and hypothesized that the rate of induction and magnitude of elicitation is greater in the crops than in the wild species. The results provide evidence of major interspecific variability in magnitude and kinetics of wounding-dependent emissions, indicating species-specific regulation of different stress pathways and overall demonstrate that wounding stress in crops can be a significant oxygenated volatile source.

2. Results

2.1. The diversity in plant photosynthetic capacity and anatomical and chemical traits

The studied five species presented different photosynthetic capacities and stomatal conductances, and both parameters were not correlated across the species ($r^2 = 0.08$, $P = 0.15$). *Amaranthus* species (*A. cruentus* and *A. hybridus*) with C_4 metabolism had similar stomatal conductance, but higher photosynthetic rate than the three C_3 species (Table 1). Mean leaf stomatal conductance and photosynthetic activity fully recovered in 30 min after wounding in all species (Student's *t*-test at 0.05 significance level) (Table 1).

The studied species varied in both leaf structural and chemical characteristics. Leaf size varied 13-fold with *Abelmoschus esculentus* having the largest and *Amaranthus cruentus* the smallest leaves. Leaf dry mass per area (LMA), varied two-fold with *Telfairia occidentalis* having the greatest LMA, and *Solanum aethiopicum* the lowest LMA (Table 1). Leaf N and P contents per area were the greatest in *Amaranthus hybridus* and *Telfairia occidentalis* (Table 1), but foliage photosynthetic N (A_{max}/N) and photosynthetic P (A_{max}/N) were the greatest in the two C_4 species. Foliage Ca and K contents varied less among species, except that *Telfairia occidentalis* had a lower K content than other species (Table 1).

Species structural characteristics and photosynthetic activity were generally weakly associated with wounding-elicited emissions. Nevertheless, the integrated and peak LOX compound hexenal emissions were the greatest in the two *Amaranthus* species with the highest photosynthetic rate (Tables 1-2). In contrast, methanol emissions were the greatest in *Abelmoschus esculentus* that had the largest leaves, but lowest photosynthesis rate (Tables 1-2). In fact, no significant correlations were found between pre-wounding photosynthetic capacity and the integrated wound-induced emissions of total VOCs (r^2 = 0.15; P = 0.16) nor VOC classes (e.g. LOCs: r^2 = 0.22, P = 0.076; LOXs: r^2 = 0.009, P = 0.74). Also, the baseline total VOC emissions were not correlated to photosynthetic capacity (r^2 = 0.008; P = 0.76).

Table 1. Average ± SE leaf photosynthetic and structural characteristics and contents of macroelements.

Characteristic	units	Abelmoschus esculentus	Amaranthus cruentus	Amaranthus hybridus	Solanum aethiopicum	Telfairia occidentalis
Photosynthesis rate before cut	µmol m^{-2} s^{-1}	4.6 ± 1.2 [a]	10.7 ± 1.0 [ab]	11.8 ± 1.6 [b]	5.7 ± 1.4 [ab]	6.2 ± 2.2 [ab]
Photosynthesis rate after cut	µmol m^{-2} s^{-1}	3.6 ± 1.1 [a]	11.1 ± 1.0 [b]	11.6 ± 1.9 [b]	5.1 ± 1.2 [ab]	5.5 ± 1.9 [ab]
Stomatal conductance before cut	mmol m^{-2} s^{-1}	66 ± 11 [a]	83 ± 8 [ab]	125 ± 6 [bc]	175 ± 23 [c]	89 ± 5 [ab]
Stomatal conductance after cut	mmol m^{-2} s^{-1}	65 ± 24 [a]	85 ± 10 [ab]	110 ± 11 [ab]	146 ± 7 [b]	88 ± 9 [ab]
Dry mass	Mg	1050 ± 130 [b]	77 ± 7 [a]	119 ± 9 [a]	230 ± 70 [a]	275 ± 33 [a]
Leaf area	cm^2	290 ± 38 [b]	28.2 ± 1.8 [a]	30.8 ± 1.9 [a]	86.1 ± 3.1 [a]	58 ± 10 [a]
LMA	g m^{-2}	36.30 ± 0.46	27.1 ± 0.6	38.5 ± 0.6	27 ± 8	53 ± 17
Pre-wound A_{max}/N	µmol s^{-1} g^{-1}	5.3 ± 2.3	11.0 ± 1.1	10.9 ± 1.4	4.6 ± 1.6	6.6 ± 1.6
Pre-wound A_{max}/P	µmol s^{-1} g^{-1}	23 ± 10	44.7 ± 4.4	34.1 ± 4.5	20 ± 7	26 ± 6
Nitrogen	g m^{-2}	0.750 ± 0.018 [a]	0.973 ± 0.010 [c]	1.083 ±0.012 [d]	0.868 ± 0.024 [b]	1.337 ± 0.027 [e]
Carbon	g m^{-2}	15.07 ± 0.11 [b]	10.07 ± 0.08 [a]	14.34 ± 0.15 [b]	10.57 ± 0.23 [a]	21.3 ± 0.8 [c]
Phosphorus	g m^{-2}	0.173 ± 0.006 [a]	0.239 ± 0.008 [b]	0.348 ± 0.007 [d]	0.219 ± 0.006 [b]	0.310 ± 0.006 [c]
Potassium	g m^{-2}	1.855 ± 0.034 [c]	1.647 ± 0.030 [b]	1.681 ± 0.020 [b]	1.549 ± 0.020 [b]	1.148 ± 0.041 [a]
Calcium	g m^{-2}	1.120 ± 0.023 [c]	0.656 ± 0.006 [a]	0.721 ± 0.005 [b]	0.952 ± 0.009 [c]	0.725 ± 0.008 [b]
Magnesium	g m^{-2}	0.633 ± 0.018 [e]	0.499 ± 0.014 [d]	0.564 ± 0.016 [c]	0.248 ± 0.007 [a]	0.383 ± 0.007 [b]

A_{max} is photosynthetic capacity at saturating light and optimal conditions.
Different letters indicate significant differences among species after Tukey HSD test (P < 0.05).
No significant differences were found between pre- and post-wounding (30 min after leaf wounding) leaf photosynthesis rate and stomatal conductance (P > 0.05 according to paired samples *t*-test; n = 3 for all species).

2.2. Wounding-induced VOC emissions, dynamics and correlations

Total emissions upon wounding varied three-fold among the plant species, with *Amaranthus cruentus* with the lowest wounding-induced VOC emissions, and *Abelmoschus esculentus* and *Telfairia occidentalis* the highest wounding-induced VOC emitters per mm of wound length (Table 2). The differences among species in LOCs emissions were 5-fold large, and 3.7-fold in the elicitation of LOXs (Table 3).

Twenty-three VOCs were identified in the emissions from the leaves of the five studied crop species (Table 2). For most compounds, the emissions prior to leaf wounding were generally at a low level, but strongly increased after mechanical wounding. The main compounds emitted after wounding (average ± SE across species) were methanol (18 ± 6 pmol mm^{-1}, 40% of total emissions), hexenal (9.3 ± 2.4 pmol mm^{-1}, 24% of total), and acetaldehyde (3.7 ± 0.5 pmol mm^{-1}, 11% of total), followed by formic acid (6.1 ± 1.3 pmol mm^{-1}) and acetone (4.73 ± 0.15 pmol mm^{-1}) (Table 2 and 3). The composition of the VOC blend by compound classes was: 28.5 ± 6.6 pmol mm^{-1} (70% of total) of LOCs, 10.7 ± 2.6 pmol mm^{-1} (29%) of LOXs, 0.153 ± 0.005 pmol mm^{-1} (0.5%) of benzenoids and jasmonates, 0.102 ± 0.009 pmol mm^{-1} (0.31%) of GDPP, 0.341 ± 0.045 pmol mm^{-1} (0.97%) of isoprenoids (8 monoterpenes, two oxygenated monoterpenes and two sesquiterpenes, Table 2 legend) (Table 3).

Table 2. Volatile organic compound (VOC) emissions (average ± SE) of five tropical crop species in (1) at optimal conditions of light, air temperature and moisture (constitutive emissions), and (2) after a 12 mm cut was made into the leaf lamina. The VOC emission rate (3) at the wounding-induced peak maximum and (4) the time to the peak maximum are also provided when the pattern of VOC emissions after wounding included a peak. Information of individual contribution to the total emissions in percentage is found in Table 3.

Compound name	Molecular formula	Protonated molecular mass	(1) Constitutive emissions (2) Wound emissions (3) Max. emission rate (4) Time from wounding	*Abelmoschus esculentus*	*Amaranthus cruentus*	*Amaranthus hybridus*	*Solanum aethiopicum*	*Telfairia occidentalis*
Formaldehyde	CH_2O	31.0178	pmol m⁻² s⁻¹	740 ± 80	612.3 ± 3.7	566 ± 41	542.6 ± 4.1	749 ± 7
			pmol mm⁻¹	0.65± 0.14	0.765 ± 0.022	0.75 ± 0.08	0.753 ± 0.049	0.66 ± 0.07
			fmol mm⁻¹ s⁻¹	-	-	-	-	-
			s	-	-	-	-	-
Methanol	CH_4O	33.0335	pmol m⁻² s⁻¹	1600 ± 100	1553 ± 28	1270 ± 160	1750 ± 250	2300 ± 140
			pmol mm⁻¹	40.2 ± 4.8	2.52 ± 0.33	14.1 ± 2.0	11.3 ± 2.6	23.2 ± 4.7
			fmol mm⁻¹ s⁻¹	275 ± 30	16.0 ± 1.6	46.7 ± 4.8	47 ± 8	331 ± 49
			s	75 ± 31	111 ± 16	130 ± 17	112 ± 10	16.2 ± 2.1
Acetaldehyde	C_2H_4O	45.0335	pmol m⁻² s⁻¹	4020 ± 330	3990 ± 90	5650 ± 1000	2750 ± 150	5310 ± 260
			pmol mm⁻¹	2.74 ± 0.20	3.0 ± 1.3	3.40 ± 0.09	3.88 ± 0.53	5.7 ± 0.6
			fmol mm⁻¹ s⁻¹	-	21.9 ± 2.1	15.9 ± 0.8	14.7 ± 1.8	17.5 ± 2.6
			s	-	733 ± 45	123 ± 32	639 ± 37	461 ± 35
Formic acid	CH_2O_2	47.0128	pmol m⁻² s⁻¹	2550 ± 70	2895 ± 30	2390 ± 60	2810 ± 80	2473 ± 19
			pmol mm⁻¹	1.73 ± 0.1	1.62 ± 0.08	2.41 ± 0.40	2.34 ± 0.27	2.2 ± 0.6
			fmol mm⁻¹ s⁻¹	-	6.3 ± 1.0	10.4 ± 1.9	7.3 ± 0.9	10.9 ± 1.3
			s	-	119 ± 21	158 ± 18	205 ± 40	96 ± 8
Ethanol	C_2H_6O	47.0491	pmol m⁻² s⁻¹	622 ± 240	404 ± 31	412 ± 22	396.2 ± 4.4	457 ± 28
			pmol mm⁻¹	0.728 ± 0.028	0.56 ± 0.13	0.68 ± 0.11	1.7 ± 1.0	0.80 ± 0.18
			fmol mm⁻¹ s⁻¹	2.813 ± 0.023	3.1 ± 0.7	3.13 ± 0.10	2.8 ± 0.05	3.44 ± 0.26
			s	78.3 ± 3.7	33 ± 7	193.5 ± 3.7	297 ± 22	100 ± 11
Acetone	C_3H_6O	59.0491	pmol m⁻² s⁻¹	1430 ± 430	864 ± 5	1160 ± 410	795 ± 75	2130 ± 210
			pmol mm⁻¹	2.3 ± 0.8	0.75 ± 0.08	1.9 ± 0.7	1.7 ± 0.6	2.6 ± 0.7
			fmol mm⁻¹ s⁻¹	5.6 ± 1.0	5.0 ± 1.7	6.3 ± 0.6	5.6 ± 2.0	7.9 ± 1.3
			s	182 ± 44	15 ± 5	123 ± 13	120 ± 50	339 ± 150
Acetic acid	$C_2H_4O_2$	61.0284	pmol m⁻² s⁻¹	1110 ± 90	1370 ± 80	881 ± 26	1800 ± 100	1010 ± 50
			pmol mm⁻¹	0.77 ± 0.10	0.71 0.15	1.17 0.23	1.38 0.20	0.77 0.16
			fmol mm⁻¹ s⁻¹	5.3 ± 1.8	5.00 ± 0.36	4.6 ± 0.6	4.7 ± 1.3	4.6 ± 1.0
			s	83 ± 35	49 ± 30	150 ± 42	172 ± 65	56 ± 22
Pentenal + 3-penten-2-one	C_5H_8O	85.0648	pmol m⁻² s⁻¹	21.4 ± 1.1	28.7 ± 0.8	17.9 ± 1.5	21.9 ± 0.7	21.2 ± 0.6
			pmol mm⁻¹	0.203 ± 0.034	0.43 ± 0.09	0.64 ± 0.05	0.56 ± 0.15	0.55 ± 0.09
			fmol mm⁻¹ s⁻¹	4.2 ± 0.9	9.8 ± 2.5	5.63 ± 0.36	12.5 ± 3.2	4.8 ± 0.9
			s	11.1 ± 1.8	9.6 ± 2.4	78 ± 12	10.8 ± 1.8	26.4 ± 3.9
Pentanal + 2-pentanone	$C_5H_{10}O$	87.0804	pmol m⁻² s⁻¹	89.3 ± 2.5	109.8 ± 2.4	75.4 ± 3.3	96.2 ± 1.4	85.7 ± 4.5
			pmol mm⁻¹	0.252 ± 0.026	0.302 ± 0.015	0.386 ± 0.017	0.333 ± 0.022	0.390 ± 0.011
			fmol mm⁻¹ s⁻¹	-	-	-	-	-
			s	-	-	-	-	-
Hexenal	$C_6H_{10}O$	99.0804	pmol m⁻² s⁻¹	41 ± 11	36.3 ± 0.9	24.5 ± 2.5	32.6 ± 2.4	28.9 ± 1.1
			pmol mm⁻¹	4.3 ± 1.6	3.7 ± 0.7	13.1 ± 1.7	9.7 ± 0.9	15.9 ± 1.9
			fmol mm⁻¹ s⁻¹	78 ± 35	85 ± 17	188 ± 43	136.7 ± 4.5	117 ± 8
			s	98 ± 11	9.6 ± 0.6	78 ± 10	15.0 ± 1.6	14.4 ± 2.1
(Z)-3-hexen-1-ol + hexanal	$C_6H_{12}O$	101.0961	pmol m⁻² s⁻¹	39.4 ± 2.6	48.7 ± 2.2	36.9 ± 1.6	48.4 ± 1.6	37.0 ± 1.7
			pmol mm⁻¹	0.210 ± 0.44	0.2045 ± 0.0042	1.11 ± 0.31	0.27 ± 0.06	0.65 ± 0.11
			fmol mm⁻¹ s⁻¹	1.88 ± 0.50	-	5.0 ± 1.3	-	3.54 ± 0.21
			s	122 ± 11	-	186 ± 8	-	70 ± 19
(E)-1-hexanol	$C_6H_{14}O$	103.1117	pmol m⁻² s⁻¹	5.68 ± 0.20	6.49 ± 0.22	4.8 ± 0.7	5.58 ± 0.30	5.58 ± 0.40
			pmol mm⁻¹	0.054 ± 0.006	0.0481 ± 0.0026	0.053 ± 0.005	0.043 ± 0.005	0.052 ± 0.005
			fmol mm⁻¹ s⁻¹	0.344 ± 0.026	0.52 ± 0.06	-	0.38 ± 0.05	-
			s	293 ± 18	304 ± 23	-	311 ± 18	-
Methyl benzoate	$C_8H_8O_2$	137.0597	pmol m⁻² s⁻¹	5.6 ± 1.0	7.0 ± 0.8	4.1 ± 0.5	4.8 ± 0.7	4.21 ± 0.28
			pmol mm⁻¹	0.059 ± 0.008	0.081 ± 0.010	0.0614 ± 0.0033	0.076 ± 0.007	0.0573 ± 0.0041
			fmol mm⁻¹ s⁻¹	0.38 ± 0.07	0.65 ± 0.06	-	0.50 ± 0.08	-

Compound name	Molecular formula	Mass	Unit	Abelmoschus esculentus	Amaranthus cruentus	Amaranthus hybridus	Solanum aethiopicum	Telfairia occidentalis
			s	284 ± 13	317 ± 18	-	322 ± 16	-
Non-oxygenated monoterpenes [a]	$C_{10}H_{16}$	137.1325	pmol m^{-2} s^{-1}	44 ± 13	33 ± 6	42 ± 8	32 ± 7	48 ± 12
			pmol mm^{-1}	0.25 ± 0.17	0.092 ± 0.011	0.094 ± 0.009	0.066 ± 0.021	0.32 ± 0.08
			fmol mm^{-1} s^{-1}	3.1 ± 1.0	-	2.5 ± 0.8	-	1.67 ± 0.42
			s	130 ± 90	-	387 ± 22	-	360 ± 120
6-Methyl-5-hepten-2-one	$C_8H_{14}O_2$	143.1067	pmol m^{-2} s^{-1}	24.2 ± 1.3	28.05 ± 0.40	19.5 ± 0.9	26.9 ± 1.6	22.1 ± 0.5
			pmol mm^{-1}	0.089 ± 0.008	0.084 ± 0.014	0.124 ± 0.022	0.124 ± 0.012	0.089 ± 0.009
			fmol mm^{-1} s^{-1}	-	-	-	-	-
			s	-	-	-	-	-
Hexyl acetate	$C_8H_{16}O_2$	145.1223	pmol m^{-2} s^{-1}	4.44 ± 0.21	4.93 ± 0.13	3.97 ± 0.12	4.69 ± 0.28	3.969 ± 0.017
			pmol mm^{-1}	0.0357 ± 0.0039	0.0402 ± 0.0023	0.0334 ± 0.0005	0.0341 ± 0.0031	0.0366 ± 0.0019
			fmol mm^{-1} s^{-1}	-	0.41 ± 0.08	0.594 ± 0.026	-	-
			s	-	338 ± 47	520 ± 90	-	-
DMNT	$C_{11}H_{18}$	151.1481	pmol m^{-2} s^{-1}	5.41 ± 0.27	6.72 ± 0.12	5.416 ± 0.035	6.1 ± 0.7	5.42 ± 0.12
			pmol mm^{-1}	0.041 ± 0.006	0.043 ± 0.006	0.040 ± 0.005	0.053 ± 0.011	0.0385 ± 0.0020
			fmol mm^{-1} s^{-1}	-	-	-	-	-
			s	-	-	-	-	-
Methyl salicylate	$C_8H_8O_3$	153.0546	pmol m^{-2} s^{-1}	6.1 ± 1.0	5.17 ± 0.26	3.50 ± 0.10	4.5 ± 0.5	5.2 ± 1.1
			pmol mm^{-1}	0.0272 ± 0.0041	0.0330 ± 0.0049	0.0426 ± 0.0013	0.0386 ± 0.0036	0.0351 ± 0.0011
			fmol mm^{-1} s^{-1}	-	-	-	-	-
			s	-	-	-	-	-
Oxygenated monoterpenes [a]	$C_{10}H_{18}O$	155.1430	pmol m^{-2} s^{-1}	4.08 ± 0.08	4.73 ± 0.43	5.8 ± 2.7	4.04 ± 0.21	3.90 ± 0.43
			pmol mm^{-1}	0.0521 ± 0.0022	0.049 ± 0.006	0.089 ± 0.010	0.0534 ± 0.0019	0.047 ± 0.006
			fmol mm^{-1} s^{-1}	-	-	-	-	-
			s	-	-	-	-	-
Sesquiterpenes [a]	$C_{15}H_{24}$	205.1951	pmol m^{-2} s^{-1}	6.19 ± 0.35	9.21 ± 0.21	6.3 ± 0.6	6.96 ± 0.17	6.9 ± 1.5
			pmol mm^{-1}	0.0357 ± 0.0018	0.0472 ± 0.0049	0.0341 ± 0.0029	0.0391 ± 0.0006	0.041 ± 0.011
			fmol mm^{-1} s^{-1}	-	-	-	-	-
			s	-	-	-	-	-
Jasmonic acid	$C_{12}H_{18}O_3$	211.1329	pmol m^{-2} s^{-1}	1.845 ± 0.022	2.286 ± 0.026	1.84 ± 0.16	2.02 ± 0.15	1.77 ± 0.17
			pmol mm^{-1}	0.0263 ± 0.0019	0.0221 ± 0.0010	0.0234 ± 0.0014	0.0243 ± 0.0035	0.0247 ± 0.0029
			fmol mm^{-1} s^{-1}	-	-	-	-	-
			s	-	-	-	-	-
TMTT	$C_{16}H_{26}$	219.2107	pmol m^{-2} s^{-1}	5.84 ± 0.06	7.55 ± 0.20	4.78 ± 0.28	6.13 ± 0.19	5.25 ± 0.24
			pmol mm^{-1}	0.0350 ± 0.0032	0.0344 ± 0.0044	0.0396 ± 0.0019	0.038 ± 0.007	0.0373 ± 0.0017
			fmol mm^{-1} s^{-1}	-	-	-	-	-
			s	-	-	-	-	-
Methyl jasmonate	$C_{13}H_{20}O_3$	225.1485	pmol m^{-2} s^{-1}	2.38 ± 0.19	2.371 ± 0.044	2.56 ± 0.13	2.52 ± 0.11	2.21 ± 0.13
			pmol mm^{-1}	0.0265 ± 0.0021	0.0292 ± 0.0018	0.0239 ± 0.0018	0.0246 ± 0.0031	0.0280 ± 0.0007
			fmol mm^{-1} s^{-1}	-	-	-	-	-
			s	-	-	-	-	-
Total constitutive emissions			nmol m^{-2} s^{-1}	12.03 ± 0.19	12.6 ± 1.5	12.4 ± 0.8	10.53 ± 0.30	14.73 ± 0.43
Total wound emissions			pmol mm^{-1}	55 ± 8	15.2 ± 2.9	40 ± 6	35 ± 6	54 ± 9

The measurements were conducted with a proton-transfer-reaction time-of-flight mass spectrometer (PTR-TOF-MS) before (constitutive emissions) and for 30 min after wounding (integrated emissions, peak emissions). Abbreviations: DMNT - (*E*)-4,8-dimethyl-1,3,7-nonatriene; TMTT - (*E*,*E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene.

[a] Thermal desorption gas-chromatography mass-spectrometry (GC-MS) analysis was used to confirm the identity of emitted compound and analyse the composition of released volatiles classes. GC-MS analysis demonstrated that monoterpenes ($C_{10}H_{16}$) were composed of camphene, Δ^3-carene, limonene, α-myrcene, α-ocimene, α-pinene, β-pinene, and α-phellandrene, oxygenated monoterpenes ($C_{10}H_{18}O$) were composed of 1,8-cineole and linalool, and sesquiterpenes ($C_{15}H_{24}$) were composed of (*E*)-β-farnesene and longifolene.

Table 3. Chemical composition of the overall blend of VOCs emitted after a 12 mm cut was made into the leaf lamina. Values are expressed as percentage contribution to the total emissions, and in pmol mm^{-1} in the summary per VOC classes. More emission peak information is found in Table 1.

Compound name	Molecular formula	Abelmoschus esculentus	Amaranthus cruentus	Amaranthus hybridus	Solanum aethiopicum	Telfairia occidentalis	Mean ± SE
Lightweight oxygenated compounds (LOCs)		49 ± 6	9.9 ± 2.1	24.4 ± 3.6	23 ± 5	36 ± 7	29 ± 7

		$pmol\ mm^{-1}$	$pmol\ mm^{-1}$	$pmol\ mm^{-1}$	$pmol\ mm^{-1}$	$pmol\ mm^{-1}$	$pmol\ mm^{-1}$
		90 %	66 %	61 %	67 %	66 %	70 ± 5 %
Formaldehyde	CH_2O	1.2	5.0	1.9	2.2	1.2	2.3 ± 0.7
Methanol	CH_4O	74	17	35	33	43	40 ± 9
Acetaldehyde	C_2H_4O	5	20	9	11	11	11.0 ± 2.5
Formic acid	CH_2O_2	3.2	11	6	7	4.1	6.1 ± 1.3
Ethanol	C_2H_6O	1.3	3.7	1.7	4.9	1.5	2.6 ± 0.7
Acetone	C_3H_6O	4.1	5.0	4.8	5.0	4.9	4.74 ± 0.15
Acetic acid	$C_2H_4O_2$	1.4	4.7	2.9	4.0	1.4	2.9 ± 0.7
Lipoxygenase pathway products (LOXs)		5.0 ± 1.7 $pmol\ mm^{-1}$ 9 %	4.7 ± 0.8 $pmol\ mm^{-1}$ 31 %	15.3 ± 2.1 $pmol\ mm^{-1}$ 38 %	10.9 ± 1.1 $pmol\ mm^{-1}$ 32 %	17.6 ± 2.1 $pmol\ mm^{-1}$ 32 %	10.7 ± 2.6 $pmol\ mm^{-1}$ 29 ± 5 %
Pentenal + 3-penten-2-one	C_5H_8O	0.37	2.8	1.6	1.6	1.0	1.49 ± 0.41
Pentanal + 2-pentanone	$C_5H_{10}O$	0.46	2.0	1.0	1.0	0.7	1.02 ± 0.26
Hexenal	$C_6H_{10}O$	8	25	33	28	29	24.5 ± 4.4
(Z)-3-hexen-1-ol + hexanal	$C_6H_{12}O$	0.38	1.3	2.8	0.8	1.2	1.29 ± 0.40
(E)-1-hexanol	$C_6H_{14}O$	0.10	0.32	0.13	0.13	0.10	0.154 ± 0.042
Hexyl acetate	$C_8H_{16}O_2$	0.07	0.27	0.08	0.10	0.07	0.116 ± 0.038
Benzenoids and jasmonates		0.139 ± 0.016 $pmol\ mm^{-1}$ 0.25 %	0.165 ± 0.018 $pmol\ mm^{-1}$ 1.1 %	0.151 ± 0.008 $pmol\ mm^{-1}$ 0.38 %	0.164 ± 0.017 $pmol\ mm^{-1}$ 0.47 %	0.145 ± 0.009 $pmol\ mm^{-1}$ 0.27 %	0.153 ± 0.005 $pmol\ mm^{-1}$ 0.49 ± 0.15 %
Methyl benzoate	$C_8H_8O_2$	0.11	0.53	0.15	0.22	0.11	0.22 ± 0.08
Methyl salicylate	$C_8H_8O_3$	0.05	0.22	0.11	0.11	0.06	0.110 ± 0.029
Jasmonic acid	$C_{12}H_{18}O_3$	0.048	0.15	0.06	0.07	0.045	0.074 ± 0.019
Methyl jasmonate	$C_{13}H_{20}O_3$	0.048	0.19	0.06	0.07	0.05	0.085 ± 0.027
Geranylgeranyl diphosphate pathway		0.089 ± 0.008 $pmol\ mm^{-1}$	0.084 ± 0.014 $pmol\ mm^{-1}$	0.124 ± 0.022 $pmol\ mm^{-1}$	0.124 ± 0.012 $pmol\ mm^{-1}$	0.089 ± 0.009 $pmol\ mm^{-1}$	0.102 ± 0.009 $pmol\ mm^{-1}$
6-Methyl-5-hepten-2-one	$C_8H_{14}O_2$	0.16 %	0.55 %	0.31 %	0.36 %	0.16 %	0.31 ± 0.7 %
Isoprenoids		0.41 ± 0.18 $pmol\ mm^{-1}$ 0.75 %	0.266 ± 0.032 $pmol\ mm^{-1}$ 1.8 %	0.297 ± 0.029 $pmol\ mm^{-1}$ 0.7 %	0.249 ± 0.041 $pmol\ mm^{-1}$ 0.7 %	0.48 ± 0.10 $pmol\ mm^{-1}$ 0.9 %	0.341 ± 0.045 $pmol\ mm^{-1}$ 0.97 ± 0.20 %
Non-oxygenated monoterpenes [a]	$C_{10}H_{16}$	0.46	0.6	0.23	0.19	0.6	0.41 ± 0.09
DMNT	$C_{11}H_{18}$	0.07	0.28	0.10	0.15	0.07	0.136 ± 0.039
Oxygenated monoterpenes [a]	$C_{10}H_{18}O$	0.10	0.32	0.22	0.15	0.09	0.176 ± 0.044
Sesquiterpenes [a]	$C_{15}H_{24}$	0.07	0.31	0.08	0.11	0.08	0.130 ± 0.046
TMTT	$C_{16}H_{26}$	0.06	0.23	0.10	0.11	0.07	0.114 ± 0.030

  VOC emissions, in particular LOC and LOX compound emissions, rapidly increased after leaf wounding, until maximum values were reached, typically 1 to 2 min after wounding (Figure 1a for a representative example of methanol and hexenal emission dynamics). Thereafter the emissions gradually decreased to pre-wounding levels. The peak shape of the summed VOCs emitted after wounding were consistent across species (Figure 1b), as well as for the individual VOCs that peaked after wounding (Figure 1c). The VOC emission rate at the maximum varied among the VOCs (average ± SE across species) from $0.41 ± 0.05\ fmol\ mm^{-1}\ s^{-1}$ for hexanol to $140 ± 70\ fmol\ mm^{-1}\ s^{-1}$ for methanol (Table 2).

  The constitutive total VOC emissions were not correlated with the level of elicitation of VOCs after wounding ($r^2 = 0.22$; $P = 0.86$). Similarly, no significant correlations were found when looking into VOC groups: e.g. LOCs inexistent correlation (Figure 1d) and the negative relationship exhibited between pre- and post-wound emissions of LOXs (Figure 2e).
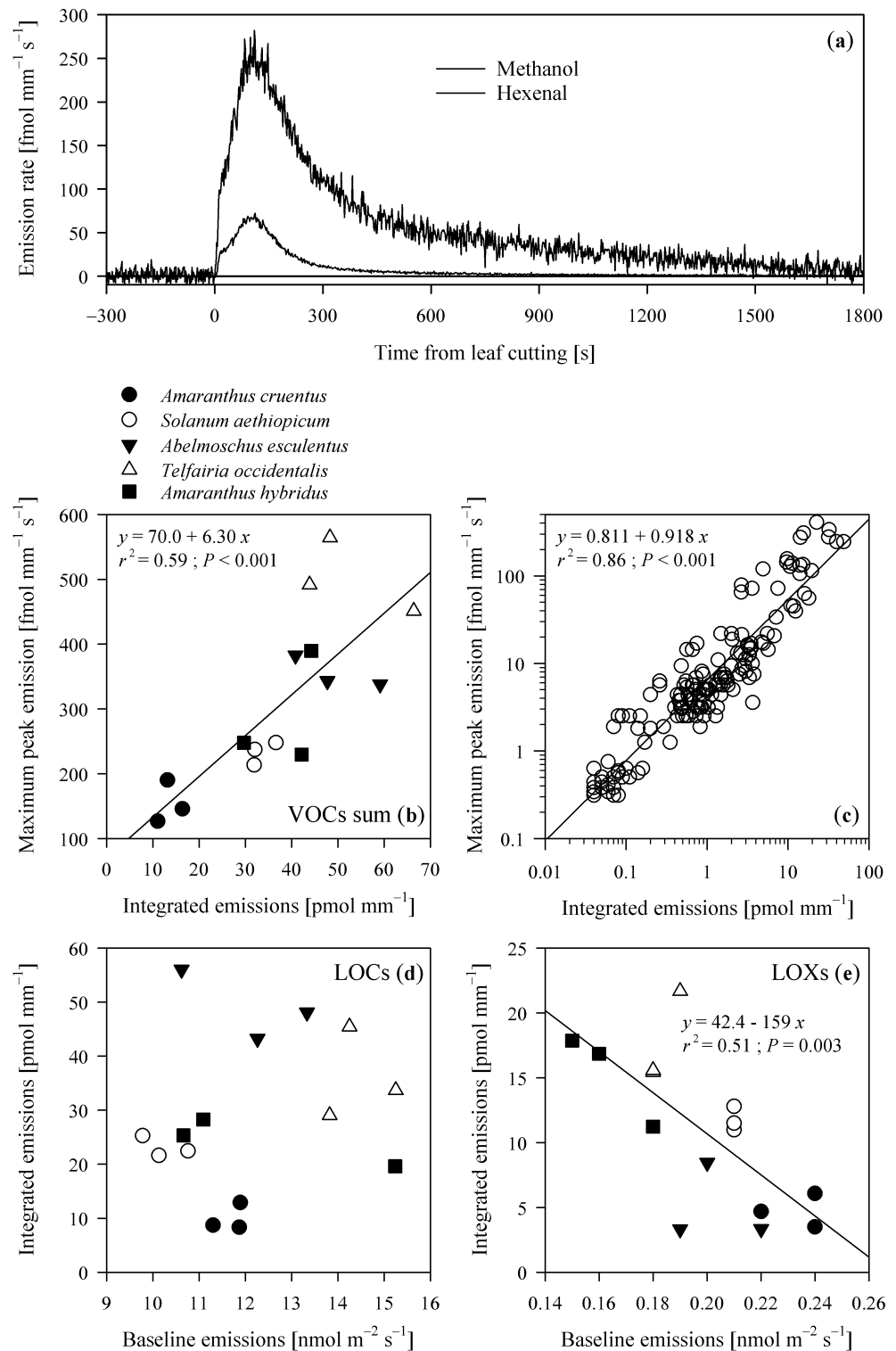
Figure 1. In (**a**), a representative time-course of wound-induced emissions of methanol (CH_4O) and hexenal ($C_6H_{10}O$) from an *Abelmoschus esculentus* leaf. The leaf was wounded at time 0 s with a 12-mm razor cut to the lamina. In the lower panels, the correlation between peak emission and (**b**) the sum of VOCs emitted and (**c**) all the VOCs individually across five tropical agricultural species. In (**d**) and (**e**) the correlation between baseline emissions of LOCs and LOXs and the integrated emissions after leaf wounding. The measurements were conducted with a proton-transfer-reaction time-of-flight mass spectrometer (PTR-TOF-MS). The values showed are corrected by the leaf constitutive emissions (Table 1) by averaging the pre-wounding emission data between −300 and 0 s and subtracting it from the emission values.

The time to peak maximum varied among the compounds (Table 2). For example, methanol and hexenal peak maxima were found 89 ± 20 s and 43 ± 19 s after wounding (means are significantly different at *P* = 0.0066 after a paired *t*-test). In addition, significant interspecific variation in the rate of emission elicitation was found, including early emissions of methanol (16.2 ± 2.1 s) in *Telfairia occidentalis,* and late emissions of hexenal in *Abelmoschus esculentus* (98 ± 11 s) and *Amaranthus hybridus* (78 ± 10 s) compared with the emissions of these compounds in the other species (Holm-Sidak pairwise comparison among the species, *P* < 0.05). After wounding, the emissions of some volatiles in several species, e.g., monoterpenes in *Amaranthus cruentus* and *Solanum aethiopicum,* presented an enhanced emission without an identifiable peak (Table 2). Compared with LOC and LOX, these compounds were emitted with a lower rate (Table 2).

Despite the interspecific variation in the emission amounts and dynamics of VOC emissions, the integrated emissions of LOC and LOX and isoprenoids and LOX and isoprenoids were positively correlated for all species pooled (*P* < 0.05) except for *Abelmoschus esculentus* (Figure 2). On the other hand, LOC, LOX and isoprenoid emissions were negatively correlated with benzenoid and jasmonate emissions (Figure 2). In these relationships, *A. esculentus* was again an outlier except for LOC and benzenoid and jasmonate emissions (Figure 2).
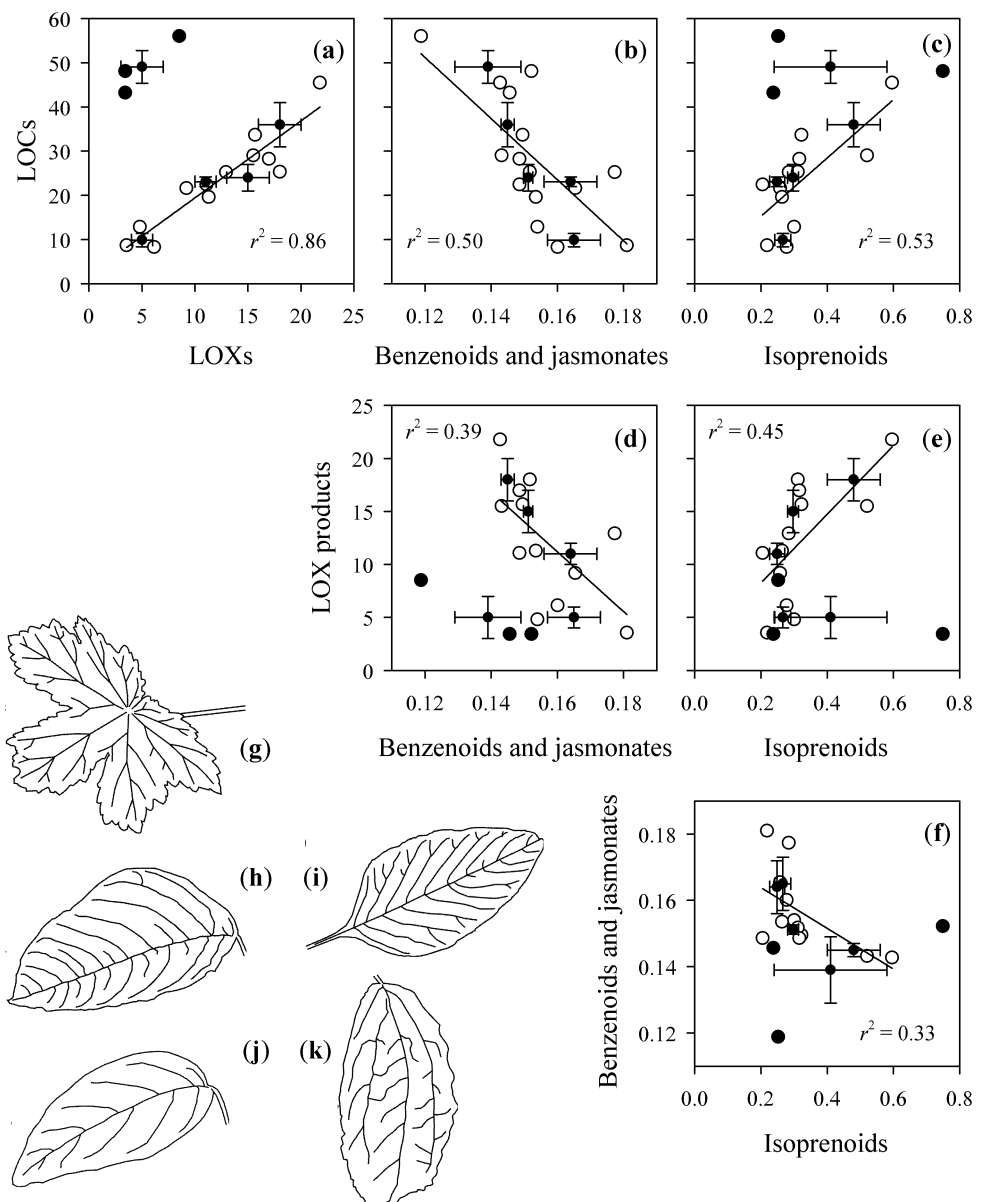
Figure 2. Correlations among (**a-f**) different classes of volatile organic compounds (VOC) emitted [pmol mm^{-1}] upon leaf wounding from leaves of five tropical crop species. Panels (**g**)-(**k**) show the outlines of leaves of studied species: (**g**) *Abelmoschus esculentus*, (**h**) *Amaranthus cruentus*, (**i**) *Amaranthus hybridus,* (**j**) *Telfairia occidentalis*, and (**k**) *Solanum aethiopicum*). Open circles are data points of replicate leaves per species. Grey circles are the data points of *Abelmoschus esculentus* that were excluded in the relationship (not in (**b**)). Filled points with standard error bar are averages for each species. Linear correlations shown are all significant at $P = 0.05$. LOX stands for volatile products of lipoxygenase pathway (also called green-leaf volatiles, typically C_5-C_6 aldehydes and alcohols, Table 1), and LOC for low-weight oxygenated compounds (C_1-C_3 volatiles synthesized through diverse pathways, Table 1).

3. Discussion

3.1 General patterns in volatile emission

Controlled leaf mechanical damage under laboratory conditions can simulate the effects of physical stress the plants undergo, particularly, under wounding by herbivory [4,5]. Real-time monitoring of different volatile emissions elicited by wounding can provide further insight into activation of different pathways in cell walls, membranes and cytosol, and can indicate the degree of degradation of cell structures and oxidation of other molecules and components [4,5] as well as changes in the activity of constitutively active metabolic pathways [9]. Furthermore, precisely controlled wounding allows quantitative and qualitative comparisons of rate of different pathway elicitation and magnitude of elicitation among different species [17,40].

Typically, wounding results in defined emission peaks of key volatiles emitted, but not for all [9]. Peaked emissions indicate *de novo* activation of stress metabolic pathways or release of stored compounds or their non-enzymatic oxidation products after sudden exposure of cellular contents to free atmosphere; or the combination of the two processes. In the case of gradual change of emissions, either increase or decrease of compounds released constitutively, wounding effect is typically indirect, through alteration of substrate availability of compounds synthesis [9]. In the five tropical species studied, we observed both peaked and non-peaked volatile emissions after wounding, although the bulk of volatiles was released in a burst-like manner (Table 2). In fact, the induction of massive emissions of volatiles upon mechanical wounding was almost immediate, peaking in 10-15 s at 85-136 fmol mm^{-1} s^{-1} for hexenal in *A. cruentus*, *S. aethiopicum* and *T. occidentalis*, or in 16 s at 331 fmol mm^{-1} s^{-1} for methanol in *T. occidentalis*; the emissions returned to the pre-stress level in less than 15 min. for most compounds, in agreement with previous studies [5,9].

We observed that methanol was generally the compound peaking among the first (Table 2). The emission of methanol to wounding and to several other abiotic and biotic stresses is an ubiquitous stress response throughout all plants due to cell wall damage and associated modification in pectin demethylation [10,41-43]. Elicitation of methanol emissions was typically followed by LOX volatiles (Table 2). The C_5 and C_6 compounds are emitted by wounded leaves due to the damage of cell membranes and the exposure to the atmospheric oxidant conditions [12,14]. The unsaturated fatty acids released from damaged membranes are broken down by LOX enzymes resulting in volatile C_5 and C_6 compounds in the hydroperoxide lyase (HPL) branch of the oxylipin pathway [19,26,44]. These can be further acetylated to compounds such as hexyl acetate and (*Z*)-3-hexenyl acetate [19,26,44]. In our study, the first LOX volatiles released were pentenal + 3-penten-2-one [14] and hexenal, followed by (Z)-3-hexen-1-ol + hexanal and (E)-1-hexanol, and ultimately by acetylated LOX volatile derivatives (Table 2) [12], quantitatively showing the activation of the sequence of biochemical events following mechanical damage.

Acetaldehyde was another key volatile released after leaf damage (Table 2) as observed in several other studies [5,12,25,26]. Acetaldehyde is mainly formed by enzymatic oxidation of ethanol, especially under anoxic conditions in the root zone [10,41,45], although some acetaldehyde can be directly formed from pyruvate [46,47], and there can be

a certain level of C_2 metabolites in leaves associated with fatty acid turnover. In fact, acetaldehyde was one of the compounds peaking the last in most species Table 2), suggesting that these emissions might be indicative of the final steps of fatty acid catabolism. On the other hand, in *A. esculentus*, there was no clear emission peak (Table 2), suggesting that wounding did not directly elicit pathways responsible for acetaldehyde release.

Apart from ubiquitous stress volatiles, we also observed the release of volatile isoprenoids and benzenoids that collectively made up a minor part of total emissions (0.49 ± 0.15% of benzenoids and 0.97 ± 0.20% of isoprenoids in all species, Table 3). Isoprene and other isoprenoids (e.g. monoterpenes, sesquiterpenes etc.), are specialized VOCs that can be emitted in large amounts in strong constitutive emitters under non-stressed conditions [40,48-50]. Terpenoid emissions can be further induced under different biotic and environmental stresses, but induction of these emissions is time-consuming taking multiple hours to days [40,48-50]. The situation is analogous with volatile benzenoids [e.g. 51]. Thus, peaked emissions of volatile isoprenoids and benzenoids should indicate release from storage and non-peaked emissions indirect modifications in pathway activity. Indeed, several plant groups store different volatiles within their leaves in specialized structures (e.g. resin ducts in conifers) or on leaf surface in glandular trichomes and upon leaf wounding, the breakage of the storage structures can expose volatiles to the atmosphere, resulting in emission bursts. We did not examine internal leaf anatomy and leaf surface characteristics in the current study, but there is evidence that all species studied have glandular trichomes on leaf surface: *A. esculentum* [52], *A. cruentus* [53], *A. hybridus* [54], *S. aethiopicum* [55] and *T. occidentalis* [56]. Different species have different density and types of glandular trichomes that store different volatile constituents [18,57], and this can be responsible for species differences in the emission dynamics of isoprenoids and benzenoids in our study. We observed that monoterpenes were released in a burst-like fashion in *A. esculentum*, *A. hybridus* and *T. occidentalis*, whereas methyl benzoate was released in a burst-like fashion in *A. esculentum*, *A. cruentus* and *S. aethiopicum* (Table 2).

3.2. Species diversity in emission responses

We observed a large interspecific variation in the rate of elicitation and emission magnitude across species (Table 2) —despite a similar constitutive VOC emission level. In particular, the emission induction rate for *Amaranthus cruentus* was 3-fold smaller as compared to the other amaranth, caused by comparably low methanol and hexenal emissions. Our hypothesis of the faster elicitation and greater magnitude of stress volatile induction upon wounding in physiologically more active leaves was partly supported. Indeed, hexenal emissions were elicited to a large degree in *Amaranthus hybridus*, and the fastest elicitation of hexenal emission occurred in *A. cruentus*, and these were the species with the highest photosynthetic rate and highest N and P use efficiency (A_{max}/N and A_{max}/P) (Tables 1 and 2). On the other hand, methanol emissions were elicited to the greatest degree in *A. esculentus* and *T. occidentalis* that had lower photosynthesis rate than the amaranths (Tables 1 and 2). It is plausible that the amount of cell walls per leaf area or their thickness was greater in species with lower photosynthetic activity [58,59], but future studies are needed to test this suggestions. Clearly, our data indicate that there is no simple correlation between leaf physiological activity (e.g. A_{max}) and wounding-induced emission response for these tropical crops. Moreover, in the same line, constitutive volatile emissions did not correlate with the power of elicitation of VOCs upon wounding.

Despite the large interspecific variability, we observed strong positive correlations among LOC and LOX compounds in the studied plant species (Figure 2), indicating coordination of the levels of elicitation for the ubiquitous volatiles with the exception of *A. esculentus*. We observed an average ± SE ratio of LOX/LOC of 0.511 ± 0.039 in the emissions for the four species, whereas in *A. esculentus* this ratio was 0.104. It is plausible that the variation in this ratio reflects overall amount of cell walls and membranes per leaf surface area and activity of enzymes responsible for LOX and LOC synthesis in membranes, cell walls and cytosol. Previous studies demonstrate that this ratio can widely vary across species: 1.23 in *Populus tremula* [5], 0.34 in *Trifolium repens*, 1.70 in *Ranunculus*

acris, and 2.64 in *Dactylis glomerata* [25]. Further research is needed to assess the variation range of this ratio across more plant families.

Interestingly, we observed negative correlations among ubiquitous LOC and LOX volatiles and terpenoids and phenolics (Figure 2), suggesting that the magnitude of emission and specificity of the emission signal are inversely related. Given that the specialized volatiles were likely mostly coming from breakage of glandular trichomes on leaf surface, such negative correlations might imply that the species with a greater constitutive defense capacity, i.e. greater density of glandular trichomes, have reduced induced defense response. Previously, such a negative correlation between glandular trichome density and stress-induced volatile release has been observed in ozone-stressed plants [57].

Regarding the levels of emissions in quantitative terms [pmol mm^{-1}], the agricultural crop species studied here emitted relatively low levels of VOCs (e.g., 3.7 to 15.9 pmol mm^{-1} of hexenal) when wounded as compared to the 283 pmol mm^{-1} of hexenal in *Dactylis glomerata* [4] and 570 pmol mm^{-1} in *Populus tremula* [5]. Similarly, the emissions of methanol (2.5-40 pmol mm^{-1}) and acetaldehyde (2.7-5.7 pmol mm^{-1}) after wounding in the studied agricultural species were smaller than those in *Populus tremula* (76 pmol mm^{-1} for methanol and 130 pmol mm^{-1} for acetaldehyde) [5]. It could be hypothesized that the agricultural plants have been selected to have high palatability as food source (among other characteristics including high nutritional value, low fiber content, reduction of secondary chemicals such as bitter- and astringent-tasting terpenes and phenolics), and this is related to the overall downregulation of secondary metabolic defenses. Nevertheless, more research into the evolutionary determinants of VOCs emissions upon wounding is needed to confirm this hypothesis.

The intraspecific variation of VOC emissions when wounding different plants was relatively low in our study (Table 2-3). In a previous study with *Populus tremula* numerous samples were taken from different leaves, and the intraspecific variability was generally low, provided fully-developed mature leaves were sampled and major veins were avoided when cutting the leaves [5,30]. Given the low intraspecific variability, we conclude that a screening study across multiple plant species can be conducted with a limited number of independent replicates, enabling large-scale investigations to find global patterns of wounding-induced VOC responses across biomes. However, such screening exercises should also consider other plant organs, because they can have different VOC compositions and emissions levels [30].

4. Materials and Methods

4.1. Plant material and growth conditions

Seeds of *Abelmoschus esculentus* (L.) Moench, *Amaranthus cruentus* L., *Amaranthus hybridus* L., *Solanum aethiopicum* L. and *Telfairia occidentalis* Hook. f. obtained from a private farm in Nigeria (9.1538 N, 7.3220 E, 445 m.a.s.l.) were sown in 3-litre plastic pots containing a 1:1:1 mixture of commercial potting soil with added balanced fertilizers (N:P:K = 10:8:16; Biolan Oy, Eura, Finland), quartz sand (AS Silikaat, Tallinn, Estonia) and vermiculite (Schetelig Group, Vantaa, Finland). The pH of soil water was 6.5. Three seedlings per species were grown in an environment-controlled plant growth room. The light period was 12 h and the light intensity at plant level was 400 – 500 µmol m^{-2} s^{-1} (HPI-T Plus 400 W, Philips, Brussels, Belgium), day and night temperatures were 28/25 °C, and relative air humidity was 60-70%. The plants grew for three months before the start of the measurements. At the start of the experiments, the plants had a similar biomass, mature stem thickness, and number of mature leaves among replicate plants.

4.2. Leaf photosynthetic capacity and stomatal conductance

This experiment was conducted with fully-developed mature attached leaves selected from the upper plant canopy. A GFS-3000 gas-exchange system (Walz GmbH, Ef-

feltrich, Germany) with the standard cuvette enclosing 8 cm^2 leaf area was used for photosynthetic measurements. The system was operated under the following conditions: leaf temperature of 25 °C, air relative humidity of 60%, chamber CO_2 concentration of 390-400 μmol mol^{-1}, incident light intensity of 500 μmol m^{-2} s^{-1} (10% blue, 90% red light), and cuvette flow rate of 750 μmol s^{-1}. Outdoor air was purified by a charcoal-filled filter to minimize its background VOC concentration.

After leaf enclosure, the photosynthetic capacity (A_{max}) and stomatal conductance (g_s) were monitored during a period of not less than 45 min to ensure leaf adaptation to the chamber conditions. After full adaptation, A_{max} and g_s were recorded, the leaf was cut as described in the next section, and A_{max} and g_s were recorded again in 30 min after wounding. Right after the cutting, the water vapour signal is strongly driven by water evaporation from the cut surface, but this initial rise is short-lived and in 30 min after leaf cutting, the values of g_s accurately reflect stomatal conductance [9]. Foliage gas-exchange rates were calculated according to von Caemmerer and Farquhar [60].

4.3. Leaf wounding and volatile organic compound emissions

The measurements of leaf volatile organic compound (VOC) emissions were conducted continuously with a proton-transfer-reaction time-of-flight mass spectrometer (PTR-TOF-MS) model 8000 (Ionicon Analytic GmbH, Innsbruck, Austria) following the setup and protocol of Rasulov et al. [9]. A part of the Walz GFS-3000 leaf cuvette exhaust air was diverted to PTR-TOF-MS during leaf measurements. The cuvette was specifically modified to sample the air as close as possible to the chamber, thereby avoiding any delays in VOC detection [9]. In addition, the background air VOC concentrations were measured before and after leaf enclosure. PTR-TOF-MS was operated as in Rasulov et al. [9]. In short, the drift tube field density ratio (E/N) was ≈130 Td, and the protonated ions exiting the tube were pulsed every 30 μs to the time-of-flight region, which resulted in spectra ranging from 1-278 *m/z*. 60,000 spectra were averaged every 1.8 s. The PTR-MS-TOF was calibrated with a standard gas mixture containing ppm level concentrations of representatives of key volatile groups (Ionimed GmbH, Innsbruck, Austria). The data of VOCs was recorded and processed using PTR-MS Viewer v3.2 (Ionicon, Innsbruck, Austria).

Volatile organic compound emissions (VOC) emissions together with leaf gas exchange rates were measured simultaneously through the leaf adaptation period, and constitutive VOC emission rates were estimated. The leaf cuvette was modified by inserting a blade attached to a fine metal rod exiting the chamber (technical drawing in [9]). A sharp and quick cut of 12 mm on the leaf lamina was obtained by rotating the blade without opening the cuvette and thereby avoiding any contamination by ambient VOC and allowing continuous VOC measurements. The leaf cuts were made in intercostal lamina areas to avoid cutting through major veins that can result in disproportionately higher emission rates than the cuts between major veins [30]. The burst of VOC emissions after the leaf wounding was recorded for 30 min (1000 data points per compound). After this period, VOC emission levels of all compounds had typically stabilized to pre-wounding levels. The VOC emission rates were calculated according to Niinemets et al. [61] considering the background VOC concentrations. The wounding stress was characterized by the integrated amount of given VOC elicited during the 30-min period expressed per wound length (pmol mm^{-1}). The wounding VOC emission peaks were fitted by a bi-Gaussian function following the protocol in [5] due to the longer tail after the maximum. A peak-like emission pattern was considered when the emission data fitted successfully the Gaussian equation at $P = 0.05$ level. Then the maximum emission rate (fmol mm^{-1} cut length s^{-1}) was reported as well as the time from wounding to the peak emission. All experiments were replicated at least thrice for each species with independent plants.

The emitted VOCs were grouped into five classes according to [5]: lightweight oxygenated compounds (LOCs) – formaldehyde, methanol, acetaldehyde, formic acid,

ethanol, acetone, acetic acid; volatile products of lipoxygenase pathway (LOXs products) - pentenal + 3-penten-2-one, pentanal + 2-pentanone, hexenal, (*Z*)-3-hexen-1-ol + hexanal, (*E*)-1-hexanol, hexyl acetate; geranylgeranyl diphosphate (GGDP) pathway – 6-methyl-5-hepten-2-one; benzenoids and jasmonates – methyl benzoate, methyl salicylate, jasmonic acid, methyl jasmonate; and isoprenoids – non-oxygenated monoterpenes, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), oxygenated monoterpenes, sesquiterpenes, and (*E,E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT). The mass ions used to identify different volatiles are provided in Table 2. Identity of compounds with C_3-C_{17} was verified by GC-MS. Although all studied species emitted trace levels of isoprene [48], isoprene and some C_5 LOX products cannot be separated during wounding by this setup.

4.4. Compound verification by gas chromatography mass spectrometry (GC-MS)

GC-MS analysis was used to verify the identity of compounds emitted constitutively and during wounding. Volatiles were collected from the cuvette exhaust air through a Teflon T-piece and PTFE tubing with a 210–1003 MTX (SKC Inc., Houston, TX, USA) air sample pump with a rate of 0.2 L min⁻¹ for 20 min, resulting in sampling of 4 L air. Volatiles were collected onto multi-bed stainless steel cartridges filled with three different carbon-based adsorbent, Carbotrap C 20/40 mesh, Carbopack B 40/60 mesh and Carbotrap X 20/40 (Supelco, Bellefonte, PA, USA), for optimal adsorption of all volatiles between C_3 and C_{17} [62]. Blank samples were taken from the cuvette air without the leaf. The analysis of cartridges was carried out with a combined Shimadzu TD20 automated cartridge desorber and a Shimadzu 2010 Plus GC-MS (Shimadzu Corporation, Kyoto, Japan) as described in detail in [62-64]. A quantitative analysis of lightweight oxygenated volatiles, fatty acid derived compounds, volatile isoprenoids, monoterpenes, and sesquiterpenes was performed to confirm the structure of the ions measured by PTR-TOF-MS. GC-MS was calibrated using authentic standards (Sigma-Aldrich, St. Louis, MO, USA) for key LOX, mono- and sesquiterpenes and benzenoids as described in detail in Kännaste et al. [62]. The identification of compounds was done on the basis of authentic standards and compound retention times and mass spectra using NIST 14 spectral library using the open-access program OpenChrom ver 1.2.0 (Alder) [36]. Three replicate measurements per plant species were conducted.

Author Contributions: Conceptualization, Ü.N., M.P.-E.; methodology, C.N.O, Y.J., E.T., E.K., Ü.N.; data analysis, M.P.-E., C.N.O; writing—initial draft preparation, M.P.-E.; writing—review and editing, Ü.N., C.NO.. All authors have read and approved the published version of the manuscript.

Data Availability Statement: The data used in this article is publicly available in a digital repository: https://doi.org/10.5061/dryad.mpg4f4qzk

Conflicts of Interest: "The authors declare no conflict of interest."

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