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Differential timing response of Herbivory-Induced Volatiles (HIPVs) in *Croton floribundus* Spreng. (Euphorbiaceae) [1]

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Short Title: Herbivory-Induced Volatiles in *C. floribundus*

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1. Part of the first author's Master Dissertation

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ABSTRACT – (Differential timing response of Herbivory-Induced Volatiles (HIPVs) in Croton floribundus Spreng. (Euphorbiaceae)). In this study, we demonstrated that *Tetranychus urticae* Koch (Acari: Tetranychidae), a generalist herbivore, induces volatile organic compounds (VOC) in *Croton floribundus* Spreng., a pioneer species widely used in Brazilian urban area. We performed experiments to evaluate the quality and quantity of VOC emission at different times (two, six and 24 hours and within four and nine days) of infestation by *T. urticae.* Results show that *C. floribundus* emitted 23 volatiles after infestation, including monoterpene, sesquiterpene and green leaf volatiles. Significant differences were only detected between infested and non-infested plants after 24 hours of treatment, in particular methyl salicylate. In contrast, 3-hexen-1ol, linaool, geranyl acetone and caryophyllene seem to be inhibited by hourly infestation. The α-farnesene, methyl salicylate, 3-carene, 3-hexen-1ol benzoate and nerolidol were the main compounds induced after four infestation-days. This study highlights that VOCs blends in *C. floribundus* is depended on the feeding time-course of *T. urticae* and suggests that the VOC-mediated ecological interaction may be less efficient in a pioneer species.
Keywords: *Croton floribundus,* herbivory, isoprenoids, pioneer tree, volatile organic compound

RESUMO – (Resposta temporal diferencial de voláteis induzidos por herbivoria (HIPVs) em Croton floribundus Spreng. (Euphorbiaceae)). Neste estudo foi demonstrado que o *Tetranychus urticae* Koch (Acari: Tetranychidae), um herbívoro generalista, promove a emissão de compostos orgânicos voláteis (COV) em *Croton floribundus* (L.) Spreng., uma espécie pioneira amplamente utilizada na área urbana brasileira. Avaliou-se a qualidade e quantidade de emissão de COV em diferentes momentos (2, 6 e 24 horas e no intervalo de 4 e 9 dias) de infestação por *T. urticae*. Os resultados mostram que *C. floribundus* emitiu 23 voláteis após infestação, incluindo monoterpenos, sesquiterpenos e voláteis de folhas verdes. Diferenças significativas entre plantas infestadas e não infestadas foram detectadas após 24 horas de tratamento, em particular foram observadas alterações nos níveis de metil salicitato. Em contraste, o 3-hexen-1ol, linaool, geranil acetona e cariofileno parecem ter sido inibidos pela infestação horária. O α-farneseno, metil salicilato, 3-careno, benzoato de 3-hexen-1ol e nerolidol foram os principais compostos induzidos após quatro dias de infestação. Este estudo demonstra que o buquet de COVs emitido por *C. floribundus* depende do tempo de infestação da *T. urticae* e sugere que a interação ecológica mediada por COVs pode ser menos eficiente em espécie pioneira.
Palavras-chave: árvores pioneiras, compostos orgânicos voláteis, *Croton floribundus,* herbivoria, isoprenoides

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Introduction

Plants produce mixtures of volatile organic compounds (VOCs), so-called herbivore-induced VOCs (HIPVs) as a natural interaction to herbivory. The amount and chemical variability of VOCs produced are specific for each insect-plant interaction and depends on the nature and the intensity of stress (Pareja and Pinto-Zevallos 2016), natural enemies and neighboring plants (Fürstenberg-Hägg et al. 2013). The chemical variability and amounts of VOCs are also dependent on the developmental stage of the plant species and the herbivore. In addition, VOCs can act as repellents for herbivores that infest and cause pests, or as attractants for their natural enemies, as well as alert intact and neighboring plants to future attacks (Maffei 2010). Literature data show that the urban environment can alter the physiology and behavior of herbivores and even substantially affect the increase in herbivory rate over time (Baldwin et al 2006, Himanen *et al*. 2010, Holopainen J. K., Blande J. D. 2013). Data on herbivory and the production of VOCs with cultivated plants, in the agricultural sector is well documented, with a significant number of herbivore-plant combinations, however, little is known about herbivory with native species, especially those that occur in urban environments. *Croton floribundus* (L.) Spreng. is a pioneer species widely distributed in the Brazilian rainforest and used in restoration plans of urban areas for being a pioneer successional species and recently considered as highly tolerant to abiotic stress (Cardoso-Gustavson *et al*. 2014, Moura *et al*. 2018). However, the effect of biotic stress in *C. floribundus* has not yet been reported, especially in the case of VOCs induced by *T. urticae*.

Our previous research has shown that *C. floribundus* emits high levels of mono and sesquiterpene constitutive volatile (Bolsoni *et al*. 2018, Moura *et. al* 2022) and under oxidative ozone stress can induce the signalling volatile, in particular methyl salicylate (MeSa) and monoterpenes that can increase the plant defence against biotic and abiotic stress (Cardoso-Gustavson *et al*. 2014). If abiotic stress, *e.g.* ozone, can induce signalling VOCs in *C. froribundus* (Pinto *et al*. 2010, Souza *et al*. 2013, Cardoso-Gustavson *et al*. 2014), would also be expected the similar behaviour in biotic stress, *e.g.* herbivory. However, there are no studies of HIPVs in *C. floribundus*. Therefore, we aimed to evaluate the effect of time infestation by *Tetranychus urticae* Koch (Acari: Tetranychidae) on volatile emission in *C. floribundus* to characterize the profile of HPIV on a pioneer species with high occurrence in an urban area and extensively used in the ecological restoration.

https://doi.org/10.1590/2236-8906-18/2022

Material and methods

Living Material - One-month old *Croton floribundus* Spreng. plants purchased from a nursery (Bioflora, Piracicaba, São Paulo) were individually sown in 10-L pots filled with a 3:1 mixture of peat and sand and watered by capillarity. Plants were kept inside the greenhouse with filtered air for four weeks and then transferred to chambers, where they were kept for 4 days before the beginning of the herbivory exposure (acclimation period). Plants were watered as needed until the VOCs collection. Fortnightly the plants received a nutritional solution. Individual of *Tetranychus urticae* Koch (Acari: Tetranychidae) were started from adults collected from *Phaseulus vulgaris* and transferred to *Croton floribundus* for adaptation. Each plant received 200 individuals of *T. urticae,* which were arranged randomly in the leaf limbs. Each individual was kept in a chamber with continuous airflow, artificial light (422 μmol.cm^{2}s^{-1}), 27.2±2 °C and 65.3± 5.2% humidity.

Plant VOC collection and analysis - VOCs from the headspace of both non-infested (control) and infested *C. floribundus* plants by *T. urticae* were collected. Induction of plants was accomplished by infesting two young leaves in two set of experiments, in which one of them was carried out in hours and another in days of infestation by *T. urticae*. The volatiles were collected from each individual after 0, 2, 6 and 24 hours (hourly experiment) and 4 to 7-9 days (daily experiment). Prior to placing the plants into the chamber, the pots were enclosed in aluminum paper to avoid the presence of volatiles from the pot or soil in the samples. The push-pull sampling system constructed worked as follows. Charcoal-filtered humidified airflow (20 L/min) was generated by a compressor. Airflow was divided into four parts (1.5 L/min) using a manifold line connection with four outlines. In this way, four replicates of treatment and control (individuals infested and non-infested) were collected simultaneously. Each individual was kept in a Teflon bag with two opens; one for inflow air and another connected to a tube for VOC collection. VOCs were collected from the out coming air onto ca. 150 g Tenax TA adsorbent (Mesh 60/80, Supelco, PA, USA) at a rate of 0.200 L/min for 90 minutes, by pulling the air with a vacuum pump (Air lite, Supelco). All the system was constructed with Teflon, and the airflows adjusted with adjustable flow meters (Cole-Parmer Instruments Co). The experiment was repeated six time.

VOCs were analyzed by gas chromatography-mass spectrometry (GC-MS) (MSD 5973; Agilent GC 6890). Trapped compounds were desorbed with a thermal desorption unit (Perkin-Elmer ATD400 Automatic Thermal Desorption system; Perkin Elmer, Waltham, MA, USA) at 250 °C for 10 min, cryofocused at -30 °C and injected into an HP-5 capillary column (50 m x 0.2 mm i.d. x 0.5 μm film thickness; Hewlett-Packard) with helium as a carrier gas. The oven

temperature program was held at 40 °C for 1 min, then raised to 210 °C at a rate of 5 °C min^{-1} and finally raised further to 250 °C at a rate of 20 °C min^{-1}. Compounds were identified by comparing their mass spectra with the mass spectra in a reference library (NIST MS 2.0), and in the literature (Adams 2007). Quantification of individual compounds was achieved by comparing the peaks with the peaks of known concentrations of synthetic compounds after the construction of calibration curves. For those compounds that synthetic standards were not available, quantification was achieved by considering their response to be the same as of α-pinene. Emission rates were calculated and expressed in ng per gram of leaf dry weight per hour (leaves dried at 60 °C for 72 hours).

Statistical Analyses - Statistical analyses were performed with Sigma plot 12.0. To detect the main compounds that contributed hourly and daily to the blend of induced volatiles, the comparison between the volatiles of *C. floribundus* infested by *T. urticae* and controls was carried out using ANOVA and *Tukey post hoc* for multiple comparisons. When normality was not achieved, the non-parametric Mann-Whitney U test was performed.

Results

Twenty-three compounds were found in the headspace of *Croton floribundus*, considering constitutive and induced VOCs. All the identified VOCs were grouped into their respective chemical classes and summarized in tables 1 and 2. No significant differences in the hourly experiment between non-infested (control) and infested plants were found except for methyl salicylate (MeSA) twhich showed higher levels after six and 24 hours and 3-hexen-1-ol after 24 hours of infestation (table 1) Although a significant difference was not detectable in others compounds, it is noteworthy that linalool was the main terpene emitted in the control of the hourly experiment (table 1), while its synthesis was almost completely inhibited in the treatment. There was also an increase in farnesene in the treated plants after 24 hours. In daily experiment, monoterpene 3-carene, α-farnesene, 3-hexen-1ol benzoate showed significant differences between the infestation and control (table 2), in which their levels were increased after four infestation-days. In additional, aldehydes such as 3-hexanal and nonanal, and alcohols such as 3-hexen-1-ol reduced their levels upon *Tetranychus urticae* Koch (Acari: Tetranychidae) infestation (table 2). Comparing the sum of all emissions in the hourly and daily experiments, there were a decrease in plant emissions on hourly treatments while an increase in plant emissions in daily treatments was observed.

https://doi.org/10.1590/2236-8906-18/2022

To demonstrate the induced, inhibited, and non-affected VOC in infested *C. florbundus* upon *T. urticae* the ration between infestation/control VOCs emissions was performed (figure 1 and 2) in logarithmic scale. To standardize the three category of infestation effect, we considered the range of experimental errors (from - 0.05 to 0.05) to non-affected VOC, logarithmic values above 0.05 to induced VOC and below -0.05 to inhibited VOC. The *T. urticae* was able to induce granyl acetone and α-cubebene at 2h and 3-hexen-1ol benzoate, 3-hexenal at 6h and 24h of exposure. Meanwhile, 3-carene, α -farnesene and β-farnesene were only induced at 24h. The beginning of infestation with *T. urticae* inhibited the syntheses of gernyl acetone, 3-hexen-1ol benzoate, n-valeric, 3-hexen 1-ol, α -farnesene, caryophelene, β-farnesene, copaene, methyl salicylate, decanal and nonanal (figure 1). In daily experiments, most of the emitted VOCs were induced; α-farnesene, 3-carene, Methyl Salicylate (MeSA), 3-hexen-1ol were induced in all days of treatment. Moreover, the sesquiterpenes, α-farnesene, were significantly induced by *T. urticae*, up to nine infestation-days. Although D-limonene and Methyl Jasmonate (MeJA) were induced in four days, they were inhibited in six and seven days of exposure. Interestingly, MeJA appeared only in the daily experiment; being induced at four days, followed by inhibition at 5 and 6 days and going through induction at 7 days. Furthermore, the induction of MeJA was higher than that of methyl salicylate after seven days of infestation (figure 2).

Discussion

Croton floribundus Spreng. is widely used in ecological restoration plans of Brazilian urban areas for being a pioneer successional species and recently considered as highly tolerant to abiotic stress (Cardoso-Gustavson *et al*. 2014, Moura *et al*. 2018). However, the effect of biotic stress in *C. floribundus* has not yet been reported, especially in the case of VOCs induced by *Tetranychus urticae* Koch (Acari: Tetranychidae). We know that *T. urticae* is a general herbivore and infests many crop plant species such as beans (Dicke *et al*. 1990*)*, apple (Takabayashi *et al*. 1991) and tomato (Dicke *et al*. 1998) and induces a variety of volatiles that are attractive to its natural predator *Phytoseiulus persimilis* (Van Den Boom *et al*. 2004), such as MeSa and α-farnesene, caryophyllene and linalool. In our study, herbivory-induced volatiles in *C. floribundus* were similar to those reported by Van Den Boom *et al.* (2004), being MeSA and α-farnesene promptly induced, indicating that responsiveness of *C. floribundus* to *T. urticae* may be similar to that in crop plants.

Previous studies with herbivores have shown that herbivore-induced volatiles are influenced by feeding time-course (Niinemets *et al*. 2013), modifying the volatile blends quantitatively (Dicke 1999, Zhang 2003). However, qualitatively differences in volatile blends

have been little reported (Himanen *et al*. 2010). In this study, the results showed that the herbivore-induced VOCs blends in *C. floribundus* varied both quantitatively and qualitatively, and that such variations depended on the feeding time-course.

In the hourly experiment, there was an inhibition of several constitutive volatiles after two and six hours of *T. urticae* infestation, leading to a qualitative change in the herbivore-induced VOCs blends. In this case, the absence of 3-hexen 1-ol, linalool, β copaene, α-farnesene in *C. floribundus* after infestation by *T. urticae* was relevant to distinguish the VOCs profiles. In contrast, quantitative changes were observed in the daily experiment, in which 3-hexen-1ol, 3-carene, linalool, MeSA, MeJA α-farnesene and nerolydol were induced and showed significant differences between undamaged and damaged plants. All of these compounds have been associated with a strategy defense of plants against herbivory (Dicke 1999, Arimura *et al*. 2000, Birkett *et al*. 2000, Baldwin *et al.* 2006, Kant *et al*. 2009, Kigathi *et al*. 2009, Pinto-Zevallos *et al*. 2013). Moreover, MeSA and MeJA, which are derived from the oxygenation of polyunsaturated fatty acids via the octadecanoid pathway, play a central role in plant defense against pathogens and herbivory. MeJA has been reported as a vital cellular regulator that mediates diverse developmental process and defenses response against biotic and abiotic stresses (Pinto-Zevallos *et al*. 2013). In our study, the syntheses of MeJA triggered only after days of exposure, indicating that that *C. floribundus* may be more tolerant to *T. urticae* during the first hours of infestation and before the synthesis of MeJA is activated, when it can diffuse through intercellular migration to induce systemic signaling (Bruinsma *et al.* 2009).

Some compounds detected here, such as 3-hexen-1ol, linalool, α farnesene and caryophyllene have been reported as volatiles induced by *T. urticae* in crop plants and, unlike those reported in this study, they are induced in shorter time of infestation (Holopainen 2004, Holopainen and Blande 2013, Zakir 2013). Also, according to Holopainen *et al*. (2013) the presence of α-farnesene and caryophyllene in the bouquet of volatiles has a relevant impact on the trophic interactions between *T. urticae* and its predators. Some studies reported that the repellency of α-farnesene and caryophyllene is directly related to the proportion of these compounds in the blend of induced volatiles (Mostafavi *et al*. 1996, Holopainen 2004). Many studies have shown that high levels of caryophyllenes can reduce the potential repellency of farnesene (Bernasconi *et al*. 1998, Mostafavi *et al*. 1996, Kant *et al*. 2004). However, the presence of caryophyllene, even in low proportions, is ecologically important because it is key volatile compound involved in the attraction of predators, such as parasitoid wasps (Dawson *et al*. 1984, Dudareva *et al*. 2013). In our study, the inducible volatiles α-farnesene and caryophyllene as well as MeSa and 3-carene were detected after 4 days of infestations, whereas

similar concentrations have been found in tomate, beans and maize at 24 hours or less (Bernasconi *et al*. 1998, Borges *et al*. 2018). Our results suggest that ecological interaction in *C. floribundus* requires more time to occur than in crop plants, in which the VOC induction often happens in the hourly infestation, acting on insect repellency, influencing oviposition and mediating the direct and indirect defenses of plants. So, these observations have important implications for understanding plant responses to herbivory attack in native species present in urban environments; in which abiotic and biotic stress act simultaneously.

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Conflicts of interest

The authors have declared that no competing interests exist.

Author Contributions

Débora Pinheiro-Oliveira: Conceived and designed the experiments; performed the experiments; analysed the data; wrote the paper.
Silvia Ribeiro de Souza: Conceived and designed the experiments; analysed the data; wrote the paper.
Giselle da Silva Pedrosa: Performed the experiments.

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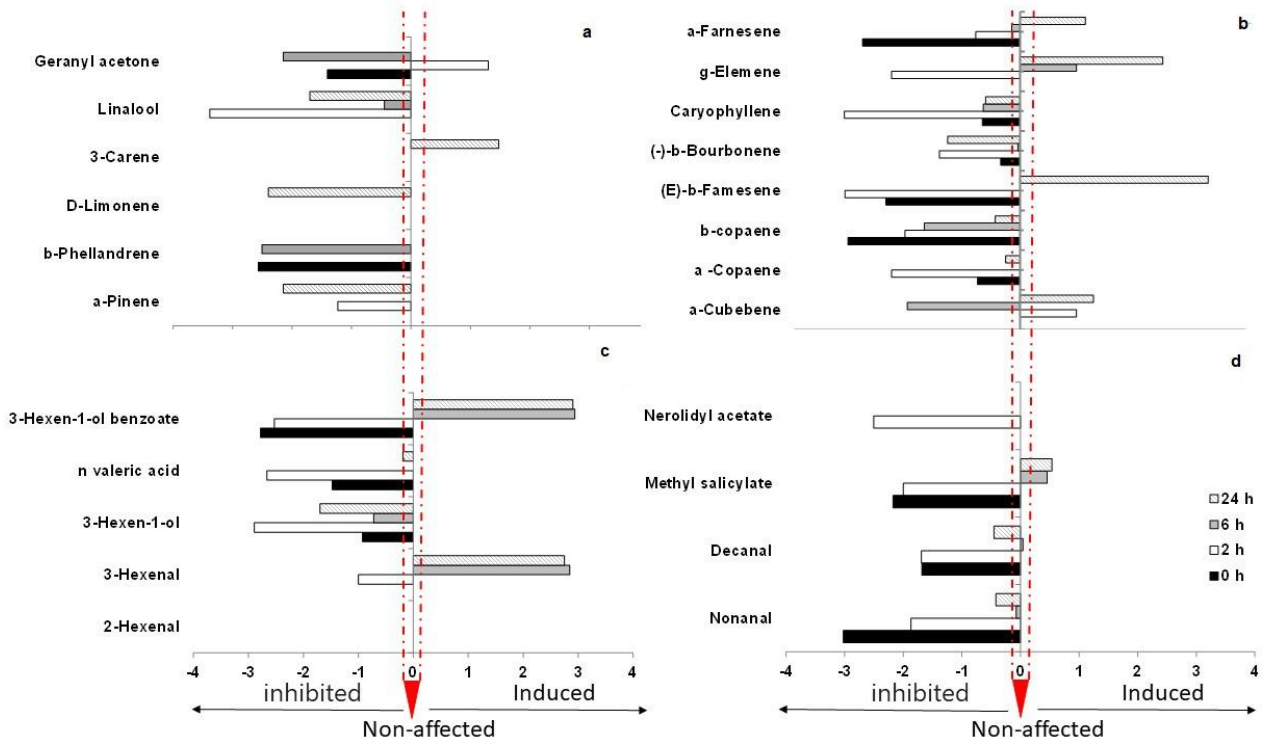

Figure 1: Induction and inhibition of Volatile Organic Compound (VOC) in *Croton floribundus* Spreng. represented as the ration between VOC emission from infested and non-infested plants by *Tetranychus urticae* Koch in the hourly experiment. The ration is expressed in the log (x). The zero value represents the equal values of control and treatment. Between -0.05 and 0.05 values represents non-affected VOC emission. Above 0.05 value is the ration of induction and below -0.05 is the ration of inhibition of VOCs emissions a: Monoterpene. b: Sesquiterpene. c: Green leaf volatiles. d: Other compounds.

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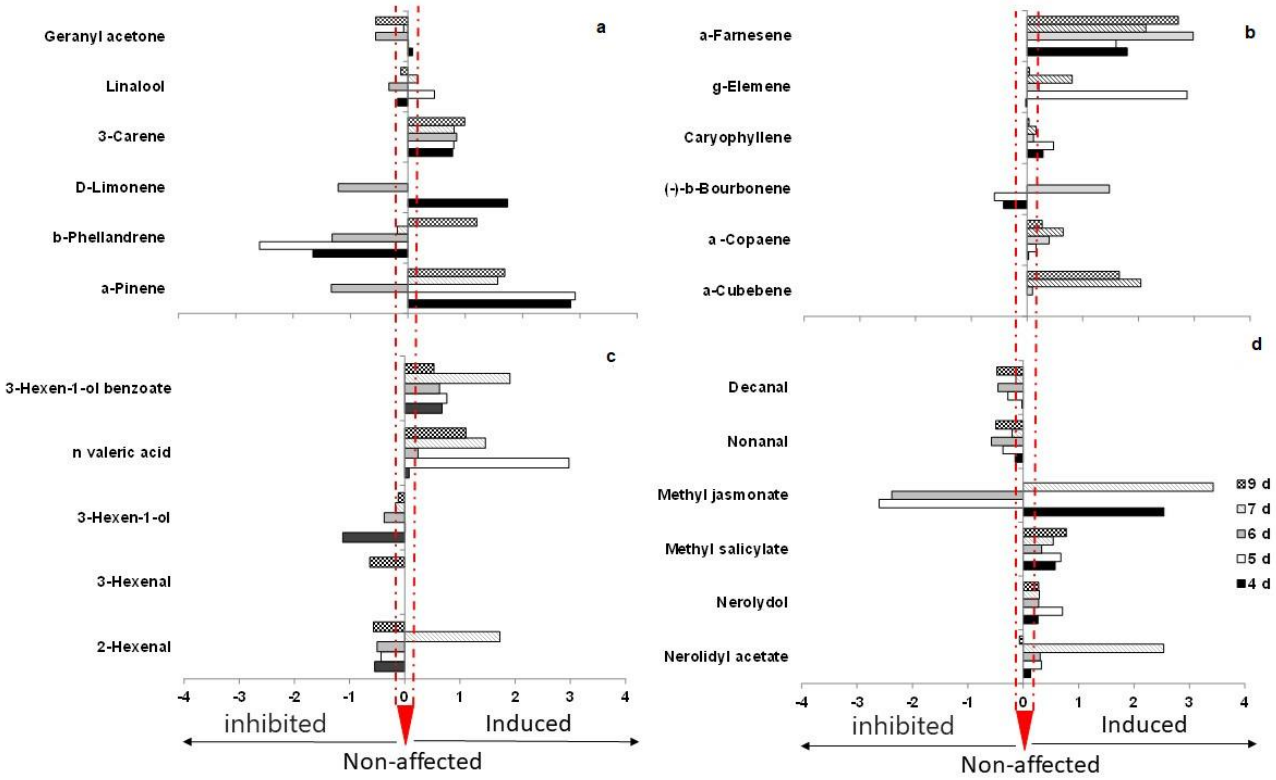

Figure 2: Induction and inhibition of Volatile Organic Compound (VOC) in *Croton floribundus* represented as the ration between VOC emission from infested and non-infested plants by *Tetranychus urticae* Koch in the daily experiment. The ration is expressed in the log (x). The zero value represents the equal values of control and treatment. Between -0.05 and 0.05 values represents non-affected VOC emission. Above 0.05 value is the ration of induction and below -0.05 is the ration of inhibition of VOCs emissions. a: Monoterpene. b: Sesquiterpene. c: Green leaf volatiles. d: Other compounds.

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Table 1. Hourly emissions of Volatile Organic Compounds (VOC) (ng g DW^{-1} h^{-1}) by non-infested (control) *Croton floribundus.* Spreng. and plants infested (Herbivory) by 200 individuals *Tetranychus urticae* Koch (Mean ± S.E.M) (*N* = 24). Statistically significant values are presented with asterisks for comparison of control and treatment.

Identified compounds	Treatment							
	Control				Herbivory			
	0 hour	2 hours	6 hours	24 hours	0 hour	2 hours	6 hours	24 hours
Monoterpenes (C10)								
α-Pinene	-	0.7±1.2	-	0.4±0.5	-	-	-	-
β-Phellandrene	3.7±3.7	-	3.2±5.5	-	-	-	-	-
D-Limonene	-	-	-	2.5±2.9	-	-	-	-
3-Carene	-	-	-	-	-	-	-	0.3±0.5
Linalool	-	24.1±20.9	8.1±11.6	20.1±10.8	-	-	2.9±3.1	0.4±0.7
Geranyl acetone	5.1±8.9	-	1.4±2.4	-	0.2±0.3	0.2±0.3	-	-
Sesquiterpenes (C15)								
α-Cubebene	-	0.2±0.4	0.2±0.4	-	-	0.2±0.5	-	0.2±0.4
α –Copaene	1.7±2.9	1.9±2.8	-	1.8±1.8	0.3±0.5	-	-	6.0±10.4
β-copaene	11.3±10.1	1.1±1.9	0.5±0.9	2.2±2.2	-	-	-	0.8±1.3
(E)-β-Famesene	2.4±4.1	12.8±12.6	-	-	-	-	-	21.7±33.6
(-)-β-Bourbonene	7.9±7.2	13.5±10.7	1.2±2.2	5.8±4.0	3.6±6.3	0.5±0.4	1.1±2.0	0.3±0.6
Caryophyllene	6.5±6.4	13.1±19.2	0.9±1.6	6.1±6.3	1.4±2.5	-	0.2±0.4	1.5±1.5
γ-Elemene	-	1.9±3.3	-	-	-	-	-	-
α-Farnesene	6.2±9.9	15.6±20.4	2.0±2.2	2.0±2.4	-	-	0.1±0.3	3.4±3.5
Green Leaf Volatiles								
2-Hexenal	-	-	-	-	-	-	-	-
3-Hexenal	-	0.1±0.3	-	-	-	-	-	-
3-Hexen-1-ol	4.2±5.3	7.8±9.0	8.9±13.9	10.0±7.9*****	0.5±0.4	-	7.0±6.5	0.6±**0.9***
n valeric acid	3.0±5.2	4.6±8.0	-	3.4±3.9	0.1±0.2	-	1.7±2.1	0.2±0.5
3-Hexen-1-ol benzoate	6.0±5.2	13.5±17.0	-	-	-	-	-	2.2±2.0
Other Compounds								
Nonanal	10.5±10.6	7.4±1.3	10.9±4.3	17.9±12.2	-	0.1±0.1	9.2±9.9	6.8±6.1
Decanal	19.2±12.3	9.8±8.7	23.6±7.0	26.±19.7	0.4±0.3	0.2±0.2	26.0±27.2	9.2±11.7
Methyl salicylate	44.9±66.7	34.3±36.5	22.8±23.4*	40.9±43.1*	0.3±0.5*	-	65.1±99.9*	141.0±90.2*
Nerolidyl acetate	-	3.2±5.6	-	-	-	-	-	-
Sum	133.0±159.1	479.4±504.7	84.2±75.8	140.1±124.5	7.0±11.3	1.5±1.8	113.7±151.7	68.2±84.4

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Table 2. Daily emissions of Volatile Organic Compounds (VOC) (ng g DW^{-1} h^{-1}) by non-infested (control) *Croton floribundus* Spreng. and plants infested (Herbivory) by 200 individuals *Tetranychus urticae* Koch (Mean ± S.E.M) (*N* = 24). Statistically significant values are presented with asterisks for comparison of control and treatment.

Identified compounds	Treatment									
	Control					Herbivory				
	4 days	5 days	6 days	7 days	9 days	4 days	5 days	6 days	7 days	9 days
Monoterpenes (C10)										
α-Pinene	-	-	5.6 ± 4.78	-	-	6.88 ± 10.36	8.28 ± 8.28	0.26 ± 0.55	0.37 ± 0.92	0.49 ± 1.05
α-Phellandrene	0.45 ± 0.64	3.86 ± 5.47	6.7 ± 9.47	0.26 ± 0.36	-	-	-	0.32 ± 1.02	0.17 ± 0.28	0.16 ± 0.52
D-Limonene	-	-	14.1 ± 3.21	-	-	0.55 ± 1.11	-	0.86 ± 1.93	-	-
3-Carene	32.69 ± 4.5	37.84 ± 41.2	9.33 ± 0.76	8.17 ± 1.21	6.3 ± 3.36	197.68 ± 80.09*	240.48 ± 106.9*	66.09 ± 57.2*	52.32 ± 51.9*	62.04 ± 53.4*
Linalool	13.53 ± 19.14	5.76 ± 4.21	8.07 ± 4.78	2.52 ± 3.56	4.68 ± 6.62	9.04 ± 8.25	16.77 ± 11.49	3.8 ± 3.02	3.76 ± 4.19	3.54 ± 2.44
Geranylacetone	2.48 ± 3.51	4.29 ± 1.67	10.1 ± 2.47	3.32 ± 0.17	9.1 ± 7.73	2.99 ± 2.02	4.39 ± 1.43	2.77 ± 1.87	2.8 ± 2.23	2.52 ± 1.86
Sesquiterpenes (C15)										
α-Cubebene	-	-	1.12 ± 1.59	-	-	-	-	1.4 ± 4.43	1.1 ± 2.71	0.45 ± 1.44
α-Copaene	5.37 ± 3.78	5.12 ± 0.53	7.84 ± 2.26	3.31 ± 0.96	4.85 ± 6.86	5.72 ± 1.33	7.41 ± 2.94	19.51 ± 50.19	14.7 ± 32.74	9.07 ± 21.28
(-)-β-Bourbonene	1.24 ± 1.76	3.38 ± 0.14	-	-	-	0.47 ± 0.94	0.88 ± 1.76	0.3 ± 0.96	-	-
Caryophyllene	12.47 ± 8.22	9.45 ± 0.55	10.37 ± 0.82	6.05 ± 3.43	9.38 ± 6.26	24.18 ± 3.52	28.16 ± 5.02	13.46 ± 6.33	8.82 ± 6.1	10.16 ± 4.76
γ-Elemene	5.87 ± 8.31	-	3.9 ± 5.52	1.09 ± 1.54	5.98 ± 6.19	5.55 ± 11.11	7.45 ± 10.5	6.36 ± 4.81	7.02 ± 4.8	6.59 ± 3.74
α-Farnesene	1.99 ± 2.81	2.07 ± 2.92	-	-	-	124.79 ± 52.9*	81.49 ± 47.2*	9.56 ± 17.45	1.35 ± 3.32	5.2 ± 6.95

Green Leaf Volatiles										
3-Hexenal	5.89 ± 8.33	1.77 ± 2.51	9.86 ± 1.26	-	10.81 ± 11.1	1.68 ± 2.28*	0.66 ± 1.32*	3.1 ± 5.82*	0.53 ± 1.31*	2.92 ± 4.2*
2-Hexenal	-	-	-	-	1.94 ± 2.74	-	-	-	-	0.45 ± 0.99
3-Hexen-1-ol	35.73 ± 57.92	22.6 ± 21.5	46.38 ± 45.3	4.25 ± 0.33	39.75 ± 34.77	8.71 ± 6.55*	22.98 ± 19.71	19.65 ± 38.86	2.89 ± 4.85	30.16 ± 33.5
Valeric acid	4.63 ± 6.56	-	2.21 ± 3.12	-	1.2 ± 1.69	5.56 ± 7.92	9.42 ± 9.63	3.83 ± 6.46	0.29 ± 0.72	15.45 ± 27.13
3-Hexen-1-ol. benzoate	2.84 ± 4.02	3.16 ± 4.47	1.7 ± 2.4	-	1.69 ± 2.39	13.42 ± 4.31*	18.27 ± 22.45	7.25 ± 10.08	0.81 ± 1.99	5.7 ± 6.78
Other compounds										
Nerolidyl acetate	4.59 ± 6.49	2.16 ± 3.06	2.36 ± 3.34	-	3.78 ± 2.20	6.25 ± 1.27	4.58 ± 3.17	4.78 ± 3.74	3.44 ± 3.04	3.24 ± 2.59
Nerolydol	9.52 ± 13.46	5.02 ± 4.01	2.35 ± 3.33	1.13 ± 1.6	1.58 ± 2.24	17.56 ± 5.13	25.77 ± 24. 85	4.48 ± 5.24	2.21 ± 4.56	3.01 ± 3.65
Methyl salicylate	32.57 ± 27.04	23.46 ± 3.89	36.64 ± 10.53	10.9 ± 0.49	6.87 ± 1.71	123.41 ± 75.5*	111.78 ± 59.1*	78.87 ± 81.9*	37.99 ± 47.6*	41.09 ± 27.2*
Methyl jasmonate	-	3.98 ± 2.03	2.36 ± 3.34	-	-	3.46 ± 4.49	-	-	26.85 ± 65.77	-
Nonanal	6.17 ± 2.92	9.52 ± 2.62	15.15 ± 6.06	4.39 ± 0.91	9.1 ± 6.34	4.4 ± 1.2*	4.1 ± 1.64*	4,05 ± 1.81*	2.77 ± 1.17*	2.92 ± 0.83*
Decanal	6.43 ± 1.23	10.87 ± 2.35	14.62 ± 5.87	5.48 ± 0.06	12.12 ± 8.38	6.11 ± 1.43	5.75 ± 1.47	5.14 ± 2.17	4.05 ± 2.04	4.06 ± 1.15
Sum of compounds	264.46± 288.78	154.31± 140.18	210.76± 161.22	50.87± 33.46	129.13± 123.21	568.41± 363.92	598.62± 400.67	255.84± 308.22	174.24± 195.37	209.22± 205.7

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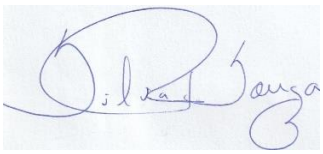

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