Supplementary Materials: Robotic Volatile Sampling for Early Detection of Plant Stress

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Robotic Volatile Sampling for Early Detection of Plant Stress

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Supplementary Material
Supplementary Figures S1-S5
Supplementary Tables S1, S2

Sampler performance under different flow rates

As mentioned in the main text (Section IV. Experimental Results, A), we produced a blend of analytical standard volatiles by mixing 20 µl of each pure substance. This corresponded to the following mass for each compound: (*E*)-2-hexenal = 16.92mg; (*Z*)-3-hexenyl acetate = 17.94 mg; linalool = 17.24 mg; b-caryophyllene = 18.04 mg; TMTT = 16.28mg; p-cymene = 17.2mg; eugenol = 21.34mg. The volatile sampling was done in the laboratory (22°C) in a semi-open setting using plastic cylinders.

The Tenax-TA™ tubes were desorbed and analyzed in a Shimadzu GCMS-QP2020NX system coupled with a thermal desorption unit (TD-30) (Shimadzu, Japan). An Rtx-wax column (Restek Corp., USA) was used (30m, 0.25mm i.d., 0.25µm film thickness). For desorption, the TD unit was heated to 220°C with a pre-purge gas flow of 20ml/min. A split injection mode was used with a split ratio of 10. The gas flow increased to 80ml/min during tube desorption for 15min. Helium was used as the carrier gas and nitrogen as the purge gas. The TD was set with following parameters: pressure of 66kPa, total flow of 16.5ml/min, column flow of 1.23ml/min, linear velocity of 40cm/s, and a purge flow of 3ml/min. Trap cooling was held at -20°C. During desorption, the trap was held at 230°C for 4 min while the sample was collected on the cold trap (Tenax-TA™ cartridge held at -20°C). The sample was then injected by rapidly heating the cold trap. The GC oven was held at 40°C for 2 min following injection, then ramped to 190°C over 11 min, held for 2 min, ramped to 230°C over 17 min, and held for 3 min. The total analysis cycle was 39 min. The GC oven cooled at a rate of 65°C/min. The temperature of the transfer line was set to 200°C and the ion source 220°C. The MS unit was set to scan at 5Hz across m/z of 40-400 after an initial delay of 3min. In our run sequence, we ran empty glass tubes (glass blanks) every 5 samples to control for potential external volatile contamination during desorption. For volatile identification and quantification, we used a method that contains a local library of target volatile compounds based on analytical-grade standards.

Overall, we found that the concentration of the GLVs, the monoterpenoid and the benzenoid p-cymene increased smoothly with increasing airflow rates, reaching a peak at 400ml/min (Figure S1). In contrast, the benzenoid eugenol showed a smooth decrease in concentration with increasing airflow rates, while the sesquiterpene and norditerpene showed a sudden drop in concentration at 300ml/min with no further decreases at 400 and 500ml/min (Figure S1). It is not clear why some compounds might decrease in concentration with increasing airflow rate. They could furthermore have lower affinity for the Tenax-TA™ matrix.

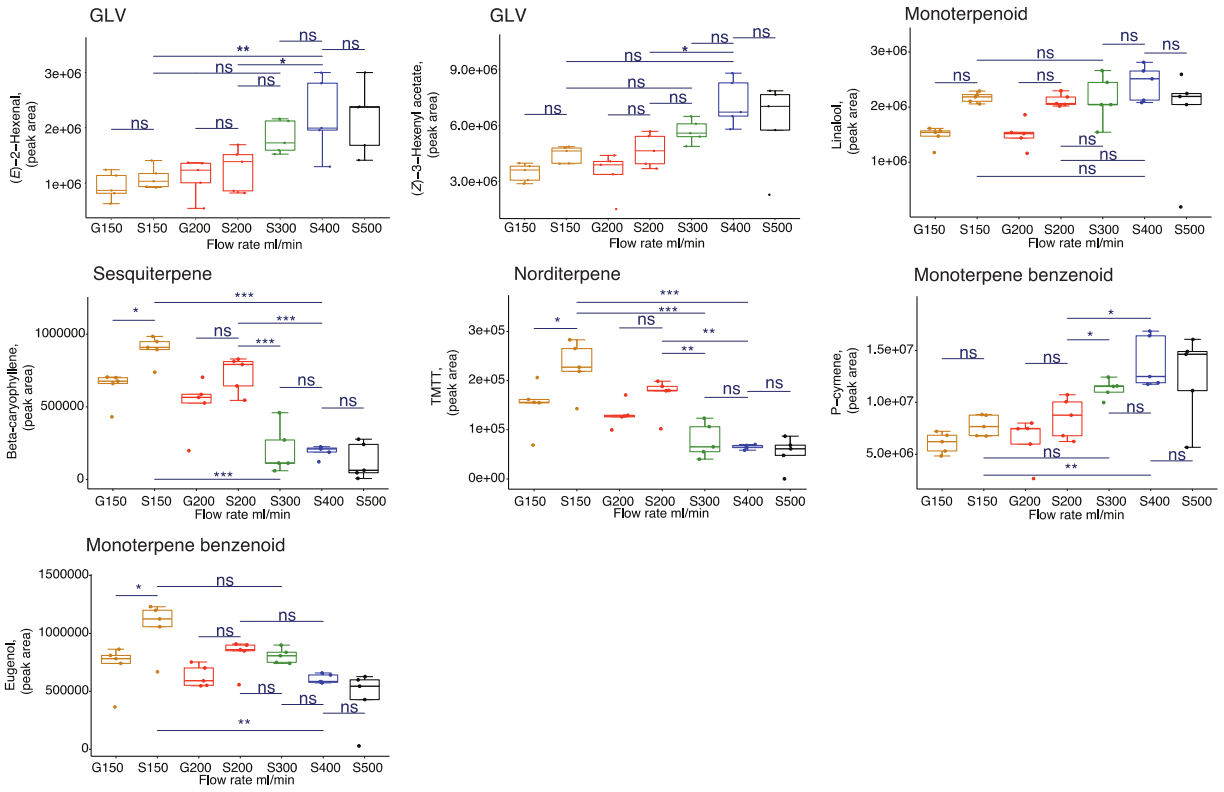

Figure S1. Patterns of volatile recovery per standard volatile compound tested in a blend with different pumps and at increasing airflow rates. In each plot, the chemical class of the volatiles is indicated. Statistical differences are denoted as *P*= 0.01 *, *P* = 0.001 **, and *P* = 0.0001 ***

Horizontal and vertical distance from volatile emission source to sampler

The horizontal distance at ground level from the sample to the volatile emission source at which it is still possible to recover volatiles has important implications for the mission planning and scalability of the drone-based volatile remote sensing. Likewise, the air downwash produced by a hovering drone above the emission source might affect the volatiles recovered if the drone is kept hovering above the sampler during the sampling period. We conducted a series of test to explore such aspects.

For horizontal distance tests, the samplers were positioned at 0, 1, 2 and 4m from the volatile emission point. The emission point consisted of a piece of Whatman filter paper with 0.5 ul of the volatile blend of six volatile compounds (the same mentioned in table 2, except for TMTT). For vertical distance tests, we simulated the air downwash of the drone by measuring the air speed of the downwash by a DJI Mavic Air Pro (743g) flying under indoor conditions of still air (mean of 15°C and 30% RH). We used an anemometer, Testo 405i, to measure the air velocity in the ground when the drone was hovering at 1m, 2m, and 4 m directly above the sensor. We then used a fan Noctua NF-A14 PPC 3000 PWM positioned above the sampler and filter paper with four 60cm legs calibrated to produce the air speed corresponding to the three vertical distances. The simulated downwash was needed, since flying the drone in the laboratory was not possible. We therefore performed the horizontal and vertical distance experiments under the same room temperature and humidity conditions to minimize sources of variation (mean of 21°C and 23% RH in the lab). In both experiments we sampled for 16.66min to collect a total air volume of 5l on Tenax-TA^{TM} at 300ml/min. The experiments were done in the laboratory space of the Spatial Genetics group in the Department of Chemistry at the University of Zurich Irchel campus. The data was analyzed with analysis of variance (ANOVA) with each volatile compound separately, as well as and the sum of all volatiles, as response variables, and distance (either horizontal or vertical) as predictor variable. All analyses were conducted using R v4.2.2; pairwise contrasts were done with the emmeans package v1.8.3, and plots were done with ggplot2 v3.4.0.

Overall, under indoor still air conditions, the results of the horizontal distance experiment suggests that volatiles can be recovered at similar quantities between 1m and 4m distance from the sampler, and less volatiles may be recovered when the emission point is next to the sampler (Figure S2). The reason for this patterns is not yet clear, but it likely has to do with how volatiles diffuse in air [1]. The vertical distance experiment indicated that the downwash can significantly reduce the quantities of total volatiles sampled compared to the positive control (Figure S2, Table S1).

Table S1. Bonferroni corrected pairwise comparisons of the peak area for total volatiles across controls and horizontal and vertical distances.

Experiment	Contrast	Estimate	DF	t-ratio	P-value
Horizontal	Ctrl-0m	-3672026	20	-5.768	0.0001
	Ctrl-1m	-4275405	20	-6.716	<.0001
	Ctrl-2m	-4009757	20	-6.299	<.0001
	Ctrl-3m	-4162424	20	-6.539	<.0001
	0m-1m	-603379	20	-0.948	0.8747
	0m-2m	-337731	20	-0.531	0.9831
	0m-3m	-490398	20	-0.77	0.9362
	1m-2m	265648	20	0.417	0.9931
	1m-3m	112982	20	0.177	0.9998
	2m-3m	-152666	20	-0.24	0.9992
Vertical	Ctrl—-Ctrl+	-1475208	20	-7.101	<.0001
	Ctrl—-4m	-487174	20	-2.345	0.172
	Ctrl—-2m	-239805	20	-1.154	0.7762
	Ctrl—-1m	-208102	20	-1.002	0.8515
	Ctrl+-4m	988034	20	4.756	0.001
	Ctrl+-2m	1235403	20	5.947	0.0001
	Ctrl+-1m	1267106	20	6.099	0.0001
	4m-2m	247369	20	1.191	0.7565
	4m-1m	279072	20	1.343	0.6686
	2m-1m	31703	20	0.153	0.9999

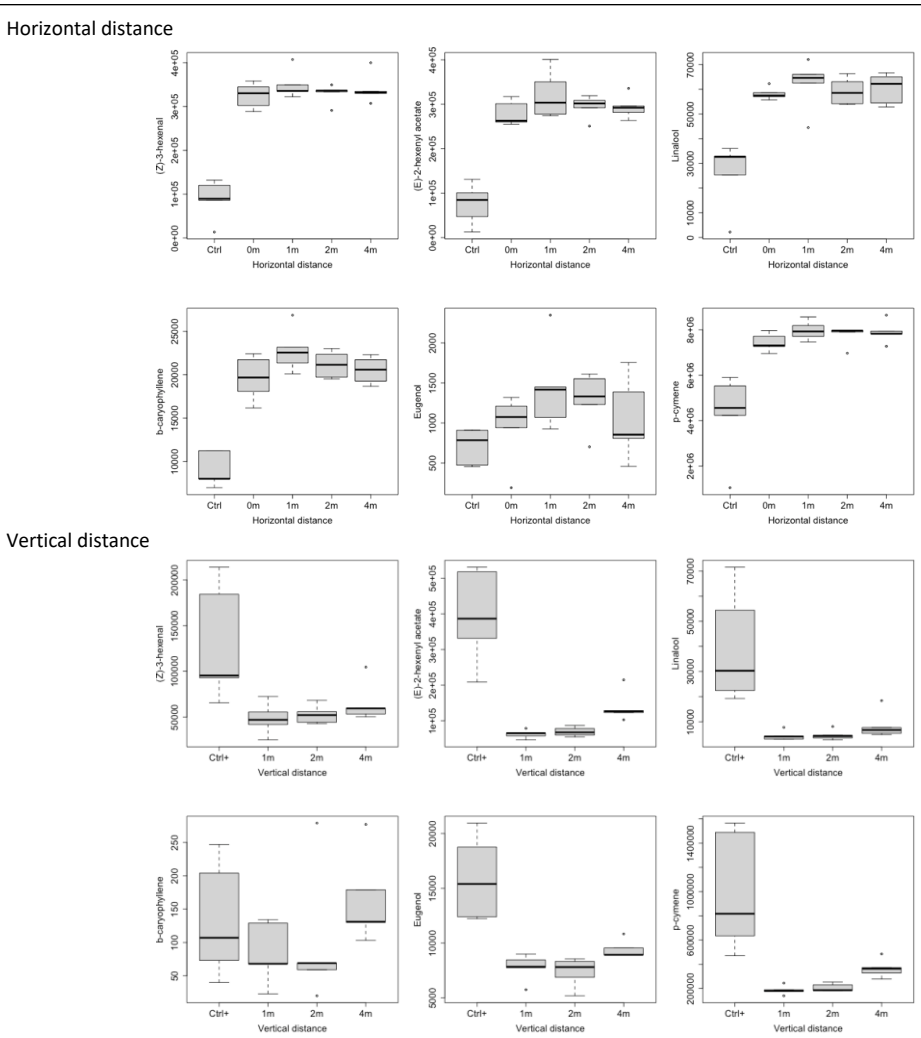

Figure S2. Patterns of volatile recovery at different horizontal and vertical distances from the emission source per each volatile organic compound in an artificial mix of laboratory standards.

Tests of outdoor volatile collection from mechanically damage and regurgitant-induced maize seedlings

We conducted an experiment where 15-day old plants were damaged, and volatiles collected under outdoor conditions with our light-weight samplers at different time periods. *Zea mais* var. Delprim was grown in individual pots at 24°C and 50% relative humidity in the greenhouse facilities at the University of Zurich Irchel campus. Leaves were mechanically damaged by scratching with fine sandpaper (#100) and fine tweezers, as shown in figure S3. The regurgitant of the Egyptian cotton leafworm *Spodoptera littoralis* from L4 and L5 larval stages were used. The regurgitant was obtained from larvae fed with the same Delprim maize variety, grown under the same conditions, during October 2022. The regurgitant was maintained in a -20°C freezer on 5 ml transparent vials divided in aliquots of 200 µl each.

The experiment took place on the 30th of May and consisted of placing 10 plants in a circle around each of the four samplers at a distance of ca- 30 cm from the sampler. This occurred at 0830 h, and the plants and samplers were placed in a green area outside the greenhouse (Figure S3). We conducted a total of six sampling periods of 30 min duration with the lightweight samplers set at an airflow of 300ml/min. The first sampling was done at time -1 (0900 h), when all plants were not yet induced with simulated herbivory. We considered this sampling as the control, and the volatiles recovered would be interpreted as volatiles emitted mostly from the surrounding maize plants, but caution is needed with this interpretation as other surrounding plants could have also contributed to the collected volatiles. The control samples are thus expected to represent constitutively emitted plant volatiles, emitted in the absence of stress, as well as background volatiles from surrounding vegetation. We consider this as the basal level from which an increase in volatile emissions – as a result of damage – can be compared. Volatiles that significantly increase their emission compared to constitutive levels are thus called induced volatiles.

The induction of plant volatiles was done at time 0 (at 1000 h) and consisted of scratching three leaves per plant with sandpaper (Figure S3). In the scratched areas (approx. 6 cm^2 per plant), a total of 10 ul of pure regurgitating was applied. The second sampling occurred 1.25 h after induction at 1125 h, the third sampling occurred 2 h after induction at 1200 h, and the fourth sampling occurred 3 h after induction at 1300 h. The first day of sampling concluded with the fourth sampling, and the plants were returned to the greenhouse and were watered. The fifth and sixth sampling was done 22 h and 24 h after the first induction, at 0800 h and 1000 h. Before these samples were taken, the plants were once again induced with regurgitant and by pinching small holes in two leaves in one half of the leaf. This was intended to replicate a second round of herbivore damage, as ongoing damage would be expected in natural conditions. Nevertheless, it is important to remember that it is not easy to fully imitate the natural patterns and timing of damage caused by real insects. Exposing plants to real herbivores

in the outdoors would be the ideal approach; however, it is generally not allowed to do this with insect pests of maize due their high risk of dispersal (e.g., *Spodoptera littoralis*), and the approach we took allowed us greater control over the timing of damage.

Figure S3. Arrangement of the plants and the samplers in the outdoor collection of induced maize plants. The middle photo shows the circular arrangement of the plants around the sampler. The right-side photo shows the damage provoked to both sides of a leaf with sandpaper to which pure regurgitant was immediately applied.

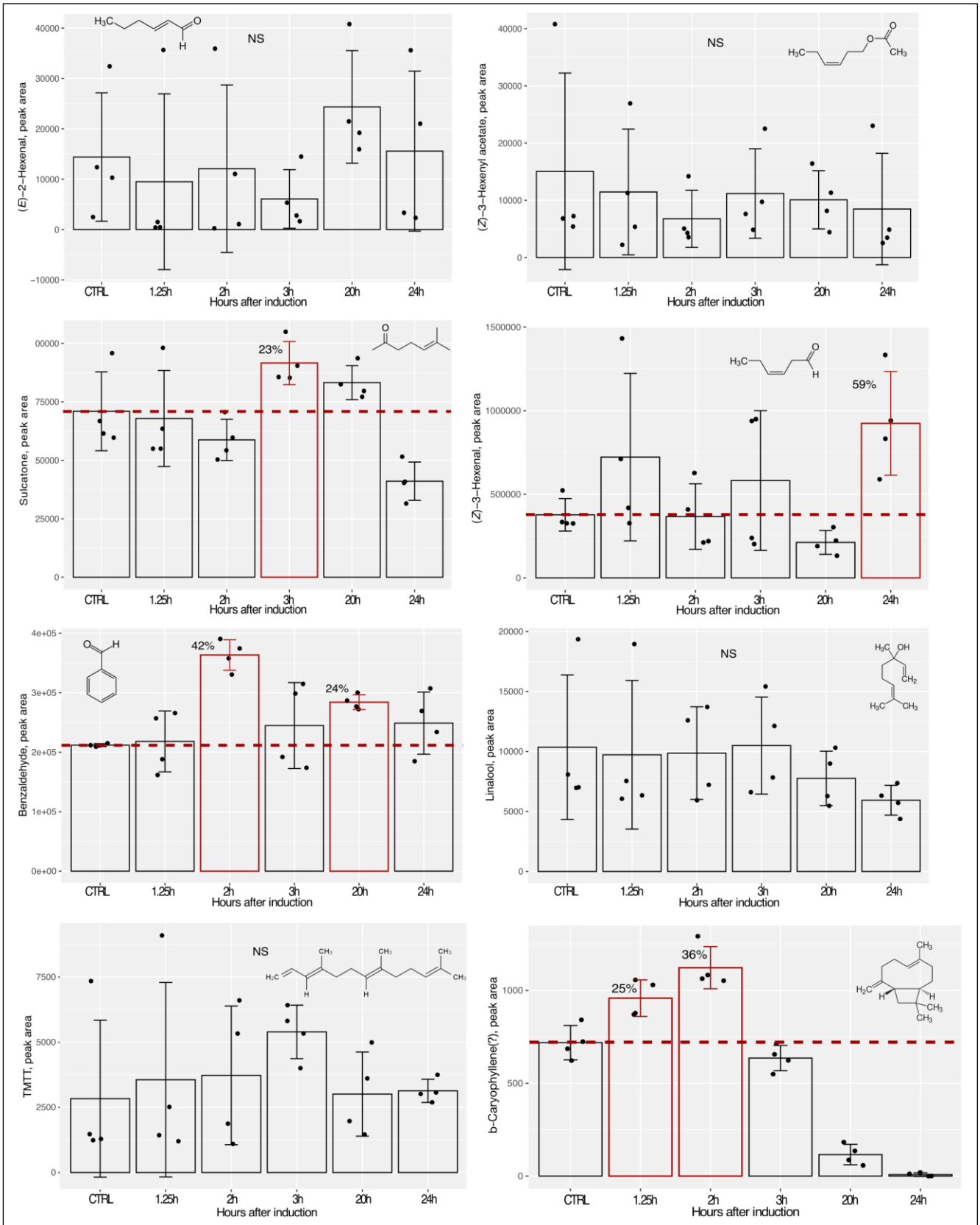

Figure S4. Volatile compounds from regurgitant-induced plants collected under open air conditions. In the barplots, bars in red color indicate a significant increase relative to the control plants (first bar in each plot), in which the percentage of increase is also noted. The dotted line across the bars indicates the mean of the control plants for better visualization of the increase in volatile emission after induction. All compounds were identified and matched with our local compound library in our TD-GCMS instrument.

Table S2. Results of statistical analyses of volatiles recovered from 15 day-old maize plants induced with simulated herbivory. To avoid false positives (error type I) due to multiple comparisons, planned pairwise contrast were performed where the control was compared to each to each of the time periods of hour after induction.

Volatile compound	Contrast	Estimate	t-value	*P*
(*E*)-2-hexenal	CTRL vs 1.25h	-4905	-0.5	0.6230
	CTRL vs 2h	-2321	-0.237	0.8156
	CTRL vs 3h	-8326	-0.849	0.4071
	CTRL vs 20h	9954	1.015	0.3236
	CTRL vs 24h	1176	0.12	0.9058
(*Z*)-3-Hexenyl acetate	CTRL vs 1.25h	-3604	-0.5	0.6228
	CTRL vs 2h	-8287	-1.151	0.2649
	CTRL vs 3h	-3880	-0.539	0.5966
	CTRL vs 20h	-4978	-0.691	0.4983
	CTRL vs 24h	-6584	-0.914	0.3726
Sulcatone	CTRL vs 1.25h	-3069	-0.338	0.7392
	CTRL vs 2h	-12231	-1.347	0.1946
	CTRL vs 3h	20645	2.274	0.0354
	CTRL vs 20h	12278	1.353	0.1929
	CTRL vs 24h	-29884	-3.292	0.0041
(*Z*)-3-Hexenal	CTRL vs 1.25h	345059	1.577	0.1322
	CTRL vs 2h	-10134	-0.046	0.9636
	CTRL vs 3h	205050	0.937	0.3610
	CTRL vs 20h	-164937	-0.754	0.4607
	CTRL vs 24h	546790	2.499	0.0223
Benzaldehyde	CTRL vs 1.25h	6286	0.204	0.8405
	CTRL vs 2h	151516	4.923	0.0001
	CTRL vs 3h	33004	1.072	0.2978
	CTRL vs 20h	72232	2.347	0.0306
	CTRL vs 24h	37047	1.204	0.2443
Linalool	CTRL vs 1.25h	-633.5	-0.207	0.8384
	CTRL vs 2h	-496	-0.162	0.8731
	CTRL vs 3h	135.7	0.044	0.9651
	CTRL vs 20h	-2599.5	-0.849	0.4070
	CTRL vs 24h	-4420.5	-1.444	0.1660
TMTT*	CTRL vs 1.25h	726.2	0.432	0.6709
	CTRL vs 2h	893.5	0.531	0.6016
	CTRL vs 3h	2560.5	1.523	0.1452
	CTRL vs 20h	175.7	0.105	0.9179
	CTRL vs 24h	300.2	0.179	0.8603
b-Caryophyllene	CTRL vs 1.25h	239.5	4.208	0.0005
	CTRL vs 2h	403.5	7.089	<.0001
	CTRL vs 3h	-83	-1.458	0.1620
	CTRL vs 20h	-602.75	-10.589	<.0001
	CTRL vs 24h	-710.75	-12.487	<.0001

*TMTT: (*E*,*E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene

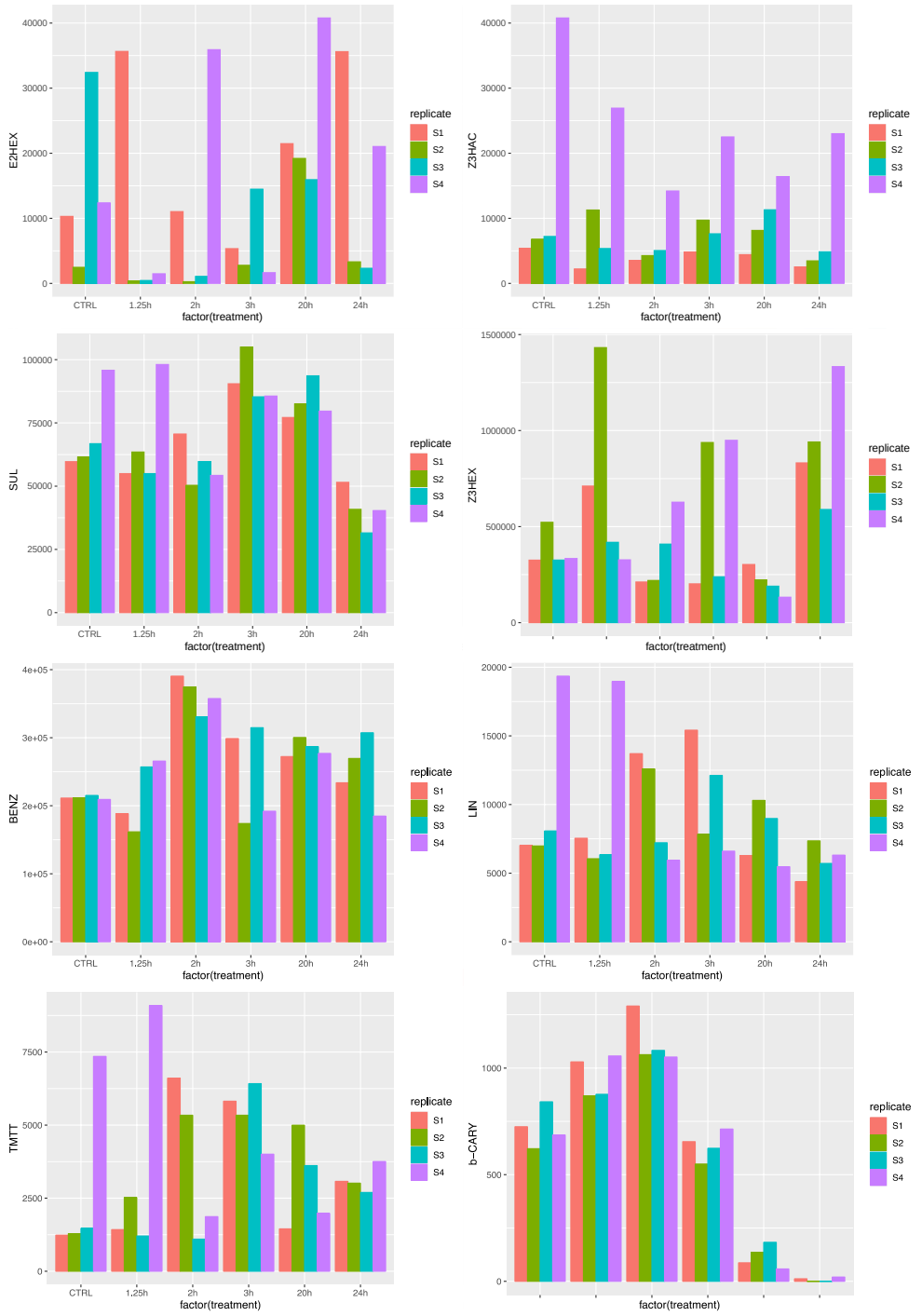

Figure S5. These barplots per volatile compound show the patterns per replicate to see the influence of some points and the consistency of the patterns observed in the previous barplot. In many cases, the replicate and pump number 4 has the most outlier behavior.