Supplemental methods and results from a common garden to test for wounding artefacts

Rationale. We observed differences across species and community types in compounds that are generally considered "green leafy volatiles" associated with plant wounding (Visser and Ave 1978; Scala et al. 2013). To determine if these patterns were the product of an experimental artefact, we conducted a second greenhouse common garden experiment to compare the volatiles emitted by wounded and non-wounded plants.

Plant source site selection. We grew individuals of both species from seeds from one single-species community per species (*C. cylindrica*: MHG; *C. unguiculata*: GRCO), one two-species community per species (SC for both species), and one four-species community per species (MCK for both species). These communities were specifically selected to represent a range in the emission rates of the "green leafy volatiles" observed in the 2018 common garden.

Plant germination and growth. Seeds were stratified and transplanted in two batches in January and February of 2019. All procedures were identical to those used in the 2018 common garden.

Quantitative scent analysis. Floral volatile samples were collected using the dynamic headspace adsorption technique between March 21, 2019 and April 6, 2019. All collection protocols were identical to those used in the 2018 common garden. To assess the potential for elevated emission of "green leafy volatiles" due to wounding, each plant was sampled twice. On the first sampling day for a given plant, we sampled the floral bouquet using the same methods as in 2018, to produce a control sample. On the second sampling day for a given plant, we sampled the floral bouquet and inflicted wounds to the plant immediately prior to the collection window. Using scissors, we snipped a piece of each leaf included in the collection chamber for *C. cylindrica*, and we snipped every other leaf included in the collection chamber for *C. unguiculata* because

C. unguiculata plants have more, larger leaves. This resulted in a mean ± 1 SE of 0.065 ± 0.005 g of fresh leaf mass removed from *C. cylindrica* and 0.049 ± 0.004 g of fresh leaf mass removed from *C. unguiculata*. The number of flowers included in each sample was recorded. We collected both non-wounded and wounded samples from 10 plants per species per site (N_{total} = 120 samples).

Scent analysis via GC-FID. Samples were analyzed using a Shimadzu GC-FID (GC 2014) with an AOC-20i auto injector. One μL aliquots of the solvent eluted samples were injected (splitless mode) at 240C onto a polar GC column (EC Wax, 30m long, 0.25 mm internal diameter, 0.25μ film thickness; BGB Analytik). The GC oven program (a 2-minute hold at 40C, followed by a 14.67C increase per minute to the maximum temperature of 260C, with a 2-minute hold at the maximum temperature) was optimized to minimize run length (for over 120 samples) while allowing for peak resolution to baseline. The “green leafy volatile” compounds of interest ((*Z*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate) were identified via direct comparison of retention time and mass spectra with those of authentic standards. Peak areas were automatically integrated by Shimadzu Postrun Analysis software.

Extraction and processing of quantitative data. Emission rates were calculated as in the 2018 common garden using response factors generated using external standard dose-response curves generated from log- and semi-log dilutions of the compounds of interest. Emission rates were related to floral masses using the floral mass data collected in 2018.

Emission rates from each sample were compared to the ambient control collected on that day, but none of the ambient control samples contained the compounds of interest.

Statistical analysis. The data were subset by species and we used paired t-tests (t.test function in R) to compare the wounded vs. non-wounded volatile emission profiles. We then examined the

2018 common garden data that had been cleaned to remove compounds with emission rates less than 5x the emission rates in the ambient control, but that had not been cleaned to remove compounds that occur in the vegetative controls. We analyzed this form of the 2018 data because we did not utilize vegetative controls in our 2019 common garden, such that the 2018 profiles prior to vegetative control screening are directly comparable to the 2019 profiles. We ran two sets of two sample t-tests for each species: 1) we compared the 2018 profiles to the 2019 non-wounded control samples, and 2) we compared the 2018 profiles to the 2019 wounded samples. Due to the variance structure of these data, we ran two sample t tests with unequal variance for (*Z*)-3-hexen-1-ol and with equal variances for (*Z*)-3-hexenyl acetate).

Expected results. If our experimental protocol induces a wounding response, we expect that the "green leafy volatile" emission rates will be equivalent across the 2019 wounded and 2019 non-wounded plants. If our experimental protocol does not induce a wounding response, we expect that the "green leafy volatile" emission rates will be higher in the 2019 wounded samples relative to the 2019 non-wounded samples. In addition, we expect that the 2018 emission rates will be roughly equivalent to the 2019 non-wounded emission rates, and lower than the 2019 wounded emission rates.

Results and Discussion. For both species and both compounds, emission rates were higher in the 2019 wounded samples relative to the 2019 non-wounded samples (Table 1), which suggests that wounding elevates emission rates of these volatile compounds. For both species and compounds, emission rates did not differ between the 2019 control samples and the 2018 samples (see 2018 – 2019 C comparisons Table 2), which suggests that our 2019 control samples are representative of the emission rates observed in 2018. For *C. unguiculata*, emissions rates of both compounds were lower in the 2018 samples relative to the 2019 wounded samples (see 2018 – 2019 W comparisons Table 2). Comparisons of emission rates for *C. cylindrica* yield similar patterns, although the comparison for (*Z*)-3-hexen-1-ol is non-significant but in the expected direction. Taken together, these results suggest that while wounding elevates the emission rates of these “green leafy volatiles,” the emission rates observed in 2018 cannot be attributed to wounding. Rather, these emission rates likely reflect constitutive emission of these compounds by flowering plants, which has been documented in other systems(e.g., Brodmann et al. 2008, 2012).

Table 1. Results of paired t-tests comparing the 2019 wounded and non-wounded plant samples. Ung refers to *C. unguiculata* and Cyl refers to *C. cylindrica*. CI.low and CI.high represent the lower and upper bounds respectively of a 95% confidence interval on the mean difference. Positive mean differences indicate higher emission rates in the wounded samples. Significant tests are bolded, and marginally significant tests are italicized.

Species	Compound	*t*	df	*P*	CI.low	CI.high	Mean difference
Ung	(*Z*)-3-hexen-1-ol	*1.7066*	*29*	*0.0986*	-0.0020	0.0226	0.0103
	(*Z*)-3-hexenyl acetate	**2.5461**	**29**	**0.0164**	0.0264	0.2421	0.1342
Cyl	(*Z*)-3-hexen-1-ol	**3.7503**	**29**	**0.0008**	0.0042	0.0145	0.0094
	(*Z*)-3-hexenyl acetate	**2.9732**	**29**	**0.0059**	0.0337	0.1825	0.1081

Table 2. Results of two sample t-tests comparing the 2018 samples to the 2019 non-wounded controls (2019 C) and to the 2019 wounded samples (2019 W). Ung refers to *C. unguiculata* and Cyl refers to *C. cylindrica*. CI.low and CI.high represent the lower and upper bounds respectively of a 95% confidence interval on the mean difference. Mean 2019 refers to the mean of the 2019 group included in the specific analysis. Significant negative *t* values indicate that the 2019 group included in the test had a higher emission rate than the 2018 samples. Significant tests are bolded.

Species	Compound	Comparison	*t*	df	*P*	CI.low	CI.high	Mean 2018	Mean 2019
Ung	(*Z*)-3-hexen-1-ol	2018 – 2019 C	-1.4014	32	0.1707	-0.0209	0.0039	0.0102	0.0188
		2018 – 2019 W	**-5.0833**	**38**	**< 0.0001**	-0.0263	-0.0113		0.0290
	(*Z*)-3-hexenyl acetate	2018 – 2019 C	-0.0258	73	0.9795	-0.1157	0.1127	0.2022	0.2036
		2018 – 2019 W	**-2.6321**	**73**	**0.0104**	-0.2385	-0.0321		0.3379
Cyl	(*Z*)-3-hexen-1-ol	2018 – 2019 C	0.6783	73	0.4997	-0.0058	0.0117	0.0156	0.0126
		2018 – 2019 W	-1.4618	73	0.1481	-0.0152	0.0023		0.0220
	(*Z*)-3-hexenyl acetate	2018 – 2019 C	-1.3750	73	0.1733	-0.1316	0.0241	0.1633	0.2170
		2018 – 2019 W	**-3.9411**	**73**	**0.0002**	-0.2437	-0.0800		0.3251