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Abstract: Interactions between plants and insect pollinators are of critical importance as the majority of flowering plants rely on animals for pollination and insects are the most diverse group of pollinators on Earth. To obtain pollination services plants must attract pollinators by signaling the presence of rewards, and chemosensory cues including floral scent are of particular importance to pollinator attraction. In highly-specialized brood-pollination mutualisms, like the yucca-yucca moth mutualism, the "reward" for pollinators is a brood site and food source for their offspring: fertilized plant ovules. Being able to distinguish among floral parts is critical for yucca moths to successfully execute the complex behaviors required for oviposition and pollination. Fine-scale, tissue-specific patterns of floral scent potentially play an important role in helping pollinators to navigate toward rewards, but such patterns and their ecological consequences remain poorly understood. To address this, I examined the floral scent of the tepals and pistils of five species of *Yucca*. All five species of *Yucca* had tissue-specific patterns of scent emission. Tissue-specific patterns of floral scent also varied among *Yucca* species, with two species *Y. reverchonii* and *Y. rupicola* producing low to nonexistent levels of a subset of compounds of known biological relevance to pollinating moths. I also observed a trend in the oviposition behavior of the common pollinator of these five species (*Tegeticula yuccasella*), wherein moths oviposited at higher rates in chemically similar yuccas and at lower rates in yuccas with reduced (or no) expression of known, biologically relevant compounds. Even though there is variation in the scent profile of tepals and pistils across *Yucca* species, *T. yuccasella* successfully uses all hosts in the wild. Our results show that moths may be using a broader, potentially redundant suite of compounds to identify yuccas and their specific tissues rather than relying on a few major compounds to determine host suitability.

The Ecology of Fine-Scale, Tissue-Specific Floral Scent Patterns in an Obligate Brood-Pollination Mutualism.

By

Gwen Bode

B.S., Eastern Washington University, 2016

Thesis
Submitted in partial fulfillment of the requirements for the degree of
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Introduction

Plants are the basis of terrestrial ecosystems— as the quintessential primary producers they play a crucial role in ecological interactions with other organisms. In particular, plants have a multitude of interactions with insects, which make up more than half of the known macrodiversity in terrestrial ecosystems (Condamine et al., 2016). Plant-insect interactions have intimately shaped the evolution of both partners (Ehrlich and Raven, 1964; Pellmyr, 1992) and these interactions encompass a spectrum ranging from antagonism to mutualism. One important, mutualistic interaction between plants and insects is pollination, wherein insects facilitate plant reproduction through pollen transfer in exchange for a reward. Roughly 87% of plants are pollinated by animals (Ollerton et al., 2011), the overwhelming majority of which are insects (Pellmyr, 1992). Because plant reproductive success often depends on attracting pollinators, studying the ecology of plant-pollinator interactions is critical to understanding the evolution of floral traits.

Flowers are incredibly diverse, varying across a suite of traits including size, shape, color, sexual function, and scent that are used to advertise rewards to pollinators (Celedon-Neghme et al., 2007; Raguso, 2008; Borghi et al., 2017; Valenta et al., 2017). The extent to which each of these traits influences pollinator attraction is system specific, varying in importance and scale (Valenta et al., 2017). Plants often have stunning visual floral displays, which most often serve to attract pollinators or influence foraging choice at relatively close ranges (Valenta et al., 2017), although there are some cases of long-distance attraction (see Lukas et al., 2020, and references therein). Alternatively, floral scent is predominantly known for its important role in long-range pollinator attraction. Many different pollinating insect groups including flies, bees,

wasps, and moths are known to be attracted to floral scent across long distances (Dobson, 2006; Raguso, 2008; Dobson, 2017). Highly specialized interactions are more likely to use floral scent for long-distance attraction than visual cues, due to the prevalence of patchy plant distributions (Williams and Dodson, 1972; Raguso, 2008; Hossaert-McKey et al., 2010; Svensson et al., 2011; Valenta et al., 2017). For example, perfume-gathering male euglossine bees are attracted to isolated orchid volatiles over distances upwards of 1 km, even across bodies of water (Williams and Dodson, 1972; Holland, 2015) and species-specific fig-fig wasp interactions depend on unique volatile cues for host recognition in dense tropical forests (Grison-Pige et al., 2002). In certain taxa, especially among night-blooming plants, floral scent may be more important for long-distance attraction than visual displays, supported by the observation that many night-blooming flowers are white and strongly scented (Knudsen and Tollsten, 1993; Raguso, 2008; Svensson et al., 2011; Borghi et al., 2017; Valenta et al., 2017).

To date, floral scent research has primarily focused on long-range attraction (Raguso, 2008), predominantly at the whole flower or inflorescence level (Raguso, 2008; Garcia et al., 2021). Getting the pollinator to the plant is of crucial importance to plant reproduction and is the first step in the pollination process. However, such a large-scale approach doesn't address the question: *what influences pollinator choice after reaching a flower*? To do so we must look to the finer scales at which insects interact with floral traits, that is within individual flowers themselves. Insect pollinators are usually much smaller than the flowers they visit, meaning fine-scale differences in the scent chemistry within a flower could be significant to pollinators. If within-flower (intrafloral) scent variation exists, it could play an important role in plant-pollinator interactions.

The existence of intrafloral scent variation has been established across multiple plant families and the patterns observed thus far are highly variable and species-specific (Bergstrom et al., 1995; Dötterl and Jürgens, 2005; Effmert et al., 2005a; Effmert et al., 2005b; Policha et al., 2016; Martin et al., 2017; Garcia et al., 2021). Floral scent can vary among floral parts in terms of which volatiles are produced (qualitatively) and the amount of volatiles produced (quantitatively). In *Ranunculus acris*, scent among most floral parts is qualitatively the same, but stamens and pistils produce significantly more scent than other floral parts, whereas pollen produces unique compounds found in no other floral parts (Bergstrom et al., 1995). The scent of floral parts can also vary temporally, such that patterns of within flower scent variation change over time or the life stage of a plant. In the case of *Aristolochia gigantea*, a type of kettle-trap flower, floral parts vary in scent spatially (among parts) and across ontogeny; when the flower changes from its receptive, female stage to its dispersive male stage the volatiles produced by different floral parts also shift both quantitatively and qualitatively (Martin et al., 2017). Variation in scent among floral parts has also been linked specifically to pollinator attraction. By decoupling visual and olfactory cues using chimeric flowers, Policha et al. (2016) showed that volatiles produced by the mushroom-scented labellum of *Dracula lafleurii* attract pollinators independently of other cues (Policha et al., 2016). Floral scent can also vary within a given floral part as demonstrated by the petals of *R. acris*, which vary quantitatively in scent from top to bottom (Bergstrom et al., 1995). In *Mirabilis jalapa*, a plant species that has funnel-shaped flowers with a fused, petal-like perianth, only the outer rim of the flower produces scent, resulting in both qualitative and quantitative differences within a single floral part (Effmert et al., 2005b).

A recent review by Garcia et al. (2021) found that to date, 57 plant species have been surveyed for intrafloral scent variation and 51 of these species showed tissue-specific scent emissions (Garcia et al., 2021). Variation in scent among floral parts may be common, but not universal for all plants and much more remains to be explored. In particular, the practical implications of tissue-specific floral scent emissions remain poorly understood, because there is a dearth of research coupling observations of pollinator behavior with intrafloral scent variation. Intrafloral scent patterns might provide a chemosensory map for insects within a flower. Such a map might help guide insects towards floral rewards or improve pollination efficiency, but there is also the potential for intrafloral scent variation to play a role in tissue defense against natural enemies of plants (Pellmyr and Thien, 1986; Irwin et al., 2004; Raguso, 2008; Raguso, 2009; Garcia et al., 2021).

The possibility that intrafloral chemical variation acts as a sensory map for insects within flowers has particular implications for brood pollination mutualisms, which often involve complex behaviors and a need for the pollinator to distinguish among floral parts (Hembry and Althoff, 2016). In one such highly specialized mutualism, female yucca moths perform active pollination and oviposition within yucca flowers, encountering specific floral anatomy through the course of pollination and reproductive behaviors (Riley, 1892; Powell, 1992). When a female moth ecloses and mates, she will first gather pollen using her specialized mouth parts, before moving to another flower. There she will circle the pistil of a flower and decide whether to oviposit. Once oviposition is complete the female will move to the top of the pistil and use tentacular mouthparts to pack pollen into the stigmatic cup. We know that yucca moths respond to yucca floral scent, as a long-distance attractant (Svensson et al., 2011; Tröger et al.,

2021). However, once the moth has reached a flower it is unclear what cues elicit pollination and oviposition behaviors. How do yucca moths navigate yucca flowers in order to correctly perform the series of behaviors necessary for both plant pollination and moth reproduction? Given the intimate nature of the interaction, wherein the female moth's abdomen, antennae, mouthparts, and ovipositor will come into contact with select floral anatomy it seems likely that floral scent volatiles are important in triggering yucca moth behavior.

Across the genus *Yucca*, whole inflorescence floral scent varies among the distantly-related *Yucca* species in the sections of Chaenocarpa (capsular-fruited), Clistocarpa (spongey-fruited, or the Joshua Trees), and Sarcocarpa (fleshy-fruited) (Pellmyr et al., 2007; Svensson et al., 2016; Tröger et al., 2021). The majority of research on yucca floral scent published to date has focused on the Chaenocarpa, which consists of three clades that roughly correspond to species groups in the eastern United States (East), the Four Corners region of the Western U.S. (West), and within Texas (Rupicolae) (Pellmyr et al., 2007). Within Chaenocarpa we see relatively little variation in floral scent among species and populations across broad geographic ranges, or among species with allopatric distributions, even when pollinator species differ (Svensson et al., 2005; Svensson et al., 2006; Svensson et al., 2011). For example, *Y. filamentosa* has a broad native range (NC-FL, United States) and experiences two different species of pollinating moths (*Tegeticula yuccasella* and *T. cassandra*) across its range, but is remarkably consistent in scent (Svensson et al., 2005). *Yucca filamentosa, Y. glauca* (Chaenocarpa East), and *Y. elata*, (Chaenocarpa West) (Pellmyr et al., 2007) which essentially replace each other geographically in a parapatric distribution, also share a common scent blend (Svensson et al., 2006; Svensson et al., 2011). Although the members of Chaenocarpa East share the moth

pollinator *Tegeticula yuccasella*, *Y. elata* is pollinated by a distinct, distantly-related yucca moth species (*T. elatella*) (Pellmyr, 1999).

Overall, the floral scent blend of yuccas in the Chaenocarpa appears to be strongly conserved, with one marked exception for species in the series Rupicolae. At least one *Yucca* sp. in the Rupicolae differs in the chemical composition of its floral scent (Tröger et al., 2021). *Yucca reverchonii* (Rupicolae) is missing a suite of novel 11-carbon terpenoids first discovered in yuccas that are known attractants of yucca moths in the genus *Tegeticula* (Tröger et al., 2021). Other species in the Rupicolae, like *Y. pallida*, produce a scent similar to species analyzed from Chaenocarpa East and West including these characteristic terpenoids (Tröger et al., 2021). The species in series Rupicolae are particularly notable because they are largely distributed sympatrically or parapatrically across Texas, but still maintain strong reproductive isolation (Darwell et al., 2017). Additionally, *Y. reverchonii, Y. pallida*, and *Y. rupicola* (Rupicolae); and *Y. filamentosa*, *Y. glauca,* and *Y. constricta* (Chaenocarpa East); share a common moth pollinator species, *T. yuccasella* (Pellmyr, 1999).

All floral scent research on yuccas published to date has been collected at the whole inflorescence scale. *Yucca* inflorescences can contain hundreds of flowers depending on the species and maturity of the individual sampled. To the best of our knowledge, no studies have analyzed yucca scent at finer scales such as individual flowers or floral parts. Additionally, all population-level and species-level comparisons were done at a whole inflorescence scale, meaning that fine-scale differences among floral parts, if present, would have been missed. Such fine-scale differences might be particularly important in mediating a moth's behavior once it has landed in a flower. To better understand the role of intrafloral scent variation in plant-

pollinator interactions, I analyzed floral scent from the tepals and pistils of five species of yuccas grown in an experimental common garden: two species from Chaenocarpa East (*Y. filamentosa, Y. glauca*) and three from Rupicolae (*Y. rupicola, Y. reverchonii*, and *Y. pallida*). For *Y. glauca*, I sampled plants sourced from three unique populations to determine if floral scent follows the trend of consistency across broad geographic ranges seen in other *Yucca* species. For each species or population, I removed and dissected individual flowers, then captured scent using a dynamic headspace method. I focused on the tepals and pistil because they are important to different aspects of the interactions with the moths—host recognition, or pollination and oviposition respectively. I also conducted a series of no-choice bioassays using locally (Geneva NY, USA) sourced moths, reared on *Y. filamentosa* to determine if moth pollination or oviposition behavior was different on non-natal yuccas. I addressed the following questions: (1) Do tepals and pistils of yucca flowers differ in scent? (2) Do tepals and pistils vary in scent among *Yucca* species? (3) Do yucca moths from NY behave differently on flowers from non-natal species?

Materials and Methods

Study System:

Yuccas are known for their obligate, active pollination mutualism with yucca moths of the genus *Tegeticula* and *Parategeticula* (Lepidoptera: Prodoxidae). Often these are species-specific, pairwise interactions (Powell, 1992; Pellmyr, 1999; Althoff et al., 2012). However, *T. yuccasella* serves as the pollinator for a group of closely related yuccas in the section Chaenocarpa and the included Rupicolae series (Pellmyr, 1999; Pellmyr et al., 2007). For this

study I focused on species in these groups, specifically: *Yucca filamentosa*, (range: Eastern United States, natively from N.C. to FL, USA), *Y. glauca* (range: Alberta, Canada to the central southwestern USA) *Y. pallida, Y. reverchonii,* and *Y. rupicola*, which grow semi-parapatrically (range: Central to West Texas, USA) (Althoff et al., 2012; Darwell et al., 2017). Plants were collected from wild populations as rhizomes and were transplanted to a common garden at Syracuse University (Syracuse, NY, US) in 2006 and 2007 (see Althoff et al., 2014 for exact locality information).

Floral Parts scent collection:

I collected scent from plants grown in the common garden at Syracuse University from June to July of 2022 (Supplemental Table 1). Virgin flowers were collected on the first night of bloom between 8 pm and 2 am, when scent emissions are strongest (Svensson et al., 2005). For each individual plant, three flowers were chosen haphazardly and transported in a cooler to a laboratory where they were dissected. The mass of the tepals and pistil from each flower were recorded for estimations of emission rates.

Floral parts were placed into the smallest possible polyvinyl acetate bag that would accommodate each floral part (ranging roughly from 60 x 60 mm to 90 x 90 mm), constructed from larger bags (406 x 444 mm) using a heat sealer (Metronic Impulse Sealer, Taizhou, China). A glass cartridge containing 10 mg Super Q adsorbent (Alltech Associates, State College, Pennsylvania, USA) was inserted into the bag and held in place with a twist tie. Air was drawn through the adsorbent cartridge for one hour by a PAS-500 personal air sample (Supelco, Bellefonte, PA, USA) calibrated to a flow rate of 200 mL/min. Scent was eluted from the trap

using 300 μL of GC-MS grade hexane and stored at -18°C until analysis. Empty bags were used as ambient controls to check for contaminants emitted from the bag as well as those potentially present in the laboratory environment. The headspace of an unopened bud was sampled for each species and used as a comparative control for any vegetative volatiles released. Prior to GC-MS analysis, samples were concentrated to 50 μL under N_2 and 5 μL of 0.03% (v/v) toluene in hexane was added as an internal standard (IS) to correct for sample volume.

GC-MS Analysis of Floral Scent

Floral volatiles were analyzed using a Shimadzu GC-17A gas chromatograph equipped with a non-polar, Shimadzu SHRXI-5MS column (30 m x 0.25 mm internal diameter, and 0.25 μm film thickness), coupled with a Shimadzu QP2010 mass spectrometer (EI: ionization energy = 0.97 kV). The carrier gas was helium (velocity 43 cm/sec), and the injection oven temperature was set to 270°C. The following temperature program for the column was used during sample runs: initial oven temperature was 50°C for 2 minutes after injection and then increased by 10°C/min until reaching 275°C, for a total run time of 29.5 min.

Compound Identification and Semi-quantitation

Compounds were identified using retention times, Van den Dool and Kratz standardized retention indices (Bicchi et al., 1999; Battaloglu, 2021), and by comparing analyte mass spectra with mass spectra from available reference standards and mass spectral libraries. Peak areas were calculated using preselected quantitative ion fragments to minimize the impact of sample noise and to aid in distinguishing analyte peaks. Semi-quantitative determination of analyte

concentration was performed using relative response factors (Rome and McIntyre, 2012). To generate relative response factors from the total ion current (TIC), peak areas of toluene at a concentration of 0.01 mg/mL and three surrogate standards ((*E*)-4,8-dimethyl-1,3,7-nonatriene, tridecane, and aromadendrene) at four concentrations (0.001, 0.01, 0.1, and 1.0 mg/mL) were collected under the same conditions as sample data. The 11-carbon terpenoid, (*E*)-4,8,dimethyl-1,3,7,-nonatriene (hereafter (*E*)-DMNT), was used as a surrogate for the novel yucca terpenoids that are not available as commercially produced standards, tridecane was used as a non-coeluting surrogate for alkanes and alkenes, and aromadendrene as a non-coeluting surrogate for sesquiterpene volatiles.

Statistical analysis

I investigated variation in floral blend composition among floral parts using a multivariate approach. For intraspecies level comparisons, I used relative peak areas (RPAs) of all identified analytes for each pair of floral parts analyzed. RPAs were calculated by dividing the raw peak area of each analyte by the total peak area of all analytes. For population and interspecies level comparisons, I generated average RPAs for each analyte across floral part samples belonging to an individual plant. I also created presence/absence data by generating a binary matrix for both RPAs and average RPAs to be used at their respective comparison levels. I chose to differentiate my approach between intra- and inter-species levels because the questions being addressed vary in scale in terms of the questions I sought to answer. I conducted intraspecies analyses that focused on testing for differences between floral parts and used individual flowers as experimental units because my questions addressed interactions

between an individual moth and the unique parts within a flower. In contrast, at the interspecies level plants were used as the experimental unit because I was addressing questions related to how moths respond to the scent blends of unique species.

All statistical analyses were performed using R Statistical Software (v4.2.1; R Core Team 2022). I performed Non-metric multi-dimensional scaling analysis (NMDS) using Bray-Curtis dissimilarity matrices calculated via the “metaMDS” algorithm in the software package “vegan” (v 2.6-2) to visualize patterns of floral scent bouquets. ANOSIM one-way permutation tests (999 random permutations, “vegan” v 2.6-2 software package) were used to determine if observed differences could be explained by pre-defined groups (e.g., floral parts, population, or species). The ANOSIM test statistic “R” compares mean ranked dissimilarities within and among groups. R-values close to 1 indicate that there are differences among groups, while values near or at 0 indicate a random distribution (Clarke, 1993). The p-value of the R statistic is the percentage of times the calculated R-value of the matrix is greater than an R-value generated via random permutations (Clarke, 1993).

To determine which compounds contributed to variation observed in the floral blend, I performed multi-level pattern analysis using the IndVal index (Dufrêne and Legendre, 1997). I calculated indicator values (IndVal) using the multipatt() function (indicspecies package, V 1.7.17) to see if specific compounds contributed strongly to the identity pre-defined groups (floral parts, populations, or species) and if their contributions were statistically significant. Each compound has a calculated indicator value showing the strength of its contribution to floral part identity, which can be subdivided into two components: specificity, or the proportion

of individuals within a defined group that produce a compound; and fidelity, or the proportion of groups in which a compound is present.

Emission rates were calculated for select compounds of known or suspected biological importance (Favaris et al., 2020; Tröger et al., 2021) and comparisons of mean emission rate were done between floral parts within species, with individual as the sample unit. I calculated emission rate using the following equation:

$$\text{Emission Rate} = \frac{\text{Analyte Concentration}}{\frac{\text{(Floral Part Mass)}}{\text{(Sampling Time)}}} \times \text{Total Sample Volume}$$

Analyte concentration was calculated using response factors and refers to the concentration of the analyte in 1 μL of sample solution or the injection volume used in analysis.

To test for differences in emission rates among species, I performed Kruskal-Wallis rank tests (“stats” package, R, v4.2.1)(Kruskal and Wallis, 1952). I chose a non-parametric approach because the data violates the assumption of normality and transformation could not correct this. Namely, certain *Yucca* species lack specific, biologically relevant compounds (Tröger et al., 2021), resulting in a high prevalence of zeros within the dataset. To determine which species were different I used Dunn’s test with a Benjamini-Hochberg adjusted p-value to correct for multiple comparisons (Benjamini and Hochberg, 1995; Dinno, 2015). Benjamini and Hochberg (1995) developed a statistical method for controlling the False Discovery Rate (FDR), or the proportion of results that are false positives (Type I error). Methods controlling for FDR have been gaining traction and support over the last two decades within the ecological research

community, over those that control for Family-Wise Error Rate (FWER), such as the highly conservative Bonferroni correction (García, 2003; García, 2004; Nakagawa, 2004; Verhoeven et al., 2005; Pike, 2011). Because the Bonferroni correction limits the chance of even a *single* Type I error by controlling FWER, it is highly conservative and often results in high rates of Type II errors, where a failure to reject the null hypothesis is false (García, 2004; Verhoeven et al., 2005). Another benefit of the Benjamini-Hochberg approach is that it doesn't strongly assume independence, making it appropriate for data with variables with some dependency (Benjamini and Yekutieli, 2001; Verhoeven et al., 2005). Because of the correlative nature of the biologically relevant compounds selected *a priori* for emission rate analysis (see discussion), I used the Benjamini-Hochberg correction.

Because emission rates appeared to differ starkly among tepals and pistils within *Yucca* species, I tested for differences *post hoc* using a series of Wilcoxon rank sum exact tests found in the "stats" package (R, v4.2.1) (Wilcoxon, 1945). A non-parametric approach was taken because the data did not meet the assumptions of normality and transformation could not correct this. For this analysis, I used the more conservative Bonferroni p-value correction. I also limited the within species, tissue-specific analysis to (*E*)-DMNT and (*E*)-nerolidol because of the correlative nature of the chosen subset of biologically relevant compounds and further tests could inflate the probability of Type I error.

Moth Behavioral and Oviposition Assays

I collected wild moths from local populations of *Yucca filamentosa* located near the Barton Laboratory at Cornell AgriTech (Geneva, NY, USA, 42°52'33.8"N 77°00'24.0"W) on July

2nd, 3rd, and 7th 2022. I used a dissecting microscope to sort female moths from males (due to the presence of pollen-collecting tentacle organs in the former (Pellmyr and Krenn, 2002)) and ascertain if pollen balls were present. Although not absolutely necessary for female moths to attempt oviposition, pollen balls are a good indicator that a female moth has mated and is ready to oviposit (Riley, 1892; Rau, 1945). Female moths were kept in 50 mL conical polystyrene tubes with a moistened kimwipe (Kimberly-Clark, Irving, TX USA) in a cool laboratory, exposed to natural light until they became active around dusk, indicating readiness for bioassays.

From July 2nd-8th, 2022 I conducted "no choice" behavioral assays wherein a female *T. yuccasella* moth was placed in a 236 mL plastic cup with an unpollinated yucca flower and was video recorded for 12 minutes using a smartphone camera. Flowers were visually assessed for pollination status using a hand lens. Using unpollinated flowers doesn't guarantee that flowers were not previously visited by moths, but it significantly reduces the likelihood that prior visitation has occurred. Flowers used in behavioral trials were labeled with a video recording number, moth ID, plant ID and species, and transported back to the lab. Each moth was used in only one trial per night. However, due to the limited availability of yucca moths, some individuals were used in one additional trial on a second night. Half of the 16 moths collected were used in an additional trial. Moths that were used in a second trial were given a flower from a different *Yucca* species than was used in their first trial. *Yucca glauca* is the only yucca species not included in moth behavior trials because all *Y. glauca* plants in the garden had ceased blooming prior to July 2nd when trials began. I compared the proportion of moths attempting oviposition and the average number of oviposition attempts across *Yucca* species

using Chi-squared tests. Female oviposition attempts observed on *Y. filamentosa* were used as positive controls to generate expected values.

Behavioral Data Analysis

To analyze video recordings, I organized behaviors of interest into categorical states: active (*crawling, alert, escape*); engaged (*antenna movement, abdomen probing, oviposition, pollination,* and *pollen gathering*); and inactive (*resting, inverted*). To extract behavioral data from recordings, I used the CowLog software program (Hänninen and Pastell, 2009) to create a customized keyboard with shortcuts assigned to unique behaviors. I used Kruskal-Wallis rank tests to determine if total oviposition time or time to first behavior (latency) differed among yucca species.

Results

Intrafloral Scent Analysis

NMDS analyses indicated that the tepals and pistils within each *Yucca* species were significantly different in overall floral volatile blend composition (Figs. 1 and 2). ANOSIM tests of dissimilarity matrices showed that tepals and pistils of *Y. glauca* were significantly different in overall scent composition (Fig. 1, R: 0.9985, P = 0.001) but populations were not (Fig. 1, R: -0.045, P = 0.852). Tepals and pistils also differed significantly in overall scent in *Y. filamentosa* (Fig. 2a, R: 0.9943, P = 0.001); *Y. pallida*, (Fig. 2b, R: 0.9991, P = 0.001); *Y. rupicola*, (Fig. 2c, R: 0.9785, P = 0.001); and *Y. reverchonii* (Fig. 2d, R: 0.7328, P < 0.001).

Multilevel pattern analysis revealed that how tepals and pistils differed varied among species (Supplemental Table 2). The suite of novel C-11 terpenoids, first identified in *Y. filamentosa* floral scent (Svensson et al., 2005) and structurally described by Tröger et al. (2021), contributed strongly to tepal identity and was statistically significant in *Y. glauca*, *Y. filamentosa*, and *Y. pallida*. In *Y. filamentosa* the full suite of novel yucca terpenoids contributed strongly to tepal identity and was statistically significant. Additionally, the monoterpene β-myrcene, and sesquiterpenes α-farnesene and germacrene-D, contributed strongly to *Y. filamentosa* tepal identity and were statistically significant. *Y. filamentosa* was also the only *Yucca* species with a compound that contributed strongly and significantly to pistil identity, the sesquiterpenoid (*E*)-nerolidol. *Y. glauca* and *Y. pallida* both had subsets of novel yucca terpenoids, as well as the sesquiterpene α-farnesene that contributed strongly and significantly to tepal identity, but only *Y. pallida* had a strong, significant association between tepal identity and β-myrcene. (*E*)-DMNT contributed strongly and significantly to tepal identity in *Y. reverchonii*. Octadecene and germacrene-D also contributed strongly and significantly to tepal identity in *Y. reverchonii*. Interestingly, no specific compounds contributed strongly or significantly to either tepal or pistil identity in *Y. rupicola*. Further analysis of indicator values showed that all compounds had a high probability of positive predictive value as an indicator of a floral part (specificity), a high probability of being found in a particular floral part (fidelity), or both (Supplemental Table 2).

Mean emission rates were calculated for select compounds of known or suspected biological importance (Tröger et al., 2021; Favaris 2020) and within-species comparisons of tissue-specific scent emission rates were performed (Fig. 3, a, b, c). I chose to analyze only (*E*)-

4,8-dimethyl-1,3,7-nonatriene (hereafter (*E*)-DMNT), and (*E*)-nerolidol emission rates at an intrafloral level because they are the compounds with the highest emission rates in the majority of *Yucca* species analyzed and I wanted to avoid unnecessary *post-hoc* testing of correlated analytes. *Yucca filamentosa* (P = 0.003; P = 0.003), *Y. glauca* (P > 0.001; P > 0.001), and *Y. pallida* ((E)-DMNT, P > 0.001; (*E*)-nerolidol, P > 0.001) tepals and pistils all differed significantly in mean emission rate of both (*E*)-DMNT and (*E*)-nerolidol. Neither *Y. reverchonii* nor *Y. rupicola* tepals and pistils, which produce very small quantities of these compounds, differed significantly in emission rate (Fig. 3c,d). One important factor to keep in mind is the large difference in mass among floral parts. Depending on the species of *Yucca,* pistil and tepal mass vary substantially. I observed a range of 0.2825 to 1.983 g for pistils (mean 0.9432 g) and 0.7446 g to 8.4964 for tepals (mean 3.7585 g). Standardizing for mass allowed us to directly compare emission rates between tepals and pistil, but these data are not representative of whole flower emission rates.

Interspecies Analysis

Because populations of *Y. glauca* did not differ in floral scent (Fig. 1) we chose to only use individuals from the Texas population for interspecies analyses for two reasons: 1) this population is geographically closest to the sampled populations for the other species; and 2) sample sizes were strongly biased in favor of *Y. glauca* (see table S1) due to population level analyses. Mean emission rates were calculated for select compounds of known or suspected biological importance (Favaris et al., 2020; Tröger et al., 2021) and comparisons of emission rates were done among *Yucca* species (Fig. 2). Mean pistil emission rate comparisons among

species were done for five unique compounds: (*E*)-DMNT, (*E*)-nerolidol, and germacrene-D, (*Z*)-filamentol, and (*Z*)-filamentolide (Fig. 4).

Mean pistil emission rates of (*E*)-DMNT (Kruskal-Wallis χ^2 = 17.349, df = 4, P = 0.002); (*E*)-nerolidol (Kruskal-Wallis, χ^2 = 17.205, df = 4, P = 0.002); and germacrene-D (Kruskal-Wallis, χ^2 = 18.381, df = 4, P = 0.001) were significantly different among species (Fig. 4). Mean pistil emission rates for both (*Z*)-filamentol (Kruskal-Wallis, χ^2 = 13.878, df = 4, P = 0.007) and (*Z*)-filamentolide (Kruskal-Wallis, χ^2 = 15.166, df = 4, P = 0.004) were significantly different among species (Fig. 4). Mean tepal emission rate comparisons among species were done for the same compounds as the pistil analysis (Fig. 4). *Yucca* species differed significantly in mean tepal emission rate for (*E*)-DMNT (Kruskal-Wallis, χ^2 = 17.174, df = 4, P = 0.002); (*E*)-nerolidol (Kruskal-Wallis, χ^2 = 18.347, df = 4, P = 0.001); germacrene-D (Kruskal-Wallis, χ^2 = 16.198, df = 4, P = 0.003); (*Z*)-filamentol (Kruskal-Wallis, χ^2 = 17.726, df = 4, P = 0.001); and (*Z*)-filamentolide (Kruskal-Wallis, χ^2 = 16.375, df = 4, P = 0.003). Dunn's tests with Benjamini-Hochberg adjusted p-value were used for pairwise comparisons among species (Table 1; Fig. S1).

Moth Oviposition and Behavior

The number of female moths that attempted to oviposit into flowers did not differ among *Yucca* species (χ^2 = 0.31, P = 0.85). Six female moths were observed and recorded searching on flowers of *Y filamentosa*. These females performed 17 oviposition attempts, producing a mean number of 2.83 ($\pm$ 1.05, SE) oviposition attempts per female per flower on the natal *Yucca* species. I used this oviposition attempt rate as the expected value for oviposition attempts on the other *Yucca* species with a null assumption of no preference for

flowers from different species. On average, female moths performed 2.5 oviposition attempts on *Y. pallida*, and 0.33 attempts each on *Y. reverchonii* and *Y. rupicola* (Fig. 5). Chi-square analysis did not detect a significant difference in oviposition attempts on different *Yucca* species ($\chi^2 = 4.451$, $P = 0.11$) although there was a clear 7.5-fold trend for reduced oviposition attempts on *Y. reverchonii* and *Y. rupicola*. Similarly, there was no significant difference in average moth latency (time to first behavior) among species of *Yucca* (Kruskal-Wallis, $\chi^2 = 2.5384$, df = 3, $P = 0.4684$), or seconds engaged in oviposition (Kruskal-Wallis, $\chi^2 = 2.5384$, df = 3, $P = 0.4684$).

Discussion

Interactions between plants and insects play a pivotal role in plant reproduction, as a majority of flowering plants rely on insects for pollination (Ollerton et al., 2011). In order to attract pollinators, flowering plants advertise the presence of rewards via a variety of floral traits. In highly specialized interactions, a patchy distribution necessitates signals that can both travel long distances and lead pollinators back to the source (Williams and Dodson, 1972; Barker, 1984; Grison-Pige et al., 2002; Raguso, 2008; Ibanez et al., 2010). In such cases, plants rely on chemical cues such as floral scent, which can be detected by the highly sensitive sensory organs of insects at remarkably low levels (Waddington, 1983), and be used to orient towards the host by following filaments of scent-laden air in natural settings (Murlis et al., 1992). The zig-zag upwind tracking flight observed for yucca moths in field settings is consistent with the proposed importance of orientation to scent from a distance as a partner-encounter mechanism in the yucca-yucca moth mutualism (Tröger et al., 2021). Floral scent also plays a

role in close-range pollinator decision-making (Wright and Schiestl, 2009) and within-flower orientation (Bergstrom et al., 1995), especially in highly specialized interactions where there is a need for pollinators to distinguish among floral parts to earn a reward (Knudsen and Tollsten, 1993). Indeed, tissue-specific variation in floral scent appears to be common if not ubiquitous among angiosperms (Garcia et al., 2021), indicating a need to explore the implications of intrafloral scent variation on plant-pollinator interactions at the actual physical scale of the interaction.

Brood-pollination mutualisms, wherein pollinators exchange pollination services for a “nursery” within the host plant where offspring develop, often involve highly specific behaviors and a need for pollinators to distinguish among floral parts. This makes them particularly good candidates for exploring the role of intrafloral floral scent variation in plant-pollinator interactions. In this study I examined variation in tissue-specific floral scent to better understand the role intrafloral scent variation plays in the brood-pollination mutualism between yuccas and yucca moths. Specifically, I examined the floral scent of the tepals and pistils of five species of *Yucca* and observed female pollinator moth behavior on flowers of four of the species. I also explored how moth behavior varied among *Yucca* species in relationship to changes in intrafloral scent patterns.

I first addressed the question, “Do *Yucca* species exhibit tissue-specific scent?” and found that tepals and pistils differed in scent for all species of *Yucca* analyzed. These differences are observed both in comparisons of the relative peak area of compounds produced (the floral scent blend) (Fig. 1 & 2, Table S2) and in the emission rate of specific biologically relevant compounds for a subset of *Yucca* species analyzed (Fig. 3 & 4). The tissue-specific differences I

observed in the floral scent blend are largely quantitative, that is tepals and pistils tend to differ in the relative amount of unique scent compounds in their floral bouquet. All species exhibited a tissue-specific division in floral scent profile when I examined the floral blend (Fig 1 & 2), but the pattern appears to be largely driven by (*E*)-DMNT, (*E*)-nerolidol, and the suite of novel yucca terpenoids (Table S2). In the species *Y. rupicola* and *Y. reverchonii*, many of these compounds are absent or only detected in trace amounts in the individual floral parts. The importance of this finding to the attraction of local populations of *Tegeticula yuccasella* in Texas remains to be determined.

In addition to examining tissue-specific floral blends, comparisons of emission rates are particularly informative because they approximate concrete concentrations of compounds emitted from a given floral part and encountered by visiting insects. The emissions rates calculations presented here accounted for both the difference in analyte response within the mass spectrometer and the difference in mass among floral parts, ensuring that final calculated rates did not reflect methodological artifacts. Comparisons of mean emission rate demonstrate that pistils emit significantly more (*E*)-nerolidol on average per gram of tissue than the tepals in *Y. filamentosa, Y. glauca,* and *Y. pallida* (Fig. 3a,b,c). Tepals in these species on the other hand, emit significantly more (*E*)-DMNT than do the pistils. However, the rates of (*E*)-DMNT and (*E*)-nerolidol emission are significantly reduced to the point of near absence in all floral parts of *Y. reverchonii* and *Y. rupicola* (Fig. 3c,d), meaning that these compounds no longer differentiate tepals from pistils in these species. Comparisons of mean emission rates for a given floral part among species showed differences among some but not all species, and the patterns observed were dependent on the floral part analyzed (Table 1, Fig 4 & S1). Among *Y. filamentosa, Y.*

glauca, and Y. *pallida* neither pistils nor tepals differed statistically in the mean emission rates of select biologically relevant compounds.

The strong reduction of (*E*)-DMNT and (*E*)-nerolidol is interesting because molecular evidence suggests that synthesis of the “novel yucca terpenoids” starts with (*E*)-DMNT. From the carbon skeleton of (*E*)-DMNT, minor modifications to, or substitutions of functional groups results in the formation of the alcohols, aldehydes, ketones, and lactones that comprise this suite of novel compounds (Tröger et al., 2021). In turn, (*E*)-DMNT is synthesized downstream of (*E*)-nerolidol (Chan et al., 2016). A common biosynthetic pathway for these compounds means that a reduction of expression or breakdown at either the point of (*E*)-nerolidol or (*E*)-DMNT synthesis would likely impact the synthesis, and ultimately concentration, of the novel yucca terpenoids. Indeed, in *Y. reverchonii* and *Y. rupicola*, the two species that produce only low level to trace amounts of (*E*)-DMNT and (*E*)-nerolidol, the novel yucca terpenoids are absent or present only in trace amounts. Contrary to Tröger’s (et al. 2021) inflorescence-scale analysis of *Y. reverchonii* floral scent showing a loss of the novel yucca terpenoids, I detected trace levels of these compounds in the floral blend of *Y. reverchonii.* However, these compounds are present at such low levels—detected in a single flower, from a single individual, and only in the tepals— that they were undetectable at the whole inflorescence scale. *Y. rupicola* was also suspected to have lost the novel yucca terpenoids, but appears to express them inconsistently at low levels. The novel yucca compounds were detected in some but not all *Y. rupicola* individuals (plants), or even flowers within the inflorescence of an individual, suggesting the question is not a matter of the pathway’s total loss but rather how consistently it is expressed within and among individuals.

Y. rupicola also produces more germacrene-D on average in both its tepals and pistils than any other species. Whether germacrene-D factors significantly into moth decision-making remains unclear, but the chemical biosynthesis relationship of this compound to (*E*)-nerolidol is worth discussing. Both (*E*)-nerolidol and germacrene-D are synthesized from farnesyl diphosphate (FDP) in the cytosol, although they differ in which stereoisomer of FDP serves as a parent molecule (Chappell and Coates, 2010). Notably, germacrene-D is a cyclic sesquiterpene, whereas (*E*)-nerolidol is a linear sesquiterpene. Because the molecular mechanics of terpene synthesis generate "variations on a theme", using targeted modifications to a shared carbon skeleton to produce a diverse array of biosynthates, a change in the production rate of various FDP stereoisomers also potentially impacts synthesis downstream (Karunanithi and Zerbe, 2019). In the case of *Y. rupicola* a reduction of (*E*)-nerolidol synthesis, and reduced emission of (*E*)-DMNT and the novel yucca terpenoids, could be due to an increase in production of the cyclic stereoisomer of FDP, resulting in increased germacrene-D expression. Interestingly, *Y. rupicola* was not the only species that showed a significant increase in mean germacrene-D emission rates. *Y. pallida* also produced significantly more germacrene-D than other *Yucca* species, though it's production of (*E*)-nerolidol was not significantly different from either *Y. glauca* or *Y. filamentosa*. Why the (*E*)-nerolidol synthesis pathway appears to be impacted in Y. *rupicola* but not *Y. pallida* remains unclear. However, the increase in mean germacrene-D emissions in *Y. pallida* and *Y. rupicola* is interesting in light of their phylogenetic relationship; these species are sister taxa within the Rupicolae series (Pellmyr et al., 2007). There is a possibility that one shared-derived trait of these species is a branch point in their FDP synthesis pathway that results in higher emissions of germacrene-D.

Regardless of the cause, emission rate of the novel yucca terpenoids in *Y. rupicola* and *Y. reverchonii* was significantly less compared to other *Yucca* species, making them difficult to detect at the whole-inflorescence scale. Headspace collection methodology only became sensitive enough to detect these compounds' in these species when scent was collected at the intrafloral level, indicating that exploration of floral scent across multiple spatial scales can improve our ability to detect chemical differences and similarities. Such a detailed characterization of the intrafloral chemical landscape is important because insects also interact with flowers at the intrafloral level. Whole inflorescence scent analysis should be combined with finer scale analyses in order to increase the chance of detecting compounds with potential biological relevance to plant-insect interactions, including both intra-individual and intrafloral scales.

The detection of low-levels of biologically relevant compounds in *Y. rupicola* and *Y. reverchonii* also has interesting implications for future work exploring the sensitivity of *Tegeticula* species using floral part extracts in electroantennogram assays. Tröger et al. (2021) performed GC-MS coupled, electroantennogram assays using whole inflorescence extracts and determined a subset of compounds in the floral blend that elicited a strong response from moth antennae, including several novel yucca terpenoids and their parent molecule (*E*)-DMNT. Comparing moth antennal responses within and among flowers could be informative regarding biologically relevant detection thresholds for yucca moths. And in the case where floral parts emit significantly more of a given compound, such as pistil emissions of (*E*)-nerolidol in *Y.* filamentosa, *Y. pallida*, and *Y. glauca* these assays could inform us of important close-range cues that influence moth behavior.

To determine if differences in scent profiles among species and between floral parts influenced moth behavior I observed female moths on whole flowers (Fig. 5). The pollinator moth *T. yuccasella* uses all of the *Yucca* species tested as a host across its geographic range. There is little evidence of genetic structure among populations using different *Yucca* species (Leebens-Mack and Pellmyr, 2004) and local moths that use *Y. filamentosa* will visit all of the *Yucca* species at the common garden (Althoff, 2016). Based on these findings, I expected subtle effects on behavior when moths were observed on flowers from non-natal hosts. There was a trend for moths to be more likely to attempt oviposition in their natal host, *Y. filamentosa* and the chemically similar *Y. pallida*, whereas attempts decreased in the chemically dissimilar species *Y. rupicola* and *Y. reverchonii* (Fig. 5). The moth observations mirror results from moth oviposition attempts in natural populations of *Y. pallida* and *Y. reverchonii*. Surveys of fruit constrictions, which are characteristic of an oviposition attempt that damages plant ovules, from 180 *Y. pallida* fruit and 298 *Y. reverchonii* fruit, showed that moths had over twice as many attempts on *Y. pallida* ($F_{1, 397}$=239.5, $P < 0.0001$, Althoff, unpublished).

I found no significant difference in the duration of time between the start of a trial and first observed behavior (latency) such as antenna movement, abdomen probing, or crawling that suggested moths were exploring the flower, among *Yucca* species. Unfortunately, there were too few oviposition attempts to determine if time to first oviposition differed when moths searched on different *Yucca* species. I did observe a trend in trials where oviposition *was* attempted wherein moths tended to accept a flower within six minutes, after which point chances of oviposition were low. The one exception to this trend was our only observation of attempted oviposition in *Y. rupicola* in which the moth took nearly 10 minutes to decide to

attempt oviposition. The lack of any clear differences in moth behavior is in some ways not unexpected given that *T. yuccasella* is successful in using all of these *Yucca* species. The results from this study suggest that large sample sizes may be necessary to find significant differences in moth behavior.

The present study demonstrates that floral parts differ in terms of both their relative blend and mean scent emissions for five species of yucca. Further, the chemical comparison of tissue-specific floral scent shows differences among plant species with a shared species of pollinator. The observation that biologically relevant compounds can be missing in certain species of *Yucca*, in conjunction with evidence that *Tegeticula yuccasella* still locates, oviposits into, and pollinates all of the *Yucca* species surveyed is particularly interesting. This behavior suggests that moths may use the entire floral blend to locate yuccas rather than relying on a few unique compounds such that the loss of (*Z*)-filamentol and (*Z*)-filamentolide does not significantly influence moth host choice. Explaining the absence of these compounds is challenging as several factors could be involved such as relaxed selection on floral blend composition resulting in loss of compounds via drift, selection to reduce production of metabolically costly compounds, or changing interactions with floral antagonists. These factors would need to be important in *Y. rupicola* and *Y. reverchonii* but not in the closely related *Y. pallida*, which produces typical levels of (*Z*)-filamentol and (*Z*)-filamentolide.

Much remains to be tested in the context of tissue-specific floral scent expression, especially as research continues to elucidate patterns of variation in an increasing number of study systems. For example, certain plant and pollinator groups are currently still underrepresented (Garcia et al., 2021). I was able to demonstrate tissue-specific scent in a

moth pollinated system, whereas to date the only plant genus with tissue-specific floral scent that is known to be pollinated by moths is *Lithophragma* (Garcia et al., 2021). It is interesting to note that *Greya,* the genus of moths that pollinates *Lithophragma* belongs to the Prodoxidae family, which includes the yucca moths (Pellmyr and Thompson, 1992). These two plant-pollinator systems are quite specialized and broader sampling across the lepidoptera, in particular in more generalist systems, would provide valuable insight into the prevalence of tissue-specific scent in moth-pollinated systems. Behavioral assays also remain in short supply, for though they are necessary to understand the biological meaning behind these patterns in the context of pollination they can be labor-intensive, time-consuming, and difficult to execute. Although I was able to identify and characterize tissue-specific scent in five species of *Yucca*, demonstrating a behavioral linkage between patterns of scent and pollinator behavior remained elusive. It is my hope that this study might serve as a call to action for those researchers interested in studying chemical ecology at biologically relevant, finer scales, namely among floral parts within individual flowers.

Table 1 Comparisons of floral parts emission rates of five biologically relevant scent compounds in *Yucca* species. Comparisons are done at the species level by floral part. Matching number pairs indicate significant differences between species, determined using Dunn tests of the ranked distribution. Species names are abbreviated as follows: FILA = *Y. filamentosa*; GLAU = *Y. glauca*; PALL = *Y. pallida*; REVE = *Y. reverchonii*; and RUPI = *Y. rupicola*.

Compound	**Floral Part**	**Comparisons among *Yucca* species**								
(*E*)- 4,8- dimethyl-1,3,7-nonatriene	Pistils	GLAU[1,2]	>	PALL[3,4]	>	FILA	>	RUPI[1,3]	>	REVE[2,4]
	Tepals	FILA[1,2]	>	GLAU[3,4]	>	PALL[5]	>	REVE[1,3]	>	RUPI[2,4,5]
(*E*)-nerolidol	Pistils	PALL[,1,2]	>	GLAU[3,4]	>	FILA[5,6]	>	REVE[1,3,5]	>	RUPI[2,4,6]
	Tepals	PALL[1,2]	>	FILA[3,4]	>	GLAU[5,6]	>	REVE[1,3,5]	=	RUPI[2,4,6]
Germacrene-D	Pistils	RUPI[1,2,3]	>	PALL[4,5,6]	>	GLAU[1,4]	>	REVE[2,5]	>	FILA[3,6]
	Tepals	RUPI[1,2,3]	>	PALL[4]	>	REVE[1]	>	GLAU[2]	>	FILA[3,4]
(*Z*)-filamentol	Pistils	FILA[1]	>	PALL[2]	>	GLAU	>	RUPI	>	REVE[1,2]
	Tepals	FILA[1,2]	>	GLAU[3,4]	>	PALL	>	RUPI[1,3]	>	REVE[2,4]
(*Z*)-filamentolide	Pistils	PALL[1,2]	>	GLAU	>	FILA	>	RUPI[1]	>	REVE[2]
	Tepals	FILA[1,]	>	GLAU[2,3]	>	PALL[4,5]	>	RUPI[2,4]	>	REVE[1,3,5]

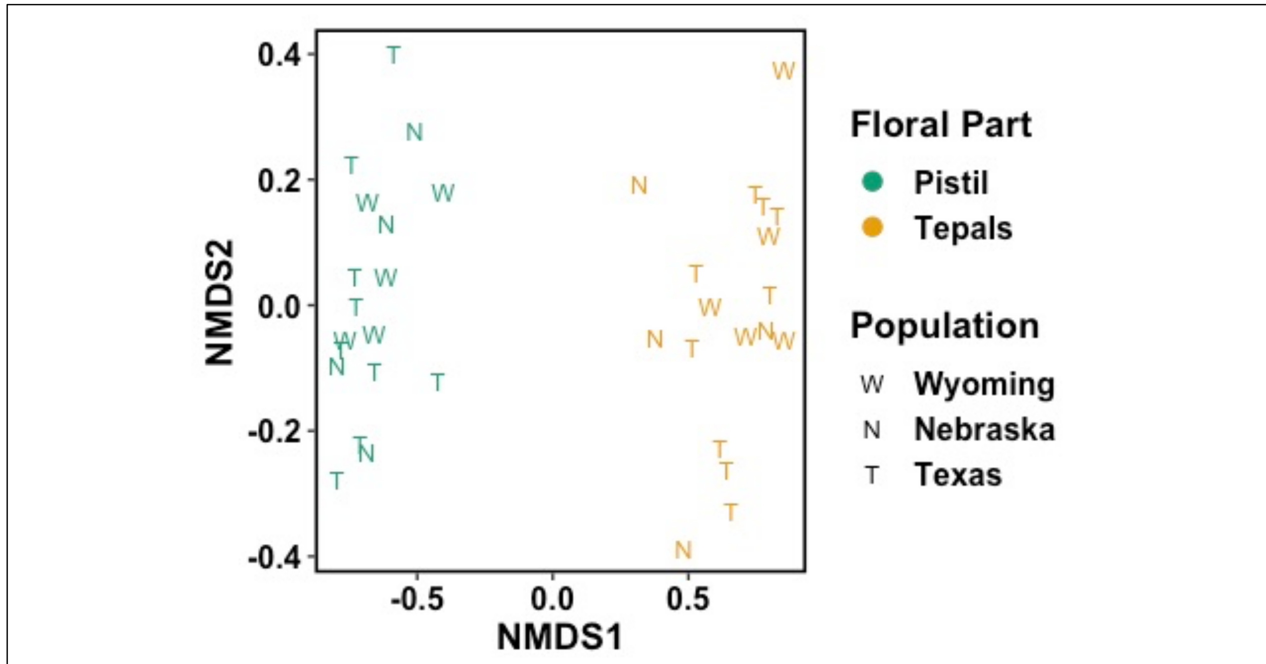


Figure 1 Non-metric multidimensional scaling (NMDS) plot of floral scent from tepals and pistils of *Y. glauca* from three populations across its native range in the United States. Analysis was performed using average relative peak area, with each point representing an average of the flowers collected from an individual plant (n = 36, r = 3). Letters correspond to population location, whereas colors correspond to floral parts (green = pistil, gold = tepals). ANOSIM tests showed that floral parts differed significantly (R: 0.9985, p = 0.001) but populations did not (R: -0.045, p = 0.852).

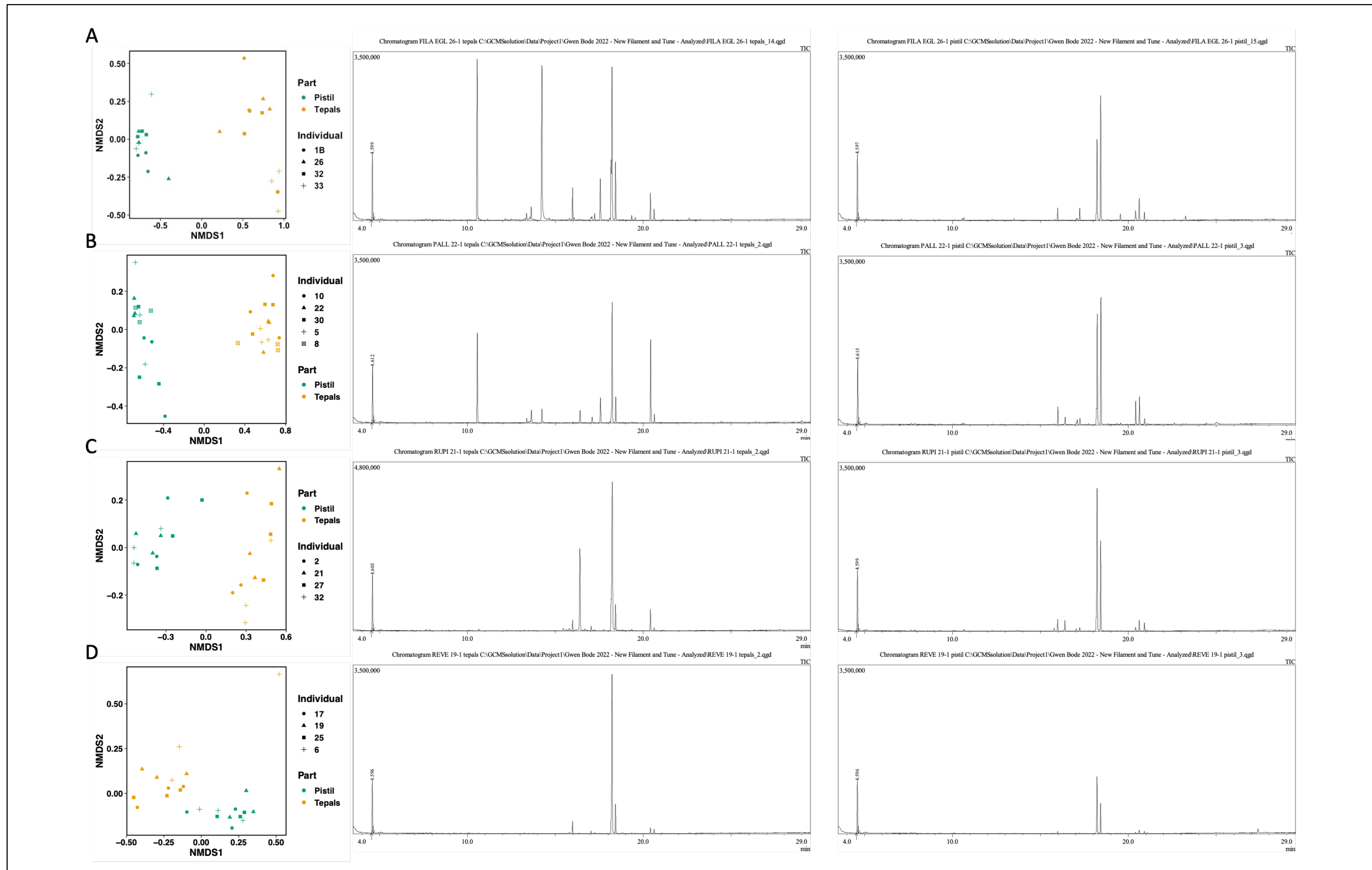


Figure 2 NMDS plots adjacent to chromatograms for a representative tepal and pistil of A) *Y. filamentosa*, B) *Y. pallida*, C) *Y. rupicola*, and D) *Y. reverchonii.* Analysis was performed using Bray-Curtis dissimilarity matrices calculated with the relative peak area of floral compounds (floral blend) collected via volatile headspace sampling. Chromatograms were generated via GC-MS. Each point represents a floral part from a single flower, either the tepals or pistil. Colors indicate floral parts (gold = tepals, green = pistils), whereas shapes indicate flowers sourced from the same individual *Yucca* plant. Note that although the majority of chromatograms share a common scale (top left-hand corner), the chromatogram for *Y. rupicola* is different to ensure all peaks were fully included.

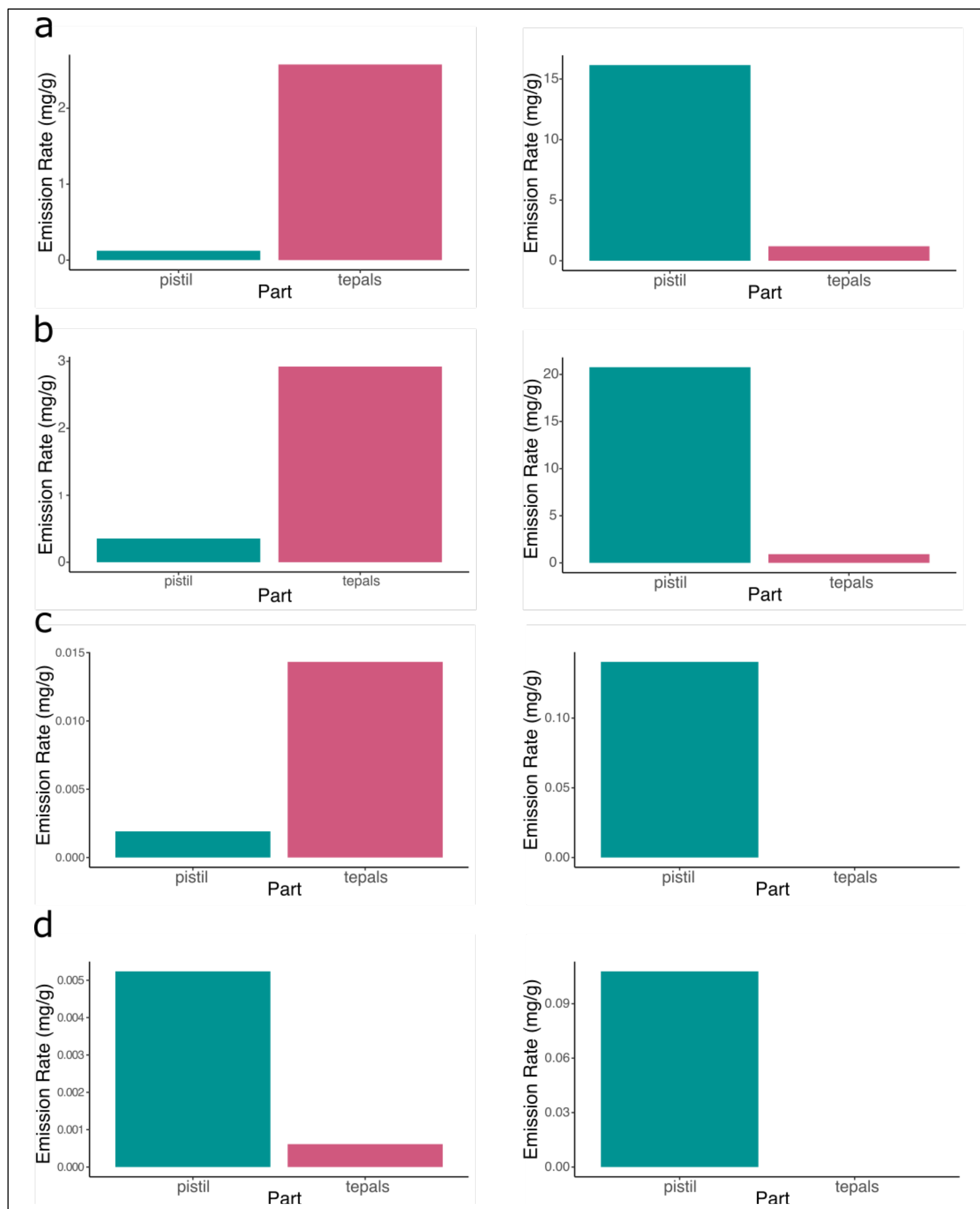


Figure 3 Comparisons of mean (*E*)-DMNT and (*E*)-nerolidol emission rates in units of mg per- g tissue per hour for the pistils and tepals of five species of *Yucca*: a) *Y. filamentosa,* b) *Y. glauca*, c) *Y. pallida*, d) *Y. reverchonii*, and e) *Y. rupicola*. Each plot shows a comparison between floral parts, indicated by colors (pistils= teal, tepals = magenta) for one compound: left= (*E*)-DMNT; right = (*E*)-nerolidol. Median values for floral parts by species for pistils and tepals respectively are as follows: *Y. filamentosa* ((*E*)-DMNT = 1.19, 2.90; (*E*)-nerolidol = 8.32, 1.13); *Y. glauca* ((*E*)-DMNT = 0.283, 1.76; (E)-nerolidol = 8.97, 0.922); *Y. pallida* ((*E*)-DMNT = 0.234, 1.58; (*E*)-nerolidol = 23.3, 1.56); *Y. reverchonii* ((*E*)-DMNT = 0, 0.00702; (*E*)-nerolidol = 0, 0); and *Y. rupicola* ((*E*)-DMNT = 0.00213, 0.000678; (*E*)-nerolidol = 0, 0).

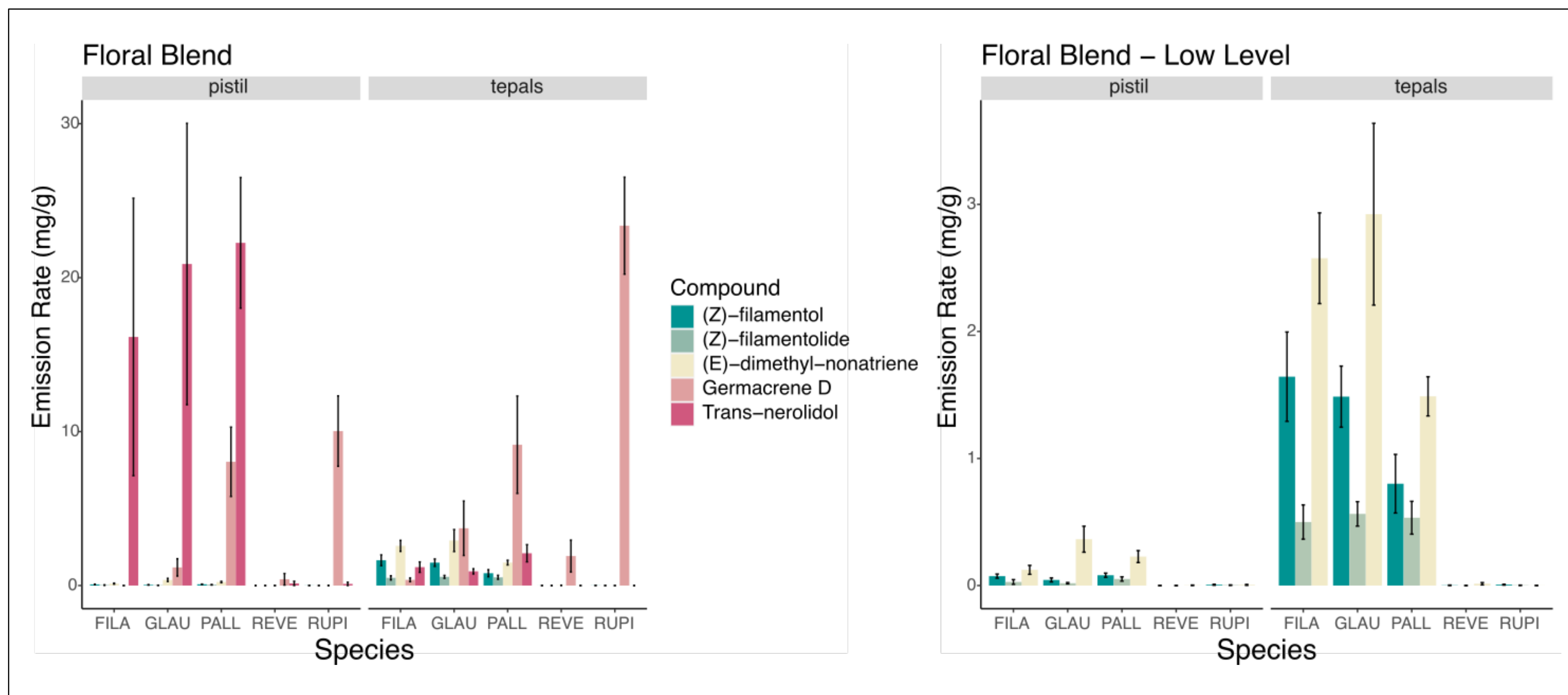


Figure 4 Mean floral scent emission rate in units of mg per- g tissue per-hour of pistils and tepals for compounds of known or suspected biological relevance across *Yucca* species. Panels show a) all compounds and b) a subset of compounds with comparatively lower per mass emission rates. Colors represent different compounds and error bars show standard error of the mean. Median values for floral parts by species for pistils and tepals respectively are as follows: *Y. filamentosa* ((*E*)-DMNT = 1.19, 2.90; (*E*)-nerolidol = 8.32, 1.13; germacrene-D = 0, 0.349; (*Z*)-filamentol = 0.0819, 1.67; (*Z*)-filamentolide = 0.0124, 0.563); *Y. glauca* ((*E*)-DMNT = 0.283, 1.76; (*E*)-nerolidol = 8.97, 0.922; germacrene-D = 0.840, 1.20; (*Z*)-filamentol = 0.0291, 1.14; (*Z*)-filamentolide = 0.0154, 0.561); *Y. pallida* ((*E*)-DMNT = 0.234, 1.58; (*E*)-nerolidol = 23.3, 1.56; germacrene-D = 7.42, 7.14; (*Z*)-filamentol = 0.0815, 0.617; (*Z*)-filamentolide = 0.0441, 0.544); *Y. reverchonii* ((*E*)-DMNT = 0, 0.00702; (*E*)-nerolidol = 0, 0; germacrene-D =0.0423 , 1.53; (*Z*)-filamentol = 0, 0; (*Z*)-filamentolide = 0, 0) ; and *Y. rupicola* ((*E*)-DMNT = 0.00213, 0.000678; (*E*)-nerolidol = 0, 0; germacrene-D = 10.2, 25.1; (*Z*)-filamentol = 0.00546, 0.00597; (*Z*)-filamentolide = 0.00244, 0.0015)

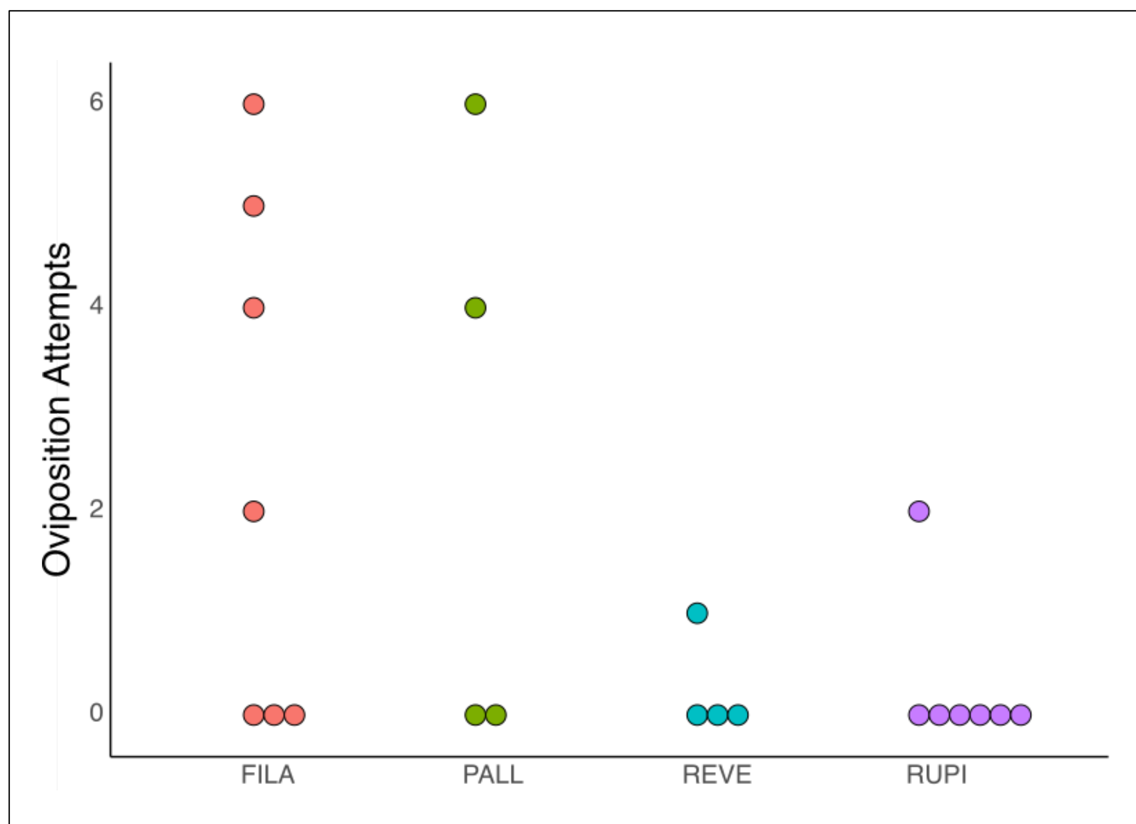


Figure 5 Count data for oviposition attempts of the pollinator moth *Tegeticula yuccasella* observed in no-choice behavioral assays on four *Yucca* species. Each point corresponds to a single moth observed in a single flower.

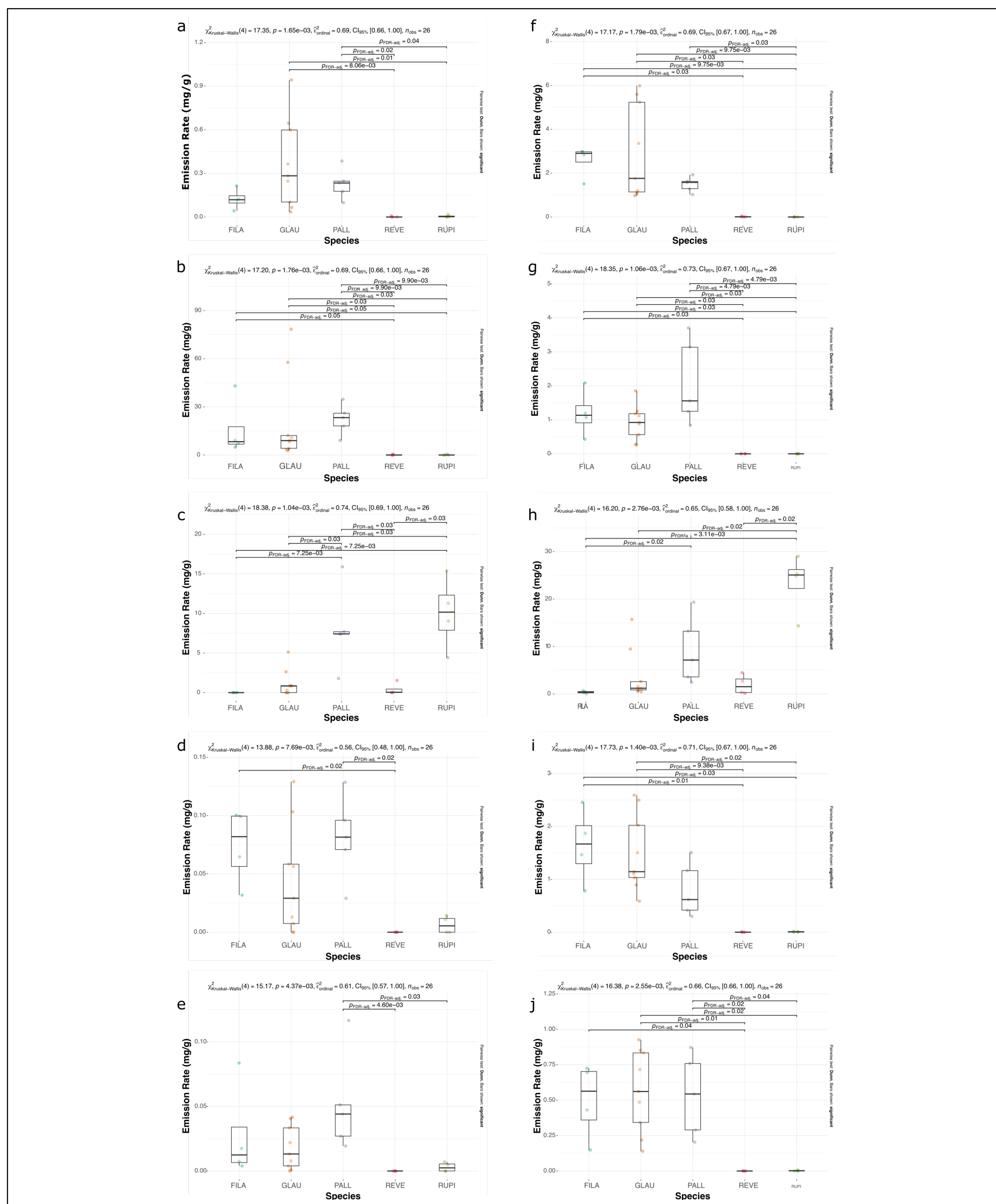
Supplemental Table 1 Names of *Yucca* species, population locations, and number of individuals per species sampled in the study. More detailed population information can be found in Althoff et al. 2014.

Species Sampled	Population Location	Individuals
Yucca glauca – WY	Clarendon, TX	5
Yucca glauca – 128	Wheatland, TX	9
Yucca glauca – NB	Lake McConaughy, TX	4
Yucca filamentosa	Lake Placid, FL and Syracuse, NY	5
Yucca pallida	Dublin, TX	5
Yucca reverchonii	Sonora, TX	4
Yucca rupicola	Kyle, TX	4

Supplemental Table 2 Compositional differences of scent from tepals and pistils of Yucca species. Significant indicator values are from a multi-level pattern analysis of the Bray-Curtis dissimilarity matrices used to generate species-level NMDS plots. Matrices were calculated using the tissue-specific floral blend of five species of *Yucca*. Comparisons were done within species, between floral parts (tepals and pistils). Compounds with high indicator values are strongly associated with the "identity" of a particular floral part in that species. Indicator values are subdivided into *specificity*, or the proportion of a given floral part that produce that compound; and *fidelity*, or the proportion of a given floral part that a compound is present in within. A permutation test (n = 999) was used to test for statistical significance of the association. No specific compounds are strongly, significantly associated with either tepals or pistils in *Y. rupicola*.

Species	Floral Part	Compound	Indicator Value	Specificity	Fidelity	p-value
Y. glauca	Tepals	Unknown (m/z 84)	1.0000	1.0000	1.0000	0.001 ***
		(*E*)-filamental	0.9930	0.9870	1.0000	0.001 ***
		Cis-unknown C11 (m/z 67)	0.9930	0.9862	1.0000	0.001 ***
		Trans-unknown C11 (m/z 67)	0.9870	0.9735	1.0000	0.001 ***
		Unknown ketone (m/z 82)	0.9730	0.9468	1.0000	0.001 ***
		(*Z*)-filamental	0.9720	0.9447	1.0000	0.001 ***
		α-farnesene	0.9220	0.8502	1.0000	0.001 ***
		(*Z*)-4,8-dimethyl-1,3,7-nonatriene	0.5770	1.0000	0.3333	0.010 *
Y. filamentosa	Pistil	(*E*)-nerolidol	0.9930	0.9858	1.0000	0.001 ***
	Tepals	β-myrcene	1.0000	1.0000	1.0000	0.001 ***
		Unknown ketone (m/z 82)	1.0000	1.0000	1.0000	0.001 ***
		(*E*)-filamental	0.9960	0.9918	1.0000	0.001 ***
		(*Z*)-filamental	0.9950	0.9896	1.0000	0.001 ***
		Cis unknown C11 (m/z 67)	0.9940	0.9873	1.0000	0.001 ***
		(*Z*)-filamentol	0.9760	0.9535	1.0000	0.001 ***
		(*Z*)-filamentolide	0.9650	0.9308	1.0000	0.001 ***
		Trans unknown C11 (m/z 67)	0.9570	1.0000	0.9167	0.001 ***
		(*E*)-filamentolide	0.9370	0.8779	1.0000	0.001 ***
		α-farnesene	0.8930	0.9577	0.8333	0.001 ***

		Unknown (m/z 84)	0.8660	1.0000	0.7500	0.001 ***
		Germacrene-D	0.8660	1.0000	0.7500	0.001 ***
Y. pallida	Tepals	(*E*)-filamental	0.9750	0.9505	1.0000	0.001 ***
		Cis unknown C11 (m/z 67)	0.9710	0.9420	1.0000	0.001 ***
		Trans unknown C11 (m/z 67)	0.9670	0.9361	1.0000	0.001 ***
		β-myrcene	0.9660	1.0000	0.9333	0.001 ***
		Unknown ketone (m/z 82)	0.9650	0.9313	1.0000	0.001 ***
		Unknown (m/z 84)	0.9570	0.9161	1.0000	0.001 ***
		(*Z*)-filamental	0.9180	0.8429	1.0000	0.001 ***
		α-farnesene	0.8720	0.8780	0.8667	0.001 ***
Y. reverchonii	Tepals	Octadecene	0.8470	0.7826	0.9167	0.005 **
		Germacrene-D	0.8290	0.9172	0.7500	0.003 **
		(*E*)-4,8-dimethyl-1,3,7-nonatriene	0.7880	0.9313	0.6667	0.005 **
Y. rupicola	-----	-----	-----	-----	-----	-----



Supplemental Figure 1 Species-level comparisons by floral part (panels (a-e), pistils; panels (f-j), tepals), of five biologically relevant compounds (panels a,f) (*E*)-4,8-dimethyl-1,3,7-nonatriene; b,g) (*E*)-nerolidol; c,h) germacrene-D; d,i) (*Z*)-filamentol; and (e,j) (*Z*)-filamentolide) (Tröeger et al., 2021), among five *Yucca* species. Plots show emission rates in milligrams of compound per gram plant tissue, per hour Brackets indicate significant differences between species.

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PUBLICATIONS

2022 *Insect performance and host plant physical defenses are altered by whole-genome duplication in the host plant*, Research Article, Submitted for Publication December 2022

2021 *Back to the Root The Role of Botany and Plant Physiology in Cannabis Testing, Part IV: The Botany of Cannabinoids or Why Do Plants Produce Cannabinoids and What's in it for Them?*Cannabis Science and Technology Magazine, February 4, 2021

2020 *Back to the Root The Role of Botany and Plant Physiology in Cannabis Testing, Part III: Genetic and Environmental Factors Associated with Terpene Synthesis in Plants*. Cannabis Science and Technology Magazine, September 24, 2020

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2018 *Optimization of Sample Preparation for Pesticide Analysis in Oil-Based Cannabis Products using LipiFiltr®*, Application Note, November 2018