WHEN FLOWERS PLAY DEAD: MICROBES AS ARCHITECTS OF A 'DECEPTIVE' FLORAL PHENOTYPE

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

Kyle Robert Martin

December 2021

WHEN FLOWERS PLAY DEAD: MICROBES AS ARCHITECTS OF A 'DECEPTIVE' FLORAL PHENOTYPE

Kyle Robert Martin, Ph. D.

Cornell University 2021

Like most entities in the phyllosphere, flowers are universally colonized by microorganisms. Because flowers are the reproductive structures of Angiosperms, microbial residence on or within floral tissues may have the potential to impact floral trait evolution and floral reproductive success by altering floral rewards and influencing pollinator behavior. To date, studies approaching these questions have mostly focused on floral microbes as larcenists of nectar rewards or as potential floral pathogens disruptive to flower-pollinator interactions. Thus, the unexplored potential for floral microbes to fundamentally contribute to extended floral phenotypes or drive evolutionary shifts to new pollinator classes constitutes a major gap in our understanding of the ecology and evolution of floral phenotypes. In this dissertation, I addressed this gap by testing the microbial impact on pollinator attraction and reward production in the flowers of the common pawpaw, *Asimina triloba*, an understory tree native to the deciduous forests of eastern North America. The small, maroon flowers of *A. triloba* produce a floral scent that is reminiscent of fermenting fruit or yeasty bread dough. Not surprisingly, the floral scent bouquet is dominated by short-chain aliphatic alcohols, acids, and esters typical of microbial metabolism, known to attract saprophilic beetles (*Glischrochilus*; Nitidulidae) and drosophilid flies that are more commonly associated with decaying organic substrates, not flowers. To test the hypothesis of microbial-mediated pollinator attraction within *A. triloba* flowers, I investigated the floral microbiome using both culture-independent and classic microbiological methods.

In addition, I performed manipulative experiments including flower sterilization, floral visitor exclusion via micromesh bagging, electroantennographic examination of pollinator chemosensory capabilities, and arthropod trap bioassays in the field. The microbiome results revealed a dynamic pattern of microbial residence that is correlated with increased fermentative volatile production in flowers, while the manipulative experiments and bioassays confirmed that microbial residence on flowers influences the attraction of wild *Glischrochilus* beetles, the most likely pollinators at my study site. Multiple sources of evidence support the hypothesis that microbial decay within pawpaw flowers represents a unique floral reward to its saprotrophic pollinators, which challenges the notion that the flowers engage in mimicry or deception for their floral advertisement.

BIOGRAPHICAL SKETCH

Born July 27th 1986, Kyle Robert Martin grew up in the foothills of western Maine where endless forest excursions, mountain hikes, and river journeys behind his home fostered a love for natural history and the outdoors. Kyle started his academic path in 2004 majoring in chemistry at the University of Maine, but promptly withdrew after one year to pursue touring full time with his punk band. After five years of travelling and playing music across all of North America with his best friends, Kyle returned to academics in 2010 and graduated from the University of New England in 2013 with a B.S. in Biology. An undergraduate honors thesis studying induced chemical defenses in marine macroalgae with advisor Dr. Ursula Röse, and a love of flowers/orchids inspired by his family led Kyle to pursue questions related to the chemical ecology of pollination for his graduate work. As a PhD student in the lab of Dr. Robert Raguso, Kyle's work has focused on examining questions related to the honesty/dishonesty of chemical signals in animal pollinated flowers, with a particular concentration on deceptive flowers that chemically mimic the egg laying sites of beetles and flies as a pollination strategy. Although science has taken recent priority, Kyle is excited now to spend more time surfing on the Maine coast, creating art, and embarking on new outdoor adventures with his fiancée Michaela.

Dedicated to the memories of

Marjorie A. Martin & Carolyn J. Witter

ACKNOWLEDGMENTS

Over the course of my graduate studies I have benefitted from the insights and assistance of many people. First, I would like to thank my PhD advisor Robert Raguso for leading by example and modeling for me what it is like to be a dedicated, enthusiastic, and honest scientist. Thank you for embracing my independence, while also having the wisdom to know when some guidance would be more beneficial. Most importantly, thank you for recognizing my humanity first and science second, and for always keeping my best interests in mind. Many thanks to my committee members Andre Kessler and Gavin Sacks for our stimulating conversations and helping me to create a logical path forward for a thesis that could easily have developed in a multitude of confusing ways. I would like to express my gratitude to Angela Douglas for graciously hosting me in her lab for the wet-lab portion of my work, and for our discussions that helped me to develop the logical framework for testing my hypotheses. I also owe my gratitude to the Doyle lab, especially Sue Sherman-Broyles. Thanks for the kindness and generosity over the years, and for tolerating my hundreds of flower samples crowding the -80 freezer. Thanks to Dan Buckley and lab, especially Roli Wilhelm, for the crucial help with microbiome analyses and results. I learned to be a true botanist from Karl Niklas and am fortunate to have taught for him for multiple years as a TA. Thank you to Jason Dombroskie for opening the doors of the Cornell Insect Collection to me and allowing me to work freely and also for helping with some particularly tricky insect IDs. Likewise to Patrick O'Grady for help with *Drosophila* identification. Thanks to Todd Bittner and the Cornell Botanic Gardens for allowing me to use Mundy Garden and the pawpaw patch for my annual spring field work. Thank you as well to Paul Cooper at the Liberty Hyde Bailey Conservatory for our hangouts/conversations when I was avoiding work in the greenhouse and also for fun

times during marathon Titan Arum events. I owe a lot to Kate Goodrich for her initial work in the pawpaw system which paved the way for me to develop these thesis questions. Thank you for your help, advice, and hospitality over the years. To all current and former members of the Raguso lab, thank you for your friendship and support during my time at Cornell. Special thanks to Kata Boroczky, Ajinkya Dahake and Sol Balbuena for being great friends and office mates, and also to Sol for electroantennography training and field assistance. To Mom, Dad, and Chelsea Martin. Thank you for embracing me as an individual and supporting me on whatever winding path I find myself exploring. Thanks as well to Jack Martin for the interest in my work and all the pertinent NYT science articles. Thanks to Mike Dignan for your interest and enthusiasm. To the entire Batstone family thank you for your encouragement and kindness over the years. Thank you to Sean Collinson and Jordan Parks for our many (maybe too many…) climbing and surf sessions that were welcomed respites from my dissertation writing. To my dearest friends Darryl Collins, Seger Dailey, Zach Haines, Mike Hoyle, and Chris Kearns. Thank you for your frequent visits to Ithaca, and for our many hangouts while I was home for vacations. I am lucky to have all of you in my corner. I love you all like brothers.

Finally, and most importantly, thank you to my fiancée and partner of 16 years Michaela Batstone. It's hard to express just how critical you were to my success and happiness during our time in Ithaca. Thank you for always having faith in my abilities when I was unsure of myself at all levels. Thank you for your endless encouragement and honest criticisms, which kept me grounded when the immensity of a PhD was overwhelming at times. Your tireless work ethic (two degrees, multiple jobs, and building a successful art business all while in Ithaca) set the bar unfathomably high and inspired me to be a better, harder-working scientist. For this and many other reasons I am eternally grateful that you are here with me. I love you very much.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1

APPENDIX

LIST OF TABLES

CHAPTER 1

APPENDIX

SUPPLEMENTARY DATA PACKAGE (.zip file)

CHAPTER 1

WHEN FLOWERS PLAY DEAD: MICROBES AS ARCHITECTS OF A 'DECEPTIVE' FLORAL PHENOTYPE

KYLE R. MARTIN[1,*], ROLAND C. WILHELM[2], CASSANDRA J. WATTENBURGER[2], GORDON C. YOUNKIN[1], SPENCER J. DEBENPORT[2], DANIEL H. BUCKLEY[2], ROBERT A. RAGUSO[3]

[1] Section of Plant Biology, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, USA

[2] Section of Soil and Crop Sciences, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, USA

[3] Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, USA

[*] Author for Correspondence: email krm243@cornell.edu

Author Contributions:
KRM and RAR conceived of and designed the study. Microbial sequencing was performed by CJW & SJD, Dr. Roland Wilhelm performed analyses of the microbiome data and wrote a portion of the microbiome results, all under guidance of DHB. GCY performed the pollinator survey data collection. Blind peak integration of volatile data was performed by Aryaman Saksena and RAR. KRM performed all other experiments and analyses. The manuscript was written by KRM. Photos of *Glischrochilus* beetles in figures 1, 12 & 13 appear courtesy of Chris Rorabaugh. The anyphenid spider photo in figure 1 appears courtesy of John Rosenfeld. Photos of dryomyzid flies were used under creative license from the Canadian Centre for DNA Barcoding.

INTRODUCTION

It is widely acknowledged that plant-pollinator interactions are a remarkable engine for biological innovation and diversification[1-6], and that floral phenotypes are famously complex[7-11]. However, it remains an open question as to how flowering plants produce floral signals that mimic distinctly non-floral substrates such as carrion, dung, or fermenting fruit. This phenotype, which is employed by many of the world's largest and most unusual flowers[12-14], is known as brood-site mimicry. Brood-site mimicry (BSM) is a form of dishonest floral advertisement in which floral form, color, and scent combine to mimic decaying organic substrates chosen by insects for mating or egg laying[15]. BSM is unique among floral mimicry systems (in contrast to food or sexual mimicry) in that the models are typically dead or inanimate, with characteristically putrid scents that result from microbial metabolism and decay[16-19]. An emerging question in pollination ecology is whether third-party organisms such as microbes can interfere with or (alternatively) synergize plant-pollinator interactions in nature. Given the obvious microbial connotation in BSM chemical space, it is logical to ask whether flower-inhabiting microorganisms contribute to floral phenotype and, by extension, plant fitness in BSM flowers.

It is by now well established that flowers are rich habitats for microbes[20,21]. However, until now studies have largely considered microbes in flowers as competitors for floral rewards like nectar, or organisms that may modify the behavior of previously established pollinators in conventional nectar markets. For example, it has been shown that increased yeast density (*Metschnikowia reukaufii*) in *Delphinium nuttallianum* nectar enhances pollen export and nectar removal by bumblebees[22]. Likewise, presence of yeasts in *Helleborus foetidus* nectar also

increases nectar consumption by bumblebees but, interestingly, at the cost of reduced plant fecundity[23]. The dynamics of microbial community ecology within nectar can also affect plant-pollinator mutualisms. In several cases it has been shown that bacteria, but not yeast, reduce nectar consumption by pollinators in both natural[24] and artificial[25] experimental conditions, which raises questions about how flowers may manipulate or influence their own microbiome to enhance pollination. Not surprisingly, it has also been shown in multiple instances that microorganisms in floral nectar can have a modest impact on floral scent[26,27], a phenomenon that is expected to be pervasive given the ubiquity of microbes in nectar-rewarding flowers[28,29].

Despite rapid growth in such studies, there has been a surprising lack of recognition for the possibility that microbial symbionts that reside on or within flowers might fundamentally shape floral phenotype and pollinator niche, for example, by driving a shift to a novel pollinator class. The suite of floral traits belonging to the generalized, nectar-producing flowers in the studies cited above do not evoke hypotheses of extended microbial phenotypes, as their colors, shapes, and (mostly) scents are not profoundly impacted by microbial residence. Alternatively, in each diverse example of BSM, the putrid (to the human nose) volatiles produced by the flowers are more typically associated with the chemical signatures produced by the microbial decomposition of organic matter[30,31]. The cognitive misclassification of these signals by pollinators (a hallmark of successful floral mimicry strategies) as reliable indicators of egg laying or mating sites[16], as well as their microbial connotation, merits further exploration into the source (plant, microbe, or both) of these chemical signals in each of the thousands of species of BSM flowers worldwide.

One current challenge in the study of BSM is that common features of many brood-site mimicking flowers, such as floral gigantism[13], remote habitats[32,33], parasitic habits[34], or long

generation times[35], tend to preclude manipulative experimental approaches. In contrast to these challenges, we present a study system that is amenable to controlled experiments based on the yeast-scented flowers of the North American pawpaw tree, *Asimina triloba* (Annonaceae). Pawpaw is native and locally abundant within the eastern United States, grows in large clonal thickets that produce thousands of small brood-site mimicking flowers in a single season[36,37], has a sequenced genome in progress, and has the potential for long term investigation across microbial/chemical, genetic, and geographic scales.

Although their floral display is more modest than the flagship examples of BSM mentioned above, pawpaw flowers exhibit an unusual and fascinating ecology (**Figure 1**). Like many Annonaceous flowers, pawpaws are protogynous, and show marked changes in floral form and scent during floral ontogeny[38,39]. Flowers open before leaf emergence, displaying immature green petals that appear odorless to the human nose, but emit background levels of sesquiterpene hydrocarbons typical of non-floral vegetation[38]. As the flowers progress through their development, the petals accumulate a maroon or wine-red pigmentation that coincides with the emission of short chain aliphatic acids, alcohols, and esters which results in a brood-site mimicking floral scent that is strongly reminiscent of fermenting fruit or yeasty bread dough[38]. As the fermentative scent is emitted, the most common visitors become drosophilid flies and several species of sap beetles of the genus *Glischrochilus* (Nitidulidae), both of which are more commonly associated with and attracted to fermenting substrates[40-42], not flowers. Perhaps the most interesting aspect of pawpaw floral display are the three inner petals that form a cup-shaped chamber around the androgynoecium. Remarkably, the nectariferous tissue at the base of these inner petals progresses through a pronounced (but apparently benign) microbial decomposition during the lifetime of the fully functional flower, a feature that is exceptionally rare in

Angiosperms (but see Sakai et al. 2000)[43] and points to the potential for an extended floral phenotype of microbial origin.

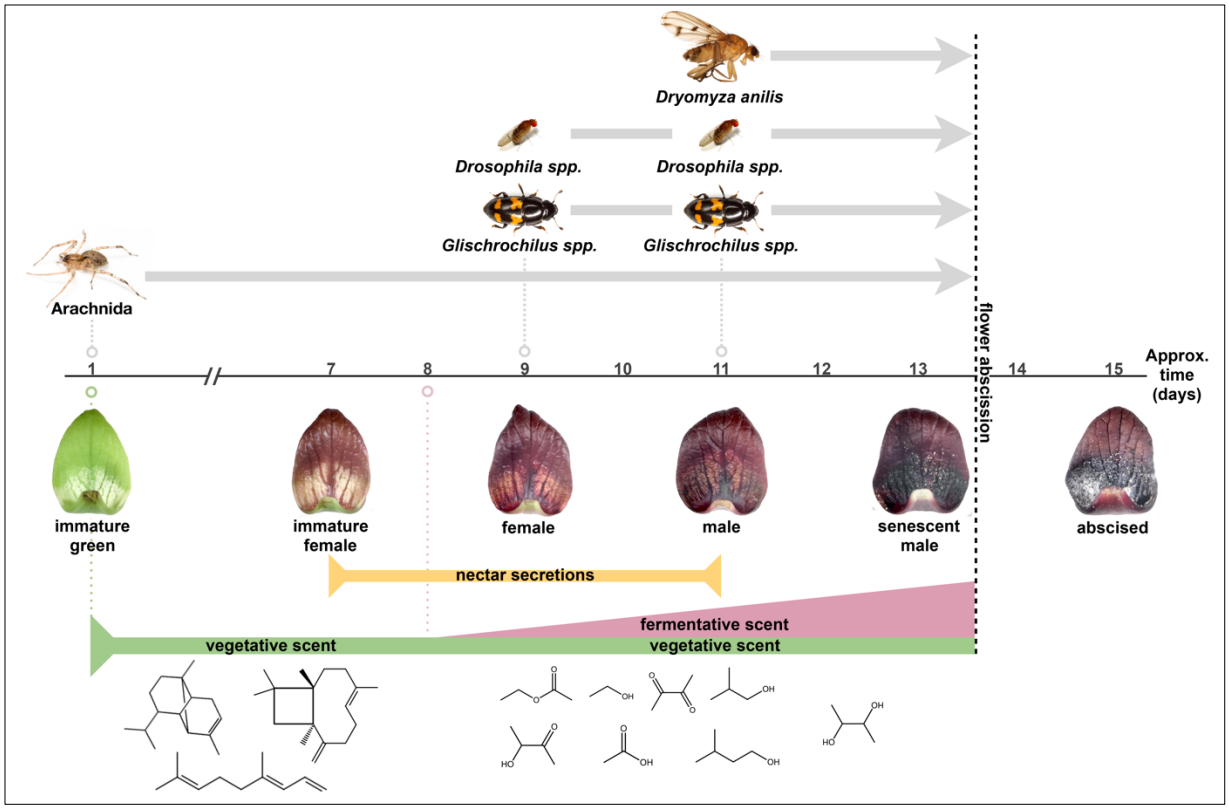

Figure 1. A timeline of ecological processes and interactions in *A. triloba* flowers over the course of floral ontogeny. Photographs of *A. triloba* inner petals show a gradual decomposition of the nectary tissue over the functional lifetime of the protogynous flowers. In green stage inner petals, nectaries are immature and appear white in color. Immature female inner petals develop anthocyanin pigmentation over several days; nectar begins to be secreted from the white tissues at the end of this stage coinciding with initial stigma receptivity. Female stage flowers begin to show a discoloration of the nectaries indicating the beginning of nectary decay. Advancing decay of the nectaries appears brown in male stage inner petals; nectar secretion has stopped but trace amounts may remain as a sheen on the nectary surface. Senescent male inner petals show advanced decay with blackened nectaries; flowers abscise from the tree at this stage. Fungal hyphae begin to emerge from abscised inner petal nectaries 24-48 hours post abscission. Floral scent chemistry begins in bud stage flowers with the emission of monoterpene and sesquiterpene (C10-C15) hydrocarbons typical of plant vegetation or immature floral tissue and continues throughout floral ontogeny until flower abscission. Short chain (C2-C4) alcohols, acids, and esters typical of microbial fermentation products begin to appear after nectar secretion and between immature and mature female stages. Emission of fermentative volatiles increases in quantity, especially from inner petals, as flowers mature. Arthropod visitation is initiated early in floral development as spiders (primarily Anyphaenidae, Thomisidae) take shelter inside immature green flowers. *Glischrochilus* beetles and drosophilid flies appear during both the female and male stages; beetles typically feed from the decaying nectariferous tissues of the inner petals or mate within the cup-shaped open chamber formed around the androgynoecium. Dryomyzid flies appear late in floral ontogeny and have only been observed visiting or mating in male and later stage flowers. Only the top three most abundant insect visitor guilds have been shown for clarity.

Previous chemical analyses of *Asimina triloba* floral scent[38,39], combined with our ongoing natural history observations concerning floral biology and insect visitation in the system, underscore the need for a coordinated study of the pawpaw floral microbiome, the microbial contribution to *A. triloba* floral phenotype, and the effect of *A. triloba* floral microbes on pollinator attraction and behavior. Therefore, in this study we have adopted the conceptual principles set forth by Douglas and Dobson[44] to test the hypothesis of microbial-mediated chemical communication between brood-site mimicking flowers and their dipteran and coleopteran pollinators (**Figure 2**; 'microbial signaling hypothesis'). In this light, we have modeled our experimental design with inspiration from Koch's postulates to examine three lines of evidence required to demonstrate this effect: 1) Are microbes present on *A. triloba* flowers and are they metabolically capable of producing the volatile chemicals that comprise the fermentative volatile signature of *A. triloba* floral scent? 2) Does the elimination or reduction of floral microbes result in an elimination or reduction of fermentative floral volatiles, and does this have a simultaneous effect on pollinator attraction or behavior? 3) Does reintroduction of microbes or microbial volatiles to microbe-free (or microbe-reduced) *A. triloba* flowers restore pollinator attraction or behavior?

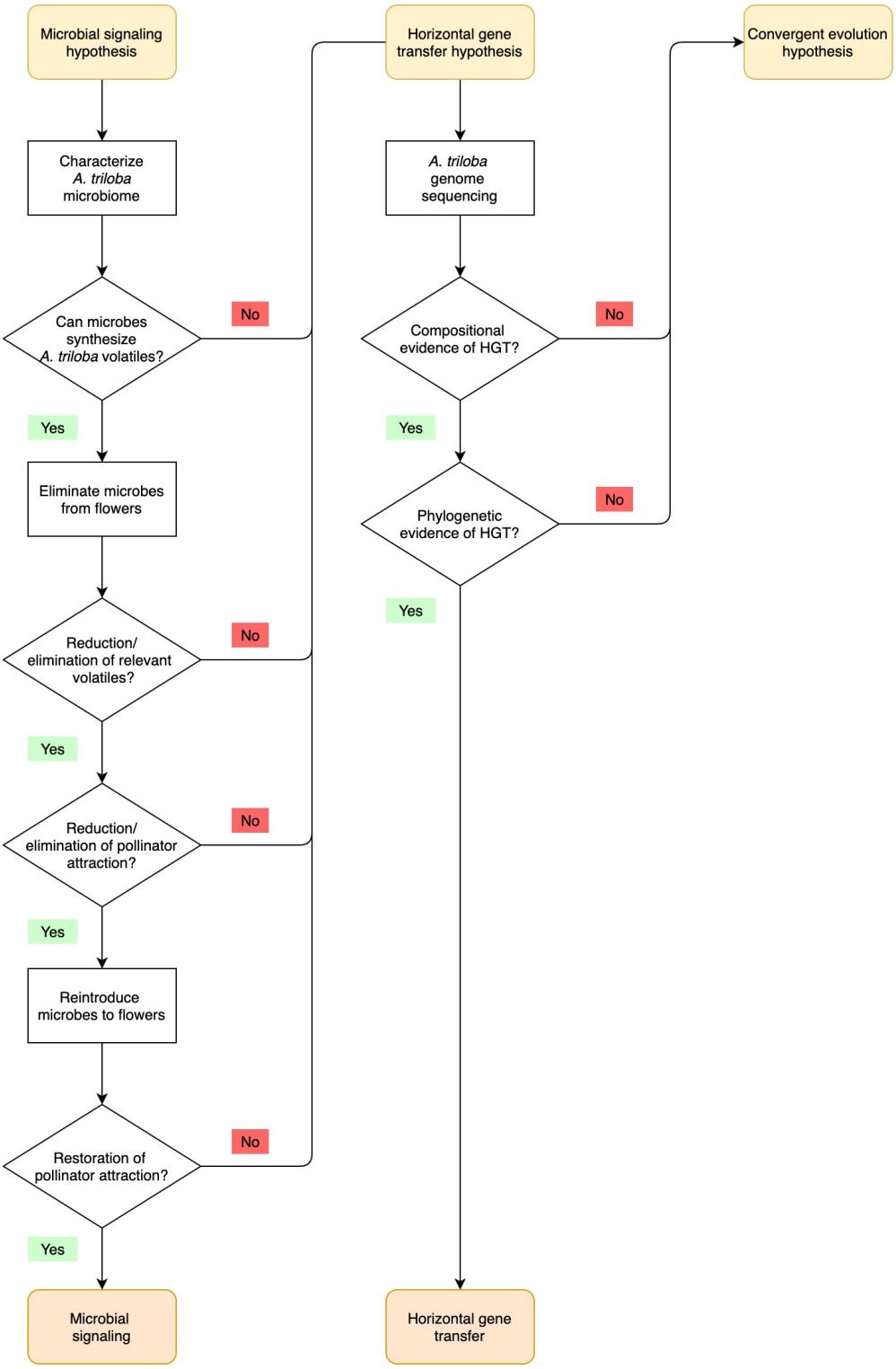

Figure 2. A flow chart showing the logical sequence of experiments to perform to support or reject our stated hypotheses. The microbial signaling hypothesis takes influence from Koch's postulates and is a modification of Douglas and Dobson's[44] criteria for testing the hypothesis of microbial-mediated chemical communication in animals.

To answer these questions, we examined the microbial community of *A. triloba* flowers across spatial and temporal scales using high throughput 16S rRNA and ITS1 gene amplicon sequencing and, using gas chromatography – mass spectrometry (GC-MS), tested whether cultured *A. triloba* floral microbes alone were sufficient to produce the fermentation volatiles present in *A. triloba* floral scent. We also subjected flowers to topical applications of conventional broad-spectrum sterilants and compared their microbiome and floral headspace to the microbiome and headspace of sham-sterilized flowers using 16S/ITS amplicon sequencing and GC-MS over three successional field seasons. In addition, we prevented *A. triloba* floral visitation using micromesh pollination bags and tested whether arthropod visitors (potential microbial vectors) affected the microbiome and floral scent of *A. triloba* flowers by comparing the microbiome and headspace of bagged and sham-bagged flowers using the same sequencing and GC-MS methods over the same three field seasons. Finally, through electroantennographic (EAG) examination of pollinator sensory capabilities as well as trap-bioassays in the field, we assessed whether microbial volatiles were detected by pollinators and whether microbial volatiles contributed ultimately to pollinator attraction.

METHODS

Floral Sterilization

In the months of May and June, during three consecutive *A. triloba* flowering seasons (2016, 2017, 2018), we conducted a floral sterilization experiment designed to test whether epiphytic microbes on *A. triloba* flowers were a potential source of fermentation volatiles emitted from female and male stage *A. triloba* flowers. Experiments were conducted on a large clonal stand of naturalized *A. triloba* trees located within a 3.5-hectare riparian woodland known as the Mundy Wildflower Garden on the Cornell University campus (Ithaca, NY, USA; 42.451505, -76.469494). At 08:00 h on each morning of the assay (conducted over two consecutive days; n=16 for all treatments), 8 female stage and 8 male stage flowers from the Mundy Garden *A. triloba* stand were chosen at random and were subjected to a sterilization procedure that consisted of a Natamycin (Natamax SF, Dupont, St. Louis, MO) spray (50 ppm aqueous solution, left for 5 minutes), followed by a spray of bleach (10% aqueous [0.825% hypochlorite], left for 2 minutes), and finished with a sterile DI H_2O rinse to remove traces of each sterilant. Eight more flowers of each sex were chosen at random for sham-sterilization which consisted of the same procedure above except that the sterilant sprays were targeted to the pedicel and abaxial surface of the sepals only to control for the potential physiological effects of each sterilant on the flowers, while simultaneously maintaining an unaffected microbiome on all other floral parts. Sham-sterilized flowers were then sprayed on all surfaces with sterile DI H_2O as above to control for any possible effects of water spray on floral volatile emissions as well as the floral microbiome. After a four-hour drying period, treated flowers were harvested into 30 ml sterile glass headspace vials and transported immediately to the lab for volatile collection (see volatile

collection/GC-MS procedures below). After volatile collection, individual flowers were transferred into sterile polypropylene tubes, weighed, flash frozen in liquid nitrogen, and stored at -80°C until further processing (DNA extraction for culture-independent microbiome analyses).

Floral Visitor Exclusion

During the same flowering seasons as the sterilization experiments, we conducted a floral visitor exclusion experiment designed to test whether microbes vectored to *A. triloba* flowers via floral visitors were a potential source of fermentation volatiles emitted from female and male stage *A. triloba* flowers. During late April of each year before floral bud break, ends of *A. triloba* branches bearing uninitiated buds were subjected to a bleach spray (10% aqueous [0.825% hypochlorite], left for 2 minutes) followed by a sterile DI H_2O rinse to remove traces of bleach. Immediately afterwards, sterilized (95% ethanol, dried) polyester micromesh pollination bags (48 cm x 30 cm, 250 μm mesh size) were placed over the ends of 16 randomly selected branches containing up to eight buds per bag. These bags were secured with zip ties to prevent any floral visitor from vectoring microbes to flowers while allowing flowers to experience abiotic microbial vectors such as rain, dust, and wind (**Figure S1**). Sixteen more branches were chosen at random for sham-exclusion, which consisted of the same procedure above except that each bag in the sham-exclusion treatment had a 15 cm diameter hole cut into it, which controlled for the effect of the bag on the flowers but allowed floral visitors (biotic microbial vectors) to reach the flowers (**Figure S1**). Flower buds were left to mature in the bags for approximately one month and were harvested for volatile collection as the flowers reached sexual maturity. Male and female flowers were harvested daily as they matured and were placed into sterile 30 ml glass headspace vials and transported immediately to the lab for volatile collection (see volatile

collection/GC-MS procedures below). After volatile collection, individual flowers were processed identically as above and stored at -80°C until microbiome analyses.

Floral Microbe Culture Plating

Floral tissues collected for culture-dependent microbiome and volatile analyses were obtained from the same location during the *A. triloba* flowering season in 2016 and 2017. Five whole flowers of each ontogenic stage (immature, female, male) were collected randomly from within the *A. triloba* stand into 30 ml sterile glass jars. Fresh floral tissue was transported immediately to the lab, individually swabbed and plated for culture-dependent microbiome and volatile analysis as follows: under sterile conditions in a laminar flow hood individual flowers were swabbed with a sterile cotton swab dipped in sterile phosphate buffered saline (PBS). The swabbing procedure consisted of five swabs of uniform pressure on the adaxial and abaxial surfaces of each inner and outer whorl petal for one flower. Once swabbed, the acquired microbial cells from one flower were streaked onto three different types of agar media plates (100mm x 15mm; TSA, MRS, YM; BD Difco, Franklin Lakes, NJ, USA) and incubated at room temperature for 96 hours. This procedure was repeated for all five replicate flowers of each ontogenic stage, resulting in 45 experimental and 3 control plates swabbed. Control plates were swabbed with sterile PBS only and showed no microbial growth. After 96 hours of growth, volatiles were collected from all control and experimental Petri dishes (see volatile collection/GC-MS procedures below), and a glycerol stock of each plate was immediately prepared by pipetting 1.5 ml of matching liquid medium onto the resultant microbial lawn, mixing the liquid medium and microbial colonies into a slurry, and transferring the mixture to a polypropylene cryotube containing 1 ml of a 50% aqueous glycerol solution to prepare a 2.5 ml

glycerol stock with a final glycerol concentration of 20%. Glycerol stocks were kept at -80°C until DNA was extracted from each stock for microbiome analyses.

Volatile Collection and GC-MS Analysis

Volatiles for all sample types were collected using micro thermal adsorbent traps constructed from cut glass microcapillary tubes (1.80 mm OD, 1.41 mm ID, 29 mm long; Drummond 200 µl,) filled with 5 mg of 60/80 mesh Tenax TA adsorbent (Supelco, Inc.) packed between plugs of silanized quartz wool (Restek, Inc.). Tenax traps were hand constructed, cleaned with methanol, oven dried at 50ºC, and desorbed at 200 ºC with a flow of helium gas for ~ 10 min before use. VOC headspace samples were collected individually using a 9V battery-operated PAS-500 vacuum pump (Spectrex, Inc., Redwood City, CA) connected to an adsorbent trap with glass Pasteur pipette and silicone reduction tubing as an adapter, with flow rates standardized to 250 ml air/min each day using a bubble flow meter (Gilmont Instruments, Barnant Company, Barrington, IL). Headspace chambers for sterilized and excluded flowers were created by sealing one whole cut flower in a 30 ml sterilized glass headspace vial with a polyester oven bag (Toppits, Minden, Germany) cap. The micro-adsorbent trap was placed 1 cm into the headspace chamber through a small hole in the oven bag cap. Headspace chambers for culture plates were created by placing an individual Petri dish (with lid attached) into a 13 cm x 13 cm oven bag. The volatile collection apparatus (micro-adsorbent trap and Pasteur pipette) was inserted into the oven bag through a 1 cm slit, and the micro-adsorbent trap was placed 1mm inside the Petri dish resting on the dish edge and covered by the dish lid. Volatile collection was initiated without equilibration for all samples and volatiles were collected for 10 min, placed into labeled 1.5 ml phenolic-capped amber glass autoinjector vials (National Scientific, Inc., Claremont, CA) and

stored at room temperature until GC-MS analysis. Volatiles were desorbed from the Tenax traps using ballistic heating (from 40 to 200°C at 20°C/sec), through the Optic 3 external flow control system and desorbed volatiles were loaded onto a polar GC column (Stabilwax-MS [polyethylene glycol], 30 m, 0.25 mm ID, 0.25 m film thickness; Restek, Inc., Bellefonte, PA) using ultra-pure helium gas (Airgas, Inc.; 99.99% pure) as a mobile phase during a 30 sec splitless injection, thereafter maintaining a constant column flow of 1 ml/min at a 20:1 split ratio. The GC oven temperature program increased from 40°C (3 min. hold) at 10°C/min. until reaching 240°C, held for 5 min. The GC was coupled to an electron-impact quadrupole MS (70 eV; 3 scans/sec. from m/z 40–350 daltons; threshold: 500). Authentic n-alkane standards (C9-C30) were injected under the same chromatographic conditions to convert volatile retention times to Kovats retention indices (KI). GC separation of volatile samples was visualized as a Total Ion Current (TIC) chromatogram for each injected sample, in which well-defined peaks were hand-integrated double-blind using GC-MS Solutions 1.2a software (Shimadzu Scientific Instruments, Inc.). Control sample chromatograms collected from un-inoculated growth medium or empty headspace vials were subtracted from true samples to avoid inclusion of background volatiles. Identification of volatile compounds was accomplished by co-injection with authentic standards and by comparison with mass spectral libraries and published chromatographic data available on the NIST Chemistry WebBook (https://webbook.nist.gov/) and websites provided by PubChem (https://pubchem.ncbi.nlm.nih.gov/) and PheroBase (www.pherobase.com/).

DNA Extraction

Plant-associated microbial DNA was extracted under sterile conditions in a laminar flow hood using the MoBio Power Plant Pro DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) according to the included protocol with the following modifications: (1) as a sample preparation step before DNA extraction, frozen plant tissue samples were forcibly shaken by hand within their respective polypropylene tubes to produce contamination-free, pulverized tissue (2) the included "phenolic separating solution" was used according to the manufacturer's suggestion due to the high anthocyanin content of *A. triloba* flowers and (3) tissue homogenization was performed on 50 mg pulverized plant tissue using a FastPrep-24 tissue homogenizer (MP Biomedicals, Solon, OH, USA) at 4 m/s for 1 min using 0.5 g of 0.1 mm diameter glass beads (BioSpec Products, Inc., Bartlesville, OK, USA) instead of the stainless steel beads included with the MoBio kit. This substitution was made because our optimization procedures consistently showed that 0.1 mm glass beads were more effective than 2 mm stainless steel beads for microbial cell lysis. Identical extraction procedures were performed as above for all sample types except that 175 µl of microbial glycerol stock or whole insects were used as starting material for tissue homogenization instead of 50 mg of plant tissue for those respective sample types. "Phenolic separating solution" was used in the extraction for all sample types to maintain consistent experimental conditions. Isolated DNA was suspended in 50 µl of solution "PD7" and stored at -20°C prior to downstream applications.

Microbiome Sequencing & Analysis

Total extracted DNA was quantified using a Quant-iT PicoGreen dsDNA Assay kit (Thermo Fisher) and a multimode microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Polymerase chain reaction (PCR) amplification was used to target bacterial (V4 region of 16S rRNA gene) and fungal communities (ITS1 hypervariable region) using universal primer sets 515f/806r and nBITS2f/58A2r, using the primers and barcode schemes described in [45] and [46]. PCR was performed in duplicate using 25 µl reaction volumes, consisting of 13.5 µl of Q5 Hot Start High Fidelity 2X Taq polymerase (New England Biolabs, Ipswich, MA, USA), 1.25 µl of each primer (10 µM) and DNA template (2 ng · μl^{-1}). The thermocycler conditions for the 515f/806r primers were as follows: 98 °C hold for 2 min, followed by 29 cycles of [30 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C], with a final extension for 5 min at 72 °C. The thermocycler conditions for the nBITS2f/58A2r primers were as follows: 98 °C hold for 2 min, followed by 29 cycles of [30 s at 95 °C, 30 s at 50 °C and 30 s at 72 °C], with a final extension for 5 min at 72 °C. Duplicates were pooled and samples were normalized using the SequalPrep normalization kit (Invitrogen) as specified by the manufacturer. Samples were then pooled into libraries and gel excision was performed using the Wizard SV Gel and PCR Clean-up System (Promega, Fitchburg, WI, USA) to isolate the 400-500 bp band region from each library. The multiplexed 16S and ITS amplicon libraries were sequenced using the Illumina Miseq platform at the Cornell Biotechnology Resource Center (Ithaca, NY, USA).

Paired-end reads of each amplicon library were merged using PEAR[47] using default settings. Merged reads were then demultiplexed using a custom script. Next, merged read quality filtering was performed using USEARCH[48] to discard reads that exceeded a maximum expected error of one. Mothur[49] referencing the SILVA SEED database (v. 132)[50] was used to perform alignment-based quality filtering on the 16S reads. Reads containing ambiguous base call "N" characters and homopolymers greater than 8 bp were discarded from 16S and ITS libraries. For ITS reads, the ITSx tool[51] was used to remove non-ITS portions of each read to improve later

taxonomic classification. All ITS reads were then set back to equal length by adding ambiguous base calls "N" to the end of each sequence to correct the effect of variable lengths on OTU clustering[52]. Pre-clustering to 98% sequence similarity was also performed on ITS reads to prior to final OTU clustering[52]. Chimeric sequences were also removed and OTUs were then binned at 97% sequence similarity. Taxonomy was assigned with VSEARCH[53] using SILVA SEED database (v. 132) and UNITE utax reference database (v. 8.2)[54] for 16S and ITS reads respectively. Sequences classified as eukaryotic (non-fungal), mitochondrial, and chloroplast were discarded. Sequencing of 16S rRNA gene amplicons produced a total of 1,593,841 reads after processing, which were assigned to 2,020 OTUs. A total of 11,692,030 ITS amplicon reads were obtained after processing, which were assigned to 5,202 OTUs.

Large proportions of amplicon sequencing libraries were comprised of plant mitochondrial or chloroplast rRNA, which were discarded prior to analyses. PCR blanks and a control swab ('sterilization.pbs.swab.ctrl', "sterilizat.pbs.swab.ctrl","Exc.pbs.swab.ctrl", "St.exp.ctrl" and "Exc.exp.ctrl.2016") were used to identify potential contaminants, which were retained in analyses, but denoted as potential contaminants. We chose not to discard OTUs also found in control samples because these could result from index hopping between samples during the sequencing process[55,56].

Microbiome composition was assessed in parallel using presence/absence (binary) and relative abundance (count) data. The count data was sparsity filtered by removing OTUs present in fewer than three samples, or at a total relative abundance < 0.1% of the whole dataset (not just an individual library). Counts were normalized to library size and reported as counts per thousand reads. Associations between OTUs and volatile production were determined in three ways: (i) a positive Pearson correlation between relative abundance and volatile production (p_{adj}

< 0.05), (ii) a significantly higher incidence in samples where production of volatiles (total or individual) was high (vs. low) according to the Chi-squared or Fisher's exact test, or (iii) any OTU which occupied greater than 95% of reads in a sample associated with high volatile production. Volatile production was divided into tertiles to designate 'high' (top tertile) versus 'low' production (bottom tertile). The abundance of volatiles was normalized to flower mass. The Chi-squared and Fisher's exact test were used to determine significant associations, the latter was used when any variable was observed fewer than five times[57]. All three methods were used to identify OTUs associated with volatile production in cultures, while only method (ii) was used to identify associated OTUs *in vivo*, due to the low read depth in these libraries (compositional biases too pronounced) and no OTU occupied > 95% of any *in vivo* library. Method (ii) was also used to identify OTUs associated with sham-sterilized vs. sterilized flowers and sham-bagged vs. bagged flowers, using experimental treatments in place of 'high' vs. 'low' volatile production. Fungi were assigned to ecological guilds with FUNGuild (v.1.1)[58].

OTUs associated with different flower stages (immature, female and male) were identified by indicator species analyses using the *indicspecies* package in R (v. 1.7.9)[59]. Indicator species analyses was performed on aggregated data across years and from whole flower tissue. Indicator OTUs exclusive to immature flowers were excluded from the volatile associated set (n_{ITS} = 9, n_{16S} = 1). Bacterial functional gene content was inferred using PICRUSt2 (v. 2.3.0)[60], targeting fourteen fermentative pathways present in metaCyc[61]. The unweighted 'genome_function_count' (the predicted copy number of a pathway per taxon) was compared between volatile-associated OTUs (n = 30) and OTUs indicative of immature flowers (n = 55) using the Wilcoxon test. Indicators of immature flowers were chosen since these taxa are not expected to be involved in fermentation volatile production. A cross-domain co-occurrence

network was constructed for volatile-associated OTUs using *in vivo* presence/absence data, excluding leaf, sterilized and bagged samples. The network graph was inferred using inverse covariance selection and sparsity corrected using Stability Approach to Regularization Selection[62] using the R package *spiece-easi*[63]. Network properties were calculated using the R package *igraph*[64].

Electroantennography

Insects

Sap beetles (*Glischrochilus fasciatus*; Nitidulidae) were obtained from a 3.5-hectare riparian woodland known as the Mundy Wildflower Garden on Cornell University campus (Ithaca, NY, USA; 42.451505, -76.469494) between July and September 2018. Male and female beetles of unknown ages were collected using makeshift cup/funnel traps baited with apple puree fermented with baker's yeast (*Saccharomyces cerevisiae*: Fleischmann's, Memphis, TN) and sexed using elytron apex shape as a criterion according to Bousquet (1990)[65]. Once collected, 10-20 beetles of mixed sex were reared together in separate 900 ml polypropylene boxes containing approximately 15 g of artificial tomato-prune diet[66] and a moist paper towel for oviposition. Beetles were maintained under a 16 h:8 h L:D photoperiod, at 23 °C, and 50% - 60% relative humidity. Individuals remained in the colony for breeding until their use in electroantennographic experiments, after which morphometric data were collected, and individual voucher specimens of each participant (n=100) were deposited into the Cornell University Insect Collection (CUIC voucher #1281).

Chemicals

Ten synthetic compounds used for electroantennography were chosen to reflect the major compounds detected in the headspace of *A. triloba* flowers: ethyl acetate (CAS# 141-78-6, Fisher Chemical, 99.9%), ethanol (64-17-5, Decon Labs Inc., 100%), 2,3-butanedione (431-03-8, Sigma-Aldrich, ≥ 99%), isobutyl alcohol (78-83-1, Sigma-Aldrich. ≥ 99.8%), 3-methyl-1-butanol (123-51-3, Aldrich, 98%), acetoin (= 3-hydroxy-2-butanone; 513-86-0, Tokyo Chemical Industry, mixture of stereoisomers, ≥ 95%), acetic acid (64-19-7, Mallinckrodt Baker Inc., ≥ 99.7%), 2,3-butanediol (513-85-9, EMD Millipore, mixture of stereoisomers, ≥ 98%), linalool (78-70-6, Aldrich, mixture of stereoisomers, 97%), and (*E*)-β-caryophyllene (87-44-5, Sigma, 98.6%). All synthetic compounds were prepared as 1 M concentrated stock solutions in 1:1 hexane:acetone, from which three ten-fold serial dilutions (0.1 M, 0.01 M, 0.001 M) were prepared.

Electrophysiology

Antennal responses from male and female *G. fasciatus* beetles to synthetic *A. triloba* volatiles were tested via electroantennography (EAG) using an IDAC-232 serial acquisition controller (Syntech, Hilversum, The Netherlands). For each EAG recording, the right antenna of the beetle was excised at the basal antennal segment (scape), and immediately placed onto a gel-prepared two-pronged fork electrode, with terminal and basal segments of the antenna inserted slightly into the electrode gel (Spectra 360, Parker Inc., Fairfield, NJ), and oriented dorsal side up with the anterior surface facing the stimulus (**Figure S2**). Once mounted to the electrode, the antenna was placed 1 mm into the opening of the air delivery tube and left for 10 minutes in the continuous 100 ml/min humidified air stream to stabilize before recording. Odor stimuli

cartridges were prepared by inserting 4 mm x 20 mm filter paper (Whatman #1) strips loaded with 1 µl of odor solution into standard size glass Pasteur pipettes capped with paraffin film. Loaded strips were held in open air for 15 seconds to ensure solvent evaporation before inserting into pipettes. Eight stimuli cartridges were prepared per antennal preparation: one paper strip negative control (= air control), one solvent negative control (= solvent control), two 1 M ethyl acetate positive controls (= EA positive control 1 and 2), and four test stimuli cartridges containing one test compound at one of four concentrations (1 M, 0.1 M, 0.01 M, 0.001 M). Cartridges were connected to the stimulus controller (Syntech CS-05) via PTFE tubing, and odor was delivered to the antenna by inserting the tip of the stimulus cartridge into the air delivery tube and puffing air through the cartridge (30 ml/min; pulse duration 0.5 s), via foot switch, into the air stream directed toward the antenna. Ten synthetic compounds were tested on both male and female antennae (each n=5; 100 total beetles). Each test compound was presented in the following order for each antennal preparation: air control, solvent control, 1 M EA positive control 1, followed by a randomly ordered dilution series of one test compound (1 M, 0.1 M, 0.01 M, 0.001 M), and concluded with 1 M EA positive control 2 to monitor antennal sensitivity over the course of each experiment. A 2-minute rest period was given between each stimulus puff to allow the antenna to stabilize, and each antennal preparation was used for no longer than 35 minutes. All EAG experiments were conducted between 11:00 h – 17:00 h from July – September 2018 to coincide with our observations of beetle activity in the field. EAG signals were measured as the change in electrical potential (-mV) between the proximal and distal ends of a single antenna. Data were processed using EAGPro software version 1.0 (Syntech).

Statistical Analyses

We tested *Glischrochilus fasciatus* antennal activity (uncorrected -mV) in response to serially diluted doses of ten volatile chemical stimuli using a linear mixed-effect model (LMM) built with the package 'lme4'[67] in the R statistical computing environment[68]. The model was constructed using natural log transformed -mV as a response variable to satisfy statistical assumptions, with 'volatile chemical', 'chemical concentration', 'sex of beetle', and their interactions as a fixed effect and 'individual beetle' as a random effect. When significant interactions were identified via the LMM, *post hoc* pairwise comparisons were explored within those significant interactions using estimated marginal means (package 'emmeans')[69] on back-transformed -mV values from the natural log scale, and using Tukey's method for *p*-value adjustment according to alpha = 0.05 as a significance threshold. The inclusion of sex as a fixed predictor was justified by our observation of sexual dimorphism in antennal size between sexes, as well as our hypothesis that male and female beetles should respond differently to volatile cues that may be used by females to locate suitable oviposition sites. We also used simple linear regressions (one for each sex) test whether 'days in captivity', 'date of EAG recording', or 'beetle collection date' were significant predictors of the magnitude of antennal response to a 1M ethyl acetate reference stimulus, with the null hypothesis being that these variables associated with beetle rearing do not have an effect on the magnitude of antennal response.

Morphometric Measurements & Statistical Analyses

After each EAG recording with the right antenna, the left antenna of each beetle was taken for antennal length and surface area measurements to correlate with the magnitude of voltage deflection in response to the first ethyl acetate reference stimulus. Measurements were made

from close-up digital photographs of the antennae taken through a dissecting microscope using Fiji software (v2.0)[70], an open source image processing package based on ImageJ[71]. Because *G. fasciatus* beetle antennae are microscopic and planar in morphology, only the dorsal planar (2D) surface area was calculated, and that value was multiplied by 2 to obtain an approximation of total surface area. Differences in the length and planar surface area between male and female antennae were compared using Welch's two-sample t-tests to account for the unequal variances found in the otherwise normally-distributed morphometric data, with the null hypothesis being that male and female beetle antennae do not differ significantly in these measurements. Simple linear regressions were also calculated to test whether antennal length or surface area were significant predictors of the magnitude of antennal response to a 1M ethyl acetate reference stimulus.

Visitor Survey

Asimina triloba visitors

We conducted daily observations of arthropod visitors to *A. triloba* flowers from May 21st to June 7th, 2019 at the Mundy Wildflower Garden pawpaw stand on Cornell University campus (Ithaca, NY, USA; 42.451505, -76.469494). The survey spanned the entirety of the *A. triloba* flowering season; flowers on May 21st were mostly immature, whereas only a few senescent flowers remained in the canopy by June 7th. Each day, surveys were conducted at 09:00 h, 14:00 h, and 19:00 h to ensure observation of visitors with different activity periods. At the beginning of the survey, we labeled 12 individual *A. triloba* trees along a transect and visited these same trees throughout the entire survey. For each tree, we observed floral visitors on up to 10 immature, 10 female, and 10 male flowers. At each time period, we recorded the number of

observed flowers per tree at each ontogenetic stage, the type of visitor taxa based on field identification and the total number of each visitor type. In order to access flowers above arms reach, we were required to bend the trunk towards the ground, which may have disturbed more sensitive visitors. When possible, a photograph or video was taken of the visitor using an iPhone 7 equipped with a 15X macro lens (Aukey Ora; Los Angeles, CA). Voucher specimens were collected whenever possible and were deposited into the Cornell University Insect Collection under CUIC voucher #1281. Floral visitors were identified to at least the family level when vouchers or high-quality photos were collected. When collections or photos in the field were not possible, identifications were made with reference to a previously collected voucher specimen or photo if available or ascribed to the lowest taxonomic rank possible by sight without reference.

Glischrochilus Beetle Egg Survey

All beetles of the genus *Glischrochilus* (Nitidulidae) found on *A. triloba* flowers during the course of the pollinator survey were collected alive, stored, and observed daily for the presence of laid eggs to assess whether female *Glischrochilus* beetles arrived gravid at *A. triloba* flowers. Females of *G. fasciatus* and *G. sanguinolentus,* and all individuals of *G. quadrisignatus* (impossible to sex without dissection) were stored individually in 60 ml (2 ounce) polypropylene cups containing artificial diet and a piece of moist paper towel for an oviposition substrate. When oviposition occurred, eggs were transferred to a new cup containing artificial diet only to monitor larval hatching as a measure of egg viability.

Insect Trap Bioassays

Study Site

During peak *A. triloba* flowering season in late May to early June 2019, we conducted a series of three-choice bioassays using baited cup traps (**Figure S3**) in the field to test whether floral microbial volatiles affected pawpaw flower arthropod visitation. Bioassays were conducted along a 120 m transect within a wetland/mesic woodland corridor on the eastern shore of the Beebe Lake Natural Area on Cornell University campus (Ithaca, NY, USA; 42.450469, -76.473807). The bioassay site was located approximately 350 m west of the pawpaw stand in the Mundy Wildflower Garden (42.451505, -76.469494) where all flowers from this study were sourced (**Figure S4A**). This site was chosen to reflect a microhabitat similar to the riparian environment experienced by pawpaw flowers and floral visitors along Fall Creek in Mundy Garden, and also to prevent competition for visitors between this bioassay and our pawpaw pollinator survey, conducted in parallel at the Mundy Garden location.

Assay Design

Each day, 15 cup traps were placed in a five by three array (five sites, three treatments at each site) along a linear transect with 30 m between each trio of traps. At each site, traps were presented 0.5 m above the ground on metal poles with a 1m distance between each trap, forming an equilateral triangle (**Figure S4B, C**). Each trio of traps consisted of two different experimental treatments presented as baits within the traps and one empty trap negative control containing 10 ml of DI H2O to control for trap visual display and humidity released from experimental traps. Traps were arranged randomly within the 1 m triangle to control for any potential positional effects at each site, and traps were cleaned and re-set with fresh bait daily.

A series of four experiments, each with different experimental treatments as baits were conducted as follows:

Experiment 1: sterile vs. sham-sterile *A. triloba* flowers) May 22nd, 23rd, 24th, 25th, and June 1st. This experiment tested differential trap capture between sterilized and sham-sterilized female stage *A. triloba* flowers used as bait. At 08:00 h on each morning of the assay, 25 female stage flowers from the Mundy Garden pawpaw stand were chosen at random and were subjected to a sterilization procedure that consisted of a Natamycin (Natamax SF, Dupont, St. Louis, MO) spray (50 ppm aqueous solution, left for 5 minutes), followed by a spray of bleach (10% aqueous [0.825% hypochlorite], left for 2 minutes), and finished with a sterile DI H20 spray to remove traces of each sterilant. Twenty-five more flowers were chosen at random for sham-sterilization which consisted of the same procedure above except that the sterilant sprays were placed on the pedicel and abaxial surface of the sepals only to control for the potential physiological effect of each sterilant on the flowers, while simultaneously maintaining an unaffected microbiome on all other floral parts. Sham-sterilized flowers were then sprayed with sterile DI H_2O as above to control for the effect of water spray on floral volatile emissions as well as to control for any effect of water spray on the floral microbiome. After a four-hour drying period, treated flowers were harvested and five cut flowers were placed into individual traps as bait, allowing volatiles to emanate from the trap's funnel opening.

Experiment 2: sterile flowers vs. sterile flowers with added microbes) May 26th, 27th, 29th, 31st. This experiment tested differential trap capture between sterilized flowers and sterilized flowers with an experimental addition of female stage *A. triloba* floral microbes. Female stage pawpaw flowers were sterilized (not sham-sterilized) and harvested using the same procedure as above and five cut flowers were used in each trap as bait. In addition to sterile flowers, traps received

either a Yeast-Mold (YM) agar plate (BD Difco, Franklin Lakes, NJ, USA) inoculated with a female stage pawpaw floral microbial community ('2017.YM.F.2') to return microbial volatiles to the trap or an uninoculated YM agar plate to control for the scent of the agar medium. Experiment 3: *A. triloba* microbes vs. microbial growth media only) June 6th, 7th, 8th, 9th. This experiment tested differential trap capture between uninoculated YM agar media and YM agar media inoculated with a female stage pawpaw floral microbial community alone. Trap baits were identical to experiment 2, except that no *A. triloba* flowers were placed into the traps as bait.

For all experiments, traps were left in the field for seven hours from 12:00 h to 19:00 h to coincide with our observations of insect activity on *A. triloba* flowers. Traps were only placed in the field on days without rain and with a forecasted temperature of 18°C or higher in an effort to standardize experimental conditions across all days/experiments and to avoid placing traps in weather too wet or cold for insect flight. Insects were collected and stored at the end of each day, thus preventing recapture. All insects captured were identified to lowest taxonomic rank possible for abundance and diversity calculations, and individual voucher specimens (n=508) were deposited in the Cornell University Insect Collection under CUIC voucher #1281.

Statistical Analyses

The effect of experimental treatment on response variables based on count data (total insect capture, species richness, and taxon abundances) was tested using generalized linear mixed-effect models using the package 'lme4'[67] in the R statistical computing environment[68]. Models were constructed using a Poisson distribution and log link function, with 'treatment' as a fixed effect and 'day' and 'site' as crossed random effects. We addressed overdispersion, when it occurred, by including observation-level random effects in the model to account for extra-

Poisson variation in response variables[72]. When response variables were ecological indices not based on count data (evenness [E_{var}][73], Simpson's Index, Shannon Index) the effect of treatment was tested using the equivalent linear mixed model with the same fixed and random effects. Each experiment was tested independently, and significance of the difference between treatment means was evaluated after adjustment of the *p*-value using the Benjamini-Hochberg correction procedure[74] for multiple comparisons. Negative control traps, containing 10 ml of DI H_2O only, did not trap a single insect over the course of all experiments and thus were excluded from our statistical comparisons. Experiment 3 (*A. triloba* microbes vs. microbial growth media only) did not trap enough insects over the course of the experiment to warrant statistical analysis and thus also was excluded from our statistical comparisons (**Table S1**).

RESULTS

Microbiome

Overview

Our *in vivo* and in culture analyses revealed that the pawpaw flower microbiome includes a total of 4,962 fungal (ITS) and 1,303 bacterial (16S) OTUs (**Figure 3**). A subset of 78 OTUs was associated with volatile production (n_{ITS} = 48; n_{16S} = 30), accounting for 14.4% and 6.3% of all ITS and 16S reads, respectively. All OTUs associated with volatile production in culture were present *in vivo*, while roughly one third were exclusively detected *in vivo*. Volatile-associated OTUs (= VAOs) encoded a significantly higher number of fermentative pathways than OTUs found primarily on immature flowers (i.e., the outgroup), which did not emit fermentative compounds ($p < 0.001$; **Figure 4**).

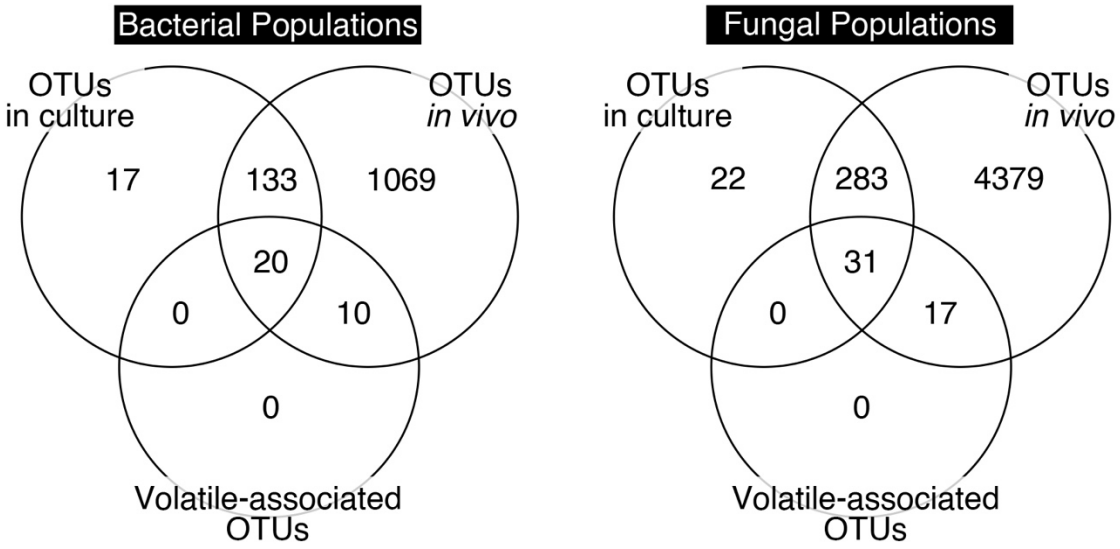

Figure 3. Venn diagram showing overlap among culturing, *in vivo* and volatile-associated OTU sets.

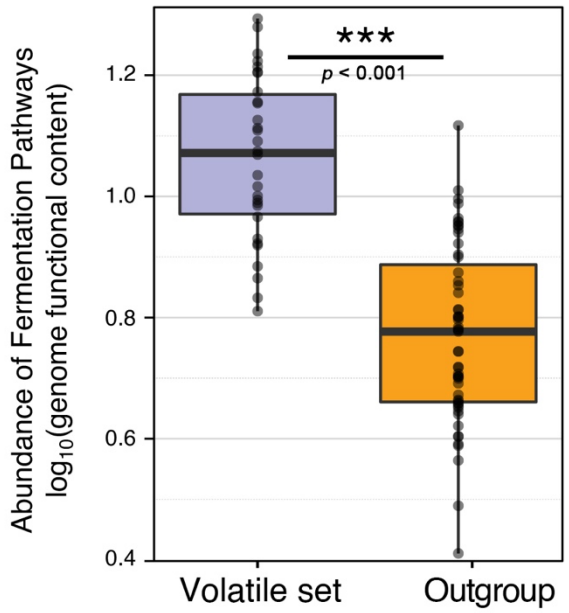

Figure 4. The total number of fermentative pathways encoded by volatile-associated OTUs (VAOs) was higher than those in OTUs indicative of immature flowers ('outgroup'). The abundance of fermentation pathways corresponds to the 'genome function count' predicted by PICRUSt2. The significant difference in means was determined using the student's t-test.

Volatile-Associated OTUs

Volatile-associated OTUs encoded a significantly higher number of 12 of the 14 fermentation pathways examined (**Figure 5**). VAOs included well-known facultatively fermentative bacteria and fungi (**Table D1**). Bacterial OTUs included *Enterobacteriaceae* (*Pectobacterium*, *Escherichia-Shigella* & *Dickeya*), *Pseudomonadales* (*Acinetobacter* & *Pseudomonas*), *Bacilli*

(*Lactococcus, Leuconostoc, Enterococcus* & *Bacillus*) and *Alphaproteobacteria* (*Acetobacteraceae*). Other fermentative bacteria were associated with male flowers, where volatile production was greatest, but occurred at too low abundance to qualify for the volatile-associated set, including *Bacilli* (*Carnobacterium, Vagococcus* & *Brochothrix*), *Clostridia* (*Lachnoclostridium*), and *Alphaproteobacteria* (*Commensalibacter*).

Of the 30 fungal VAOs assigned to guilds by FUNGuild, most were saprotrophic (n = 9), pathotrophic (n = 8) or both (n = 5), with other VAOs associated with a broad range of trophic relationships ('saprotroph-pathotroph-symbiotroph'; n=6). Twelve of the 30 fungal VAOs were classified to genera of yeast-like fungi, including fermentative ascomycotal *Torulaspora, Starmerella* and *Kluyveromyces* (*Saccharomycetaceae*), and basidiomycotal *Udeniomyces*[75], *Genolevuria*[76] and *Vishniacozyma* (*Tremellomycetes*) genera[77] as well as those known to express pectinolytic (*Filobasidium*)[78] or xylanolytic (*Papiliotrema*)[79] activity. One yeast-like fungal VAO (OTU.3; unclassified *Aureobasidiaceae*) was ubiquitous (detected in 97.5% of samples), highly abundant (μ = 14.5% of libraries) and was a dominant presence in one of the 'high' producing volatile cultures (99% of ITS reads in 'MRS.WF.f4'). The genus *Aureobasidium* includes taxa that are common colonizers of fruit trees, exhibit broad extracellular enzyme activity, and are fermentative species also capable of cellulose and hemicellulose-degradation used in industrial applications[80,81]. Two *Saccharomycetaceae* (*Kluyveromyces* and *Torulaspora*) were central nodes in the VAO co-occurrence network (OTU.1442 and OTU.1533) due to their co-occurrence with several major bacterial VAOs (**Figure 6**). The *Kluyveromyces* OTU exhibited the highest degree (n = 12) and the second highest betweenness centrality (154) of all identified VAOs (**Table D2**). The only fungal VAO with higher centrality (242) was a *Penicillium* (OTU.1181), which co-occurred with an independent set of fungal VAOs. Two bacterial OTUs (OTU.64 and

OTU.634) that exhibited high connectedness (n = 8) and centrality (120 and 96, respectively) were identified as *Hymenobacter* (*Bacteroidetes*) and unclassified *Enterobacteriaceae*. *Hymenobacter* are known to exhibit the capacity to degrade plant polysaccharides[82], but are not known to be fermentative[83]. Accordingly, in the PICRUSt2 data analysis, *Hymenobacter* OTU.64 ranked 28th out of 30 in fermentative pathway content. Thus, the set of VAOs contain predominantly fermentative fungi and bacteria, some of which are capable of degrading plant matter, but also other primary decomposers that may perform important metabolic roles upstream of volatile production, given that their capacity to produce fermentation volatiles is less clearly established.

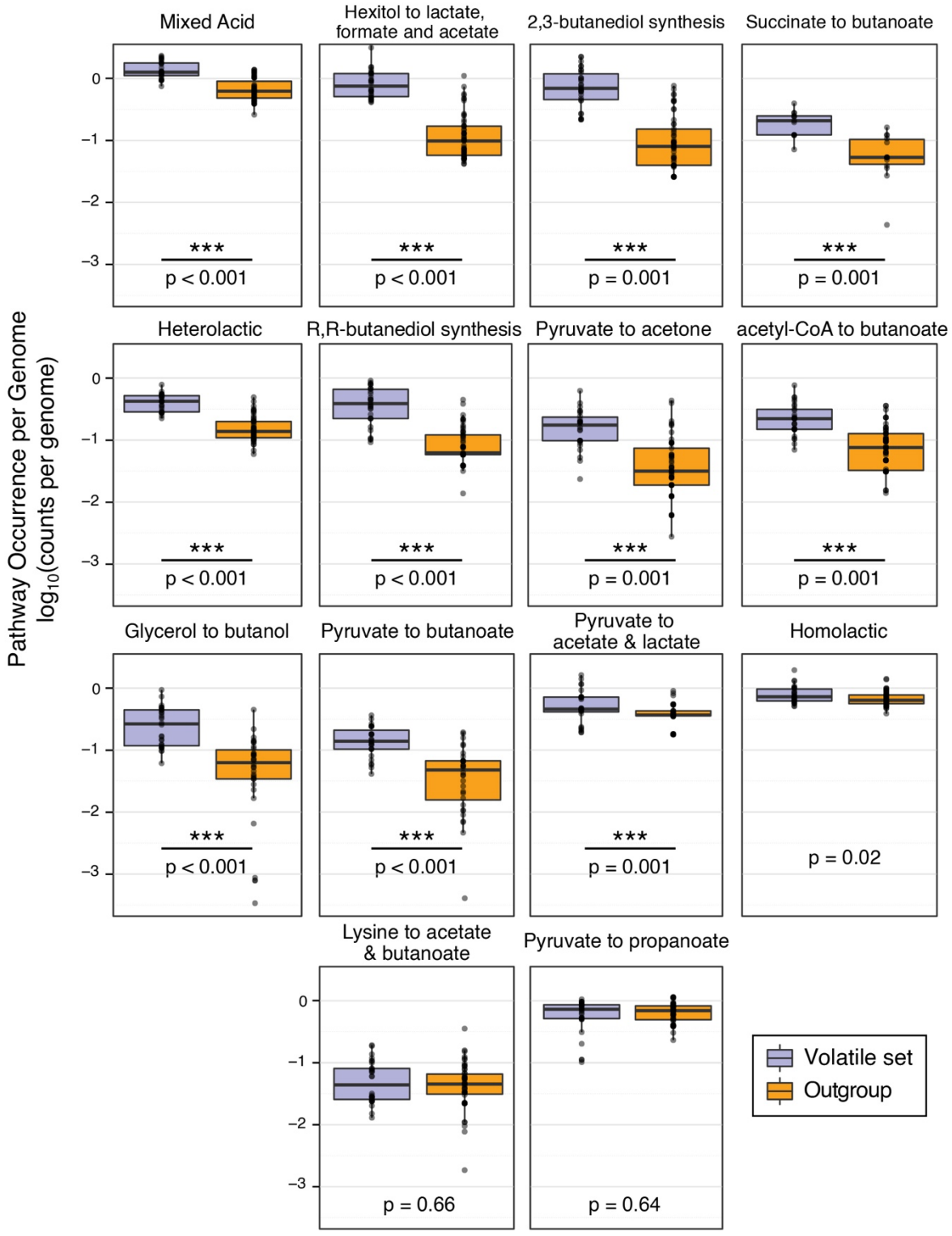

Figure 5. Differences in individual fermentative pathways encoded by volatile-associated and immature flower OTUs ('outgroup') predicted by PICRUSt2. The significant difference in means was determined using the Wilcoxon test.

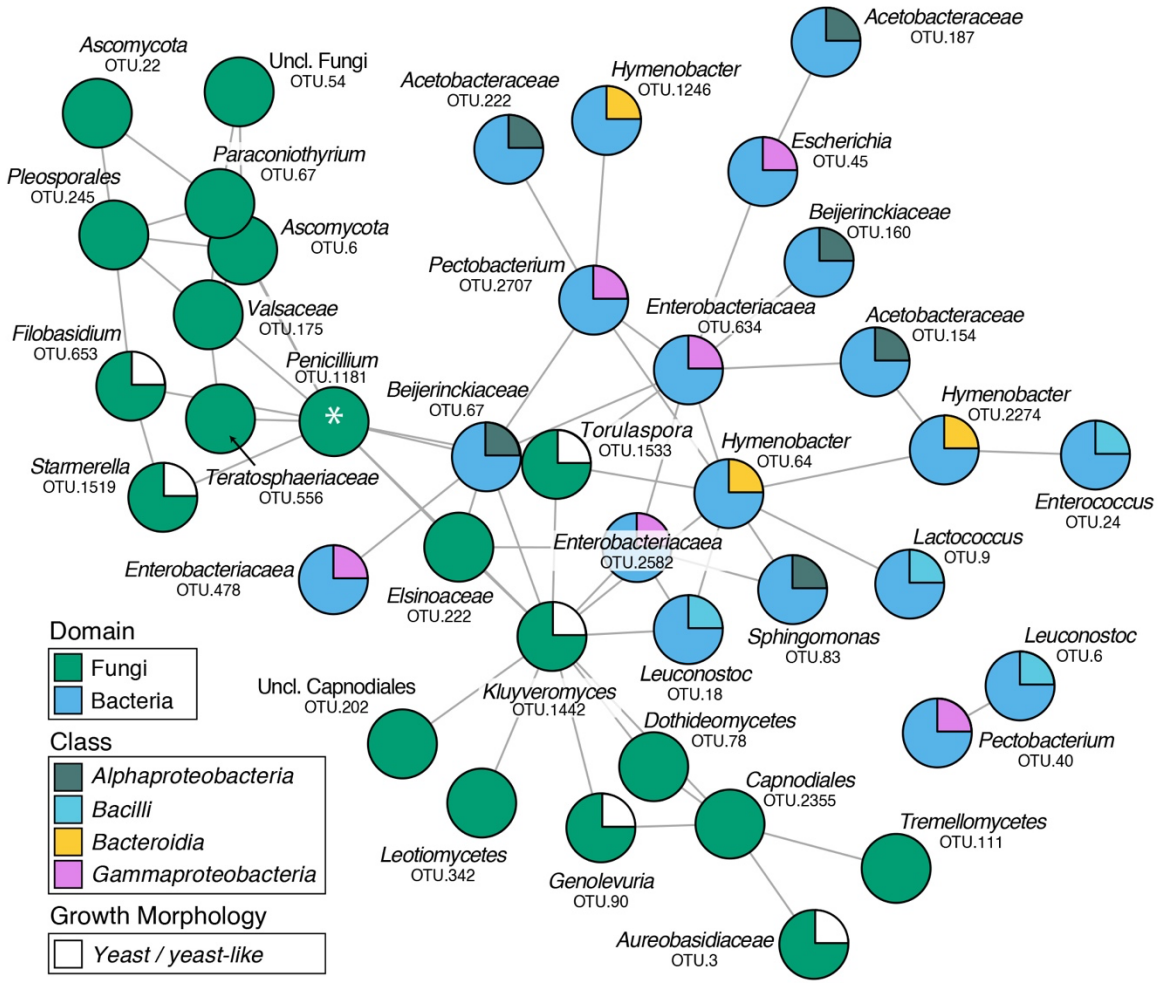

Figure 6. A co-occurrence network based on the presence/absence of bacterial and fungal VAOs. Nodes represent OTUs and edges represent consistent co-occurrence in *in vivo* data. Nodes are colored by domain and the taxonomy of bacterial OTUs were denoted using colored wedges. Fungi classified as yeast or yeast-like in FUNGuild were denoted with a white wedge. The white asterisk denotes the node with the highest degree of edges.

Microbial Community Composition Over Floral Ontogeny

The composition of volatiles varied most between female and male flowering stages ($R^2 = 0.14$), followed by year-to-year variation ($R^2 = 0.08$) and treatment ($R^2 = 0.01$; e.g., sterile vs. sham-sterile), according to PERMANOVA analyses. Total volatile production was 1.6-times higher in male flowers, corresponding with increased bacterial biomass also on male flowers based on trends in the proportion of plant and bacterial reads (**Figure 7**). The identity of volatile-associated OTUs differed substantively between male and female stage flowers (**Figure 8**). This pattern was especially true for bacteria, for which populations of *Bacilli* and *Gammaproteobacteria* dominated the male flowering stage, whereas *Alphaproteobacteria*, *Bacteroidia* and *Actinobacteria* were more abundant on female flowers. In terms of *in vivo* volatile production, female stage flowers produced significantly more (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and isobutyl alcohol (Wilcoxon test; $p_{adj} < 0.001$) (**Table D3**). Correspondingly, only taxa affiliated with female stage flowers were significantly associated with the abundance of DMNT, 2,3-butanedione, and (*E*)-β-caryophyllene (**Table D1**) despite our expectation that DMNT and (*E*)-β-caryophyllene should be exclusively plant-produced volatiles. Only fungal VAOs were associated with isobutyl alcohol emissions. Male stage flowers produced significantly more acetic acid, ethyl acetate, 3-methyl-1-butanol, 2,3-butanediol and dimethyl disulfide (DMDS) than did female stage flowers.

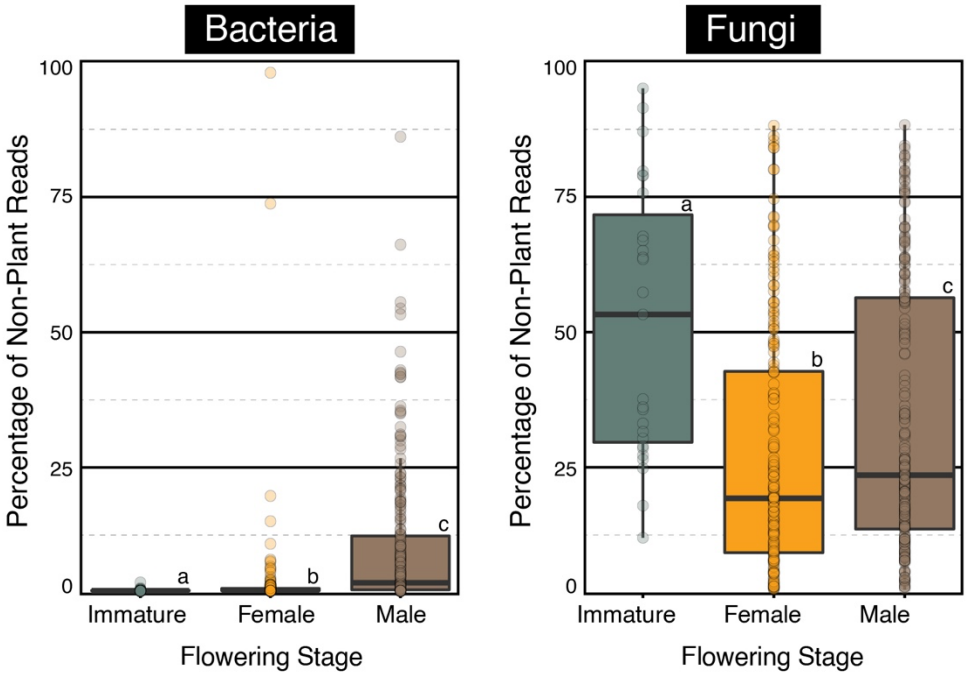

Figure 7. The difference in the proportion of plant and non-plant reads in amplicon libraries during flower development. The amount of plant DNA extracted from tissues remained constant, with plant tissue weighed prior to DNA extraction. Therefore, the increased proportion of non-plant (bacterial or fungal reads) reflects changes in microbial biomass. Pairwise significant differences among flowering stages are denoted by lettering ($p < 0.05$).

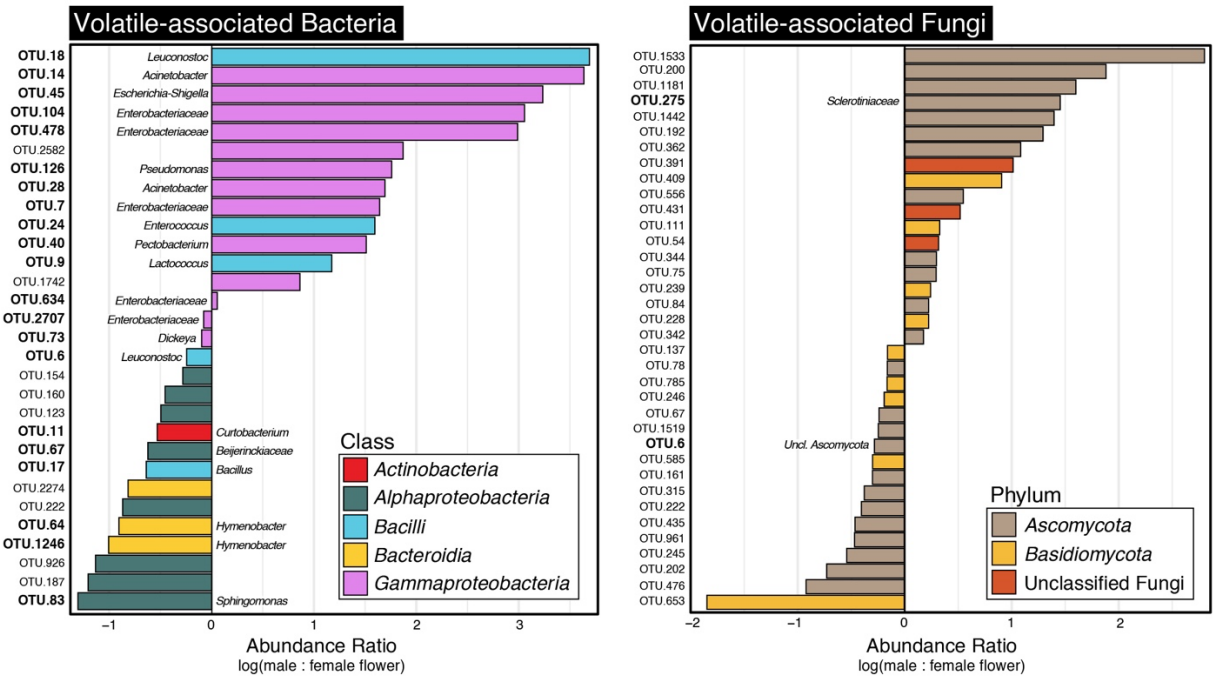

Figure 8. A comparison of the relative abundance of VAOs in female and male flowers. The x-axis corresponds to the log ratio of average relative abundance in male versus female flowers. The names of OTUs occupying, on average, greater than 1% of total reads are bolded (y-axis) and the lowest taxonomic classification is provided. Fungal VAOs with a | ratio | < 0.15 are not displayed.

The pathway to ferment hexitol to lactate, ethanol and acetate was more prevalent in VAOs affiliated with male stage flowers (Wilcoxon test; $p_{adj} = 0.01$) – the only significant difference in pathway content between the two flowering stages. No significant trends associated with volatile production were observed in culture plates differentiated by the abundance of female- versus male-affiliated VAOs (**Table D6**).

Two of the most abundant and commonly observed male-stage affiliated VAOs (OTU_{40} & OTU_{2707}), classified as *Pectobacterium*, were linked with acetic acid, ethyl acetate and 2,3-butanediol in culture and *in vivo* (**Table D1**). The incidence of OTU_{40} was also higher on sham-sterilized than on sterile flowers (Odds Ratio = 3.2; $p < 0.01$; **Table D5**) where acetic acid and ethyl acetate were measured at significantly higher abundances, and which were among the relatively few volatiles that differed for male stage sterilized flowers. According to an NCBI

BLAST search, the closest described relative of OTU_{40} is *Pectobacterium aroidearum*, (99.2% identity), a plant pathogen that can cause soft rot[84]. *Pectobacterium* – related degradation of plant tissue is associated with high levels of butanediol production[85], which is a dominant volatile in male-stage flowers. A significantly higher production of DMNT, 2,3-butanedione and isobutyl alcohol was observed in sham-sterilized female flowers, coinciding with a greater incidence of VAOs (*Alphaproteobacteria* and *Bacteroidia*) affiliated with female stage flowers (**Table D5**).

Changes in community composition from year-to-year accounted for the most variation in the flower microbiome ($R^2 = 0.10 – 0.32$) according to PERMANOVA (**Table D6**). Flower developmental stage was a significant explanatory variable only for understanding bacterial community composition. The inclusion of relative abundance data greatly improved the amount of variation explained by the model for bacterial communities (from $R^2_{binary} = 0.14$ vs. $R^2_{abund} = 0.3$) and had the opposite effect for the ITS model, intended to explain fungal communities ($R^2_{binary} = 0.34$ vs. $R^2_{abund} = 0.3$). Although variation in volatile-associated populations was greatest year-to-year, developmental stage also was a significant explanatory variable for both domains (**Table D6**). Volatile-associated bacteria were more consistently present across all four sampling years (29/30; 97%) than were fungi (22/48; 46%), although a higher proportion of abundant fungal VAOs (> 1% rel. abund.) were present across all years (4/5 OTUs). Taken together, these trends indicate that between-year variation in bacterial populations corresponded to shifts in relative abundance while fungal populations were more variable in the identity of OTUs present. Fungi with a growth morphology characterized as tremelloid, tremelloid-yeast and facultative yeast-microfungus-tremelloid were 2.5-fold more common in fungal populations that were consistent between years. One of the most abundant groups of tremelloid-yeasts in the dataset (max. abundance = 56.4%; $\mu = 3.3\%$, frequency = 89% of samples) was the

Bulleribasidiaceae, a group known to tolerate antibiotic antagonism in the phyllosphere[86]. OTUs classified to *Saccharomycetaceae* were present in all years except 2015, which had the fewest of all samples (n = 48 vs. 79 [2016], 194 [2017] & 157 [2018]).

The extent of between-year variation is evident in the hierarchical clustering of samples based on the presence/absence of VAOs (**Figure S5**). The heatmap also revealed clustering of VAOs present on immature and female flowers (**Figure S5**). A much smaller proportion of total bacterial OTUs (10%) than fungal OTUs (40%) was present on immature flowers. The proportion of fungi common to immature and sexually mature flowers was greatest for female flowers (**Figure S6**). The predominance of fungi on immature flowers is also evident in greater proportions of fungal-to-plant sequences, which were lower in female and male flowering stages. Together, this evidence suggests that volatile-associated fungi tend to already reside on flowers prior to sexual maturation, although they are unlikely to be endophytic/plant mutualists given the high interannual variation of fungal populations. There were no significant associations between bacteria or fungi with inner vs outer whorl petals, though the data here may be insufficient to draw conclusions (n_{inner} = 16 vs. n_{outer} = 17).

The Microbiome of Pawpaw Arthropod Visitors

Overall, sizeable proportions of bacterial (65%) and fungal (12%) OTUs present in the flower microbiome also were detected in the arthropod populations sampled (**Figure 9**). Even larger proportions of bacterial (90%) and fungal (67%) VAOs were present in the arthropod microbiome. OTUs present in the arthropod microbiome were significantly more abundant in male and female stage flowers than in immature flowers (**Table D7**).

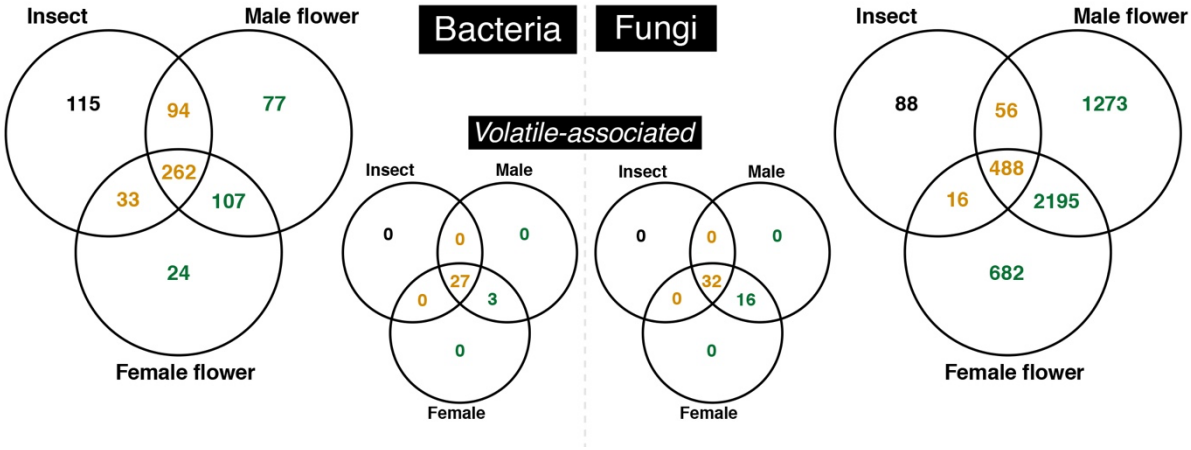

Figure 9. Venn diagrams showing overlap among insect and flower (female and male) microbiomes for whole and volatile-associated microbial communities.

Male stage flowers also were significantly more likely than female stage flowers to harbor OTUs found in arthropods (Odds Ratio = 1.6; $p < 0.001$). The increase in putatively 'arthropod-borne' populations in latter stages of flower development is consistent with the increased frequency of visitation to flowers over floral ontogeny (see visitor survey results section). Patterns in VAO populations also tracked the temporal order of insect visitation. The dominant bacterial VAOs from Arachnida were typically affiliated with the female flowering stage, while bacterial VAOs with a higher prevalence in late arriving insects in the orders Coleoptera and Diptera (**Figure S24**), were affiliated with male stage flowers (**Figure 10A**). Four of the five fungal VAOs identified in Arachnida were associated with female flowers, following the trend in bacteria (**Figure 10C**). Arthropods were more likely to carry *Gammaproteobacteria* and *Bacilli* (Odds Ratio. = 1.91; $p = 0.05$) than *Alphaproteobacteria* and *Bacteroidia* (Odds Ratio = 1.05; $p = 0.45$). The prevalence of *Gammaproteobacteria* and *Bacilli* was greater in late-arriving insects, consistent with their increased OTU relative abundance on male stage flowers (**Figure 10B**). All

bacteria associated with the sham-bagging condition (flower access allowed to visitors) were members of *Enterobacteriaceae* (*Gammaproteobacteria*) or *Enterococcaceae* (*Firmicutes*; **Table D8**), which were found primarily in Diptera and at lower levels in Coleoptera. The prominence of enteric bacterial VAOs, and the deleterious effect of bagging, suggests that arthropod frass may provide an important source of fermentative bacteria[87]. These trends support the hypothesis that arthropods could serve as vectors for sizeable microbial populations in the flower microbiome, including key volatile-producing OTUs.

Fungal VAOs present in the insect microbiome were primarily found in Coleoptera, though those classified as yeast or yeast-like tended to be shared between *Drosophila* and Coleoptera. The two *Saccharomycetaceae* OTUs central to the co-occurrence network (OTU.1533 & OTU.1442; **Figure 6**) were equally abundant in *Drosophila* and Coleoptera. Notably, the *Saccharomycetaceae* OTU (OTU.1519) that was present in the tangential cluster in the network was absent from the insect microbiome. Almost none of the volatile-associated fungi (1/48 OTUs; 2%) were affected by flower bagging, whereas volatile-associated bacteria were affected to a greater extent (6/30; 20%; **Table D8**).

Pectobacterium were prevalent in the arthropod microbiome (max. abundance = 61% of an arthropod sample; $\mu = 3.5\%$, frequency = 60% of insects), were absent from immature flowers, were present at a greater incidence on sham-bagged versus bagged flowers and were most prevalent in *Drosophila* but were also detected in Coleoptera and Arachnida (**Figure 10B**). Our findings are consistent with the occurrence of *Pectobacterium* in insect guts[88,89] and with previous reports of their transmission via insects in agricultural settings[90]. Three OTUs classified as *Wolbachia* were markedly abundant in arthropod microbiomes (max. abundance = 99.6%; $\mu = 7.5\%$, frequency = 40% of arthropods) and, to a lesser extent, were detected in flower

microbiomes (max. abundance = 32.6%; μ = 3.0%, frequency = 12% of flower). *Wolbachia* were primarily detected in Arachnida, and to a far lesser extent in Coleoptera. The presence of *Wolbachia* visitation in flower microbiomes may been seen as a clear signature of arthropod visitation, though *Wolbachia* were not volatile associated. The relative abundances of *Pectobacterium* and *Wolbachia* were not correlated in the insect microbiome (Pearson's r = -0.13, $p = 0.5$), nor in the flower microbiome (r = -0.07, $p = 0.41$), consistent with these taxa being transported by different arthropod vectors, namely *Drosophila* and Arachnida, respectively.

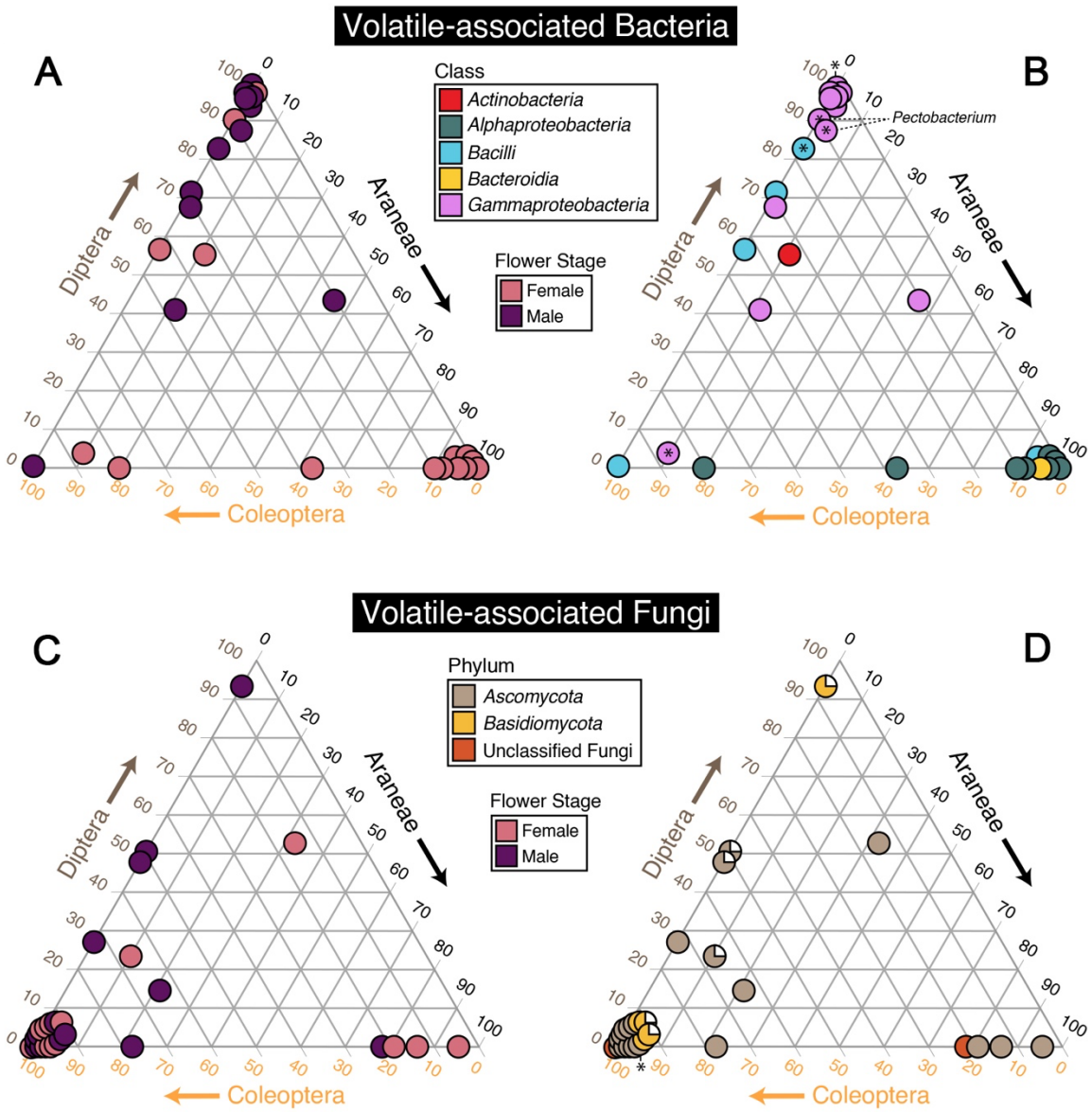

Figure 10. The prevalence of volatile-associated OTUs differed by insect order with road differences in proportioned relative abundance among *Diptera*, *Coleoptera* and *Araneae* evident in ternary plots. In (**A**) and (**C**), each volatile associated OTU is colored by its association with flowering stage and volatile-associated OTUs that were significantly impacted by bagging treatment were denoted with an asterisk. In (**B**) and (**D**), each volatile-associated OTU is colored by taxonomic classification. In (**D**), fungi classified as yeast or yeast-like in FUNGuild were denoted by a white wedge. All dots piled in the corner of a triangle (top corner in bacteria and lower-left corner in fungi) were manually jittered to visualize fill information.

Electroantennography

Antennal Morphology & Measurements

The antennae of *G. fasciatus* present with a general appearance that is typical of most Nitidulidae. Antennae are comprised of 11 antennomeres including the scape, pedicel, 6 flagellar segments, and an enlarged capitate club comprised of the distal 3 antennomeres which is diagnostic for the family. In contrast with the glabrous flagellum, the segments of the antennal club are more than twice the diameter and covered with fine hairs. Antennae are small (~ 0.9 mm – 1.4 mm observed in this study) but track overall with beetle size. Sexual dimorphism was present in the size (but not form) of antennae, with males tending to have antennae that were greater on average than females in length (1.205 mm vs. 1.052 mm, $p = 2.855\text{x}10^{-11}$; **Figures S7, S8**) and surface area (0.303 mm^2 vs. 0.255 mm^2, $p = 6.209\text{x}10^{-9}$; **Figures S9, S10**), which was a trend also seen in body length (unmeasured). Despite this difference, antennal length and surface area for both sexes were not correlated with the magnitude of EAG response (see **Table 1**; **Figures S11, S12**) when presented with a pre-test control stimulus (1M ethyl acetate control).

Electrophysiology

As compared with air and solvent controls, all tested volatile compounds elicited significant electrophysiological responses (uncorrected -mV) from *Glischrochilus fasciatus* beetle antennae to at least one concentration within a 0.001 M-1 M serial dilution (**Figures 11, S13**). Significant antennal responses to volatile chemicals were dependent on chemical concentration ($F_{45, 400} = 11.4206$, $p < 2.2\text{x}10^{-16}$), and generally were observed only at the higher end (0.1 M-1 M) of the dilution series for most compounds. The exceptions to this trend were ethyl acetate and (*E*)-β-caryophyllene, each of which elicited significant antennal responses at the 0.01 M concentration

(mean ± SE: 1.84 ± 0.18 & 1.15 ± 0.16 respectively). No compounds elicited significant responses above the air/solvent controls when presented at 0.001 M. 3-methyl-1-butanol and acetoin elicited the strongest responses (mean ± SE: 8.92 ± 1.59 & 7.19 ± 1.15 respectively) from antennae, evoking magnitudes of more than double those of all other volatiles tested when presented at the 1 M concentration. At lower concentrations, 3-methyl-1-butanol and acetoin evoked magnitudes within a similar range of all other volatiles (mean ± SE: 1.043 ± 0.259 to 1.996 ± 0.394), indicating that the double or greater antennal sensitivity to these compounds at 1 M was dependent on the interaction between dose and chemical, but not chemical alone.

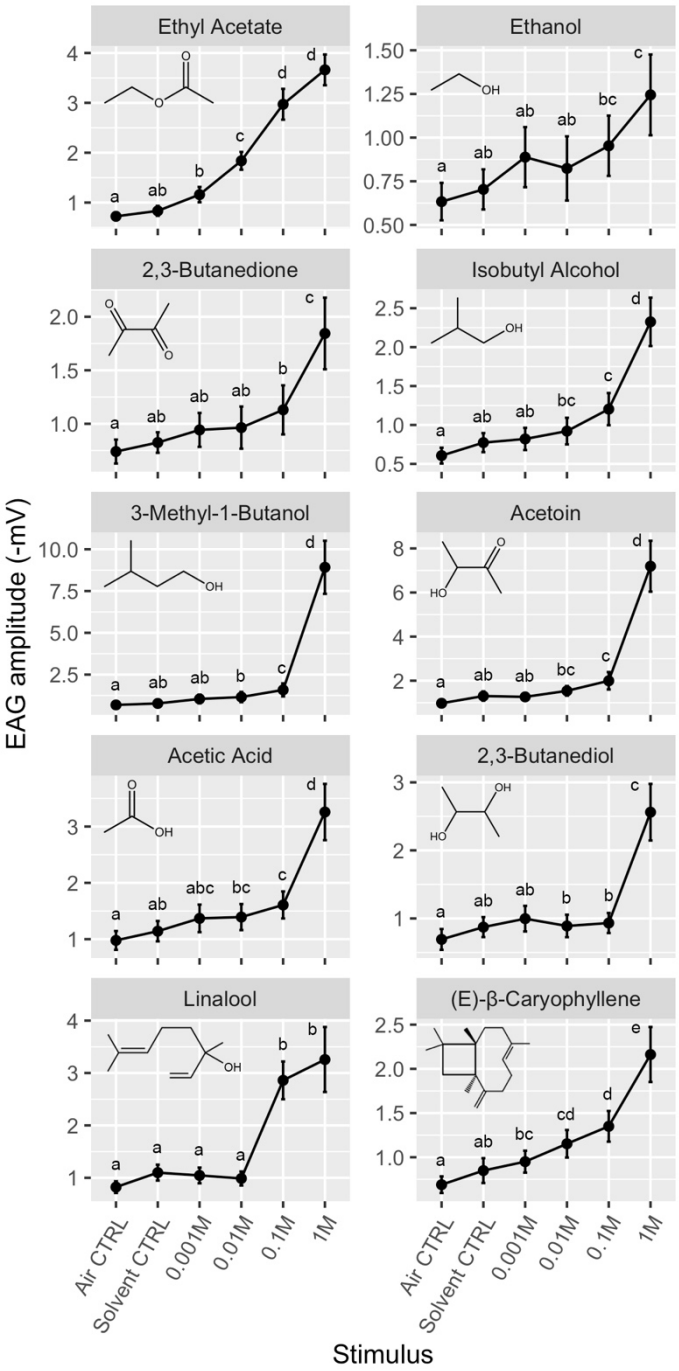

Figure 11. Mean ± SE uncorrected electroantennographic responses from *G. fasciatus* beetle antennae to serial dilutions of ten volatile compounds found in the headspace of *A. triloba* flowers. Significant pairwise differences between stimuli are indicated by labels that do not share a letter (*post hoc* pairwise analysis via estimated marginal means with Tukey's *p*-value adjustment). Mean values for each stimulus represent pooled data from male and female beetles (n=10 beetles per chemical) because sex was not a significant predictor of -mV response ($F_{1, 80}$ = 1.841, p = 0.179). Note different y-axis scales for each volatile.

Table 1.

Regression analysis for rearing conditions/antennal morphometrics predicting EAG response (-mV). n=50 beetles for each sex.

	Female			Male		
Predictor Variable	R^2	$F_{1, 48}$	*p* -value	R^2	$F_{1, 48}$	*p* -value
Antenna length	0.0002	0.0115	0.9151	0.0068	0.3280	0.5695
Antenna surface area	0.0024	0.1174	0.7334	0.0015	0.0742	0.7864
Days in captivity	0.0009	0.0409	0.8406	0.0029	0.1414	0.7085
Date of EAG recording	0.0400	2.0000	0.1638	0.0037	0.1792	0.6739
Beetle collection date	0.0234	1.1500	0.2889	0.0002	0.0108	0.9177

Sex was not a significant predictor of differential antennal response ($F_{1, 80} = 1.841$, $p = 0.179$) despite significant dimorphism in antennal length and surface area between male and female beetles (**Figures S7, S9**). Therefore, the results above are based on the average of both sexes (n=10 beetles per chemical). When presented with a 1 M ethyl acetate pre-test reference stimulus, antennal response was not correlated with the amount of time beetles spent in captivity before the EAG test, the date of EAG recording, or the date the beetles were collected from the wild (see **Table 1**, **Figures S14, S15, S16**).

Field Bioassays

Capture Survey

A total of 503 insects and 5 arachnids were caught over the course of all bioassay experiments, representing 61 species in 29 different arthropod families (**Table S1**). Trap capture was dominated by three insect families (Drosophilidae, n=258; Nitidulidae, n=87; Dryomyzidae, n=75) which accounted for 83% of total capture over all experiments and were among the most common families of insects observed on *A. triloba* flowers from a simultaneous pollinator survey at the Mundy Garden field site (see visitor survey results section) located 350 m east of the bioassay location.

Scent Preference

Experiment 1 – sterilized flowers vs. sham-sterilized flowers choice assay

In a two-choice comparison between traps baited with sterilized vs. sham-sterilized flowers, sterilization did not have a significant effect on total arthropod capture per trap ($F = 1.2496$, $p = 0.2629$; **Figure 12A**). Although traps of both treatments caught similar numbers of total arthropods, traps baited with sterilized flowers caught significantly fewer *Glischrochilus* beetles (Nitidulidae) per trap ($F = 5.0232$, $p = 0.0232$; **Figure 12C**). Similarly, sterilized flowers attracted fewer nitidulid beetles overall ($F = 5.9204$, $p = 0.0109$; **Figure S17A**), but the significance of this result was nullified by the removal of *Glischrochilus* abundance from the analysis ($F = 1.0542$, $p = 0.2920$; **Figure S17C**). This result indicates that the reduced attraction of the family Nitidulidae to sterilized flowers was largely driven by the reduced attraction of *Glischrochilus* beetles, which comprised a large proportion (60%) of total nitidulid capture.

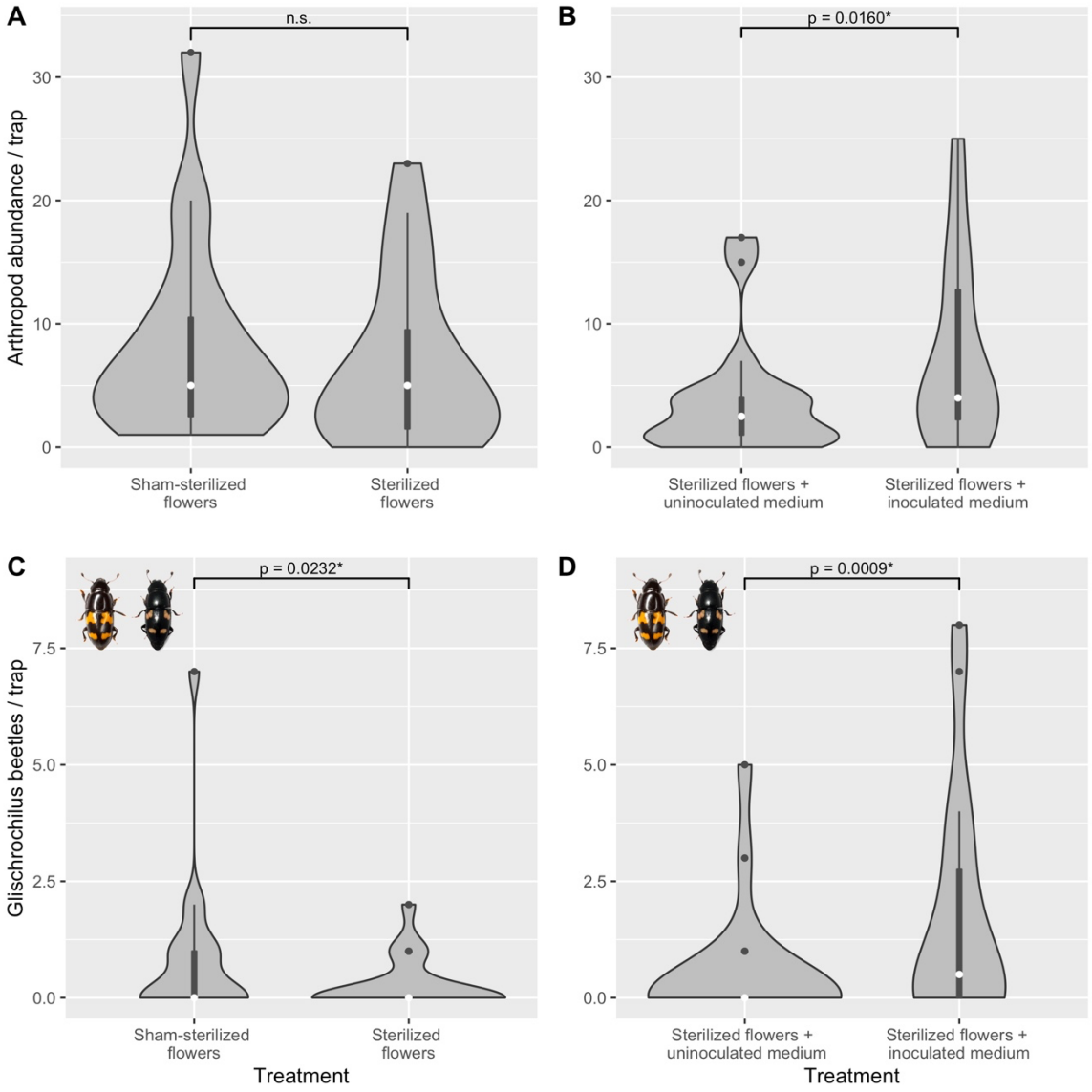

Figure 12. The effect of flower sterilization (**A**, **C**; experiment 1) or experimental addition of floral microbes to sterile flowers (**B**, **D**; experiment 2) on the number of total arthropods (**A**, **B**) or *Glischrochilus* beetle individuals (**C**, **D**) captured in cup-funnel traps. Traps baited with sham-sterile flowers vs. traps baited with sterile flowers were tested in one experiment, while traps baited with sterile flowers + uninoculated agar medium vs. traps baited with sterile flowers + floral microbe-inoculated medium were tested in a separate experiment. Negative control traps did not capture any insects over the course of the entire experiment and have been excluded from analyses and figures. *p*-values are given to indicate significant differences between means (GLMM). Asterisks indicate *p*-values robust to FDR multiple comparisons correction (**Table 2**); n.s. = not significant.

In addition to our *a priori* choice to examine total arthropod and *Glischrochilus* abundance in response to floral sterilization, we also conducted *post hoc* statistical analyses to test the effect of sterilization on select taxon capture at the generic level (*Drosophila*, *Dryomyza*), as well as on several measures of ecological diversity including species richness, species evenness (E_{var}), and Gini-Simpson and Shannon diversity indices, none of which were significantly affected by flower sterilization (see **Table 2**; **Figures S17, S18**).

Experiment 2 – sterilized flowers with uninoculated growth medium vs. sterilized flowers with inoculated growth medium choice assay

Traps baited with sterilized flowers + microbe inoculated medium trapped significantly more total arthropods than traps baited with sterilized flowers + uninoculated medium ($F = 5.734$, $p = 0.0160$; **Figure 12B**). Likewise, traps baited with sterilized flowers + added microbes caught significantly more *Glischrochilus* beetles ($F = 10.253$, $p = 0.0009$; **Figure 12D**), with the differential abundance between treatments for both experiments (exp. 1 & 2) occurring at the same ratio (1:3.2). Again, significantly more nitidulid beetles were caught with sterilized flowers + added microbes ($F = 4.0612$, $p = 0.0489$; **Figure S17B**), but not after subtraction of *Glischrochilus* abundance from the analysis ($F = 0.000$, $p = 1.000$; **Figure S17D**). *Post hoc* analyses show that sterilized flowers with added microbes caught significantly more *Drosophila* individuals, and showed a significant increase in species richness, and a significant decrease in species evenness (see **Table 2**; **Figures S17, S18**). Gini-Simpson and Shannon diversity indices were not significantly affected by adding microbes to sterile flowers, nor was the total abundance of *Dryomyza* individuals (see **Table 2**; **Figures S17, S18**).

Table 2.

Results from mixed effect models testing the effect of flower sterilization (experiment 1) or experimental addition of floral microbes (experiment 2) on the response variables listed. Generalized mixed effect models (GLMM) were used to analyze the effect of treatment on count-based response variables, and the equivalent linear mixed effect models (LMM) on non count-based ecological indices. Multiple comparisons corrected (FDR) p-values are given to indicate significant differences between treatments.

			Experiment 1			Experiment 2		
	Response Variable	Model type	*F*-value	*p*-value	FDR adjusted *p*-value	*F*-value	*p*-value	FDR adjusted *p*-value
a priori	Total arthropod abundance	GLMM	1.2496	0.2629	0.2629	5.7340	0.0160*	0.0160*
	Glischrochilus abundance	GLMM	5.0232	0.0232*	0.0464*	10.253	0.0009*	0.0018*
post hoc	Nitidulidae abundance	GLMM	5.9204	0.0109*	0.0872	4.0612	0.0489*	0.0978
	Nitidulidae - *Glischrochilus* abundance	GLMM	1.0542	0.2920	0.5544	0.0000	1.0000	1.0000
	Drosophila abundance	GLMM	0.5453	0.4534	0.6045	7.4291	0.0054*	0.0432*
	Dryomyza abundance	GLMM	0.0242	0.8717	0.8717	1.8220	0.1598	0.1826
	Species richness	GLMM	2.6072	0.1029	0.4116	4.7631	0.0271*	0.0722
	Species evenness (Evar)	LMM	0.2203	0.6440	0.7360	7.2683	0.0128*	0.0512
	Gini-Simpson Index	LMM	1.0742	0.3140	0.5544	3.2796	0.0850	0.1180
	Shannon Index	LMM	0.9285	0.3465	0.5544	3.2059	0.0885	0.1180

Experiment 3 – Uninoculated growth medium vs. inoculated growth medium

Over the course of the entire 4-day experiment, neither treatment trapped enough arthropods to warrant statistical analysis. Traps baited with uninoculated growth medium caught 11 total arthropods (8 insects, 3 arachnids; **Table S1**), while traps baited with inoculated growth medium trapped 9 total arthropods (8 insects, 1 arachnid; **Table S1**).

Asimina triloba Visitor Survey

Overview

A total of 842 arthropods (782 insects, 60 arachnids) were observed over the entire *A. triloba* visitor survey, representing 28 different arthropod families, and 10 arthropod orders (**Table D9**). Floral visitors were dominated by 4 insect families (Drosophilidae, n=253 [30%]; Nitidulidae, n=47 [6%]; an unidentified hemipteran family, n=114 [14%]; Formicidae, n=56 [7%]) which accounted for 57% of total visitor observations during the survey period. The most represented order by far were Diptera, with 328 individuals observed (39% of all visitors).

Flowering Phenology and a Dynamic Visitor Spectrum

A. triloba flowers follow a protogynous ontogeny, starting as overwintered buds and proceeding through a female to male transition in a single flower in late spring. Our visitor survey started at the first notice of a mature female flower. At this time (day 1, May 21st), the proportion of both immature and female flowers was close to twice that of male flowers (**Figure S19B**). By day 3, an inflection point was reached early in the blooming period where the proportion of all flower stages was equal, and after day 3 male flowers dominated the canopy in total numbers and proportion to other floral stages (**Figure S19A, B**). Total arthropod visitation to flowers was greatest at the time of this inflection point with 133 and 120 total arthropods observed on days 2 and 3 (May 22nd, May 23rd) respectively. The total number of arthropods observed slowly waned as the proportion of flowers shifted to a male majority, however another spike in total arthropods was observed on May 27th when the proportion of male to other flowers was near its highest (**Figure S20**). The trends for total Diptera observed were largely the same as for all arthropods with visitation waning over time and peak numbers coinciding with early blooms and a large

proportion of male flowers (May 22nd, May 27th respectively, **Figure S21A**). Coleopteran visitation followed a slightly different pattern, with beetles arriving in their greatest numbers a day later than Diptera, when more male flowers were in the canopy (**Figure S21B**). A rarefaction curve of the number of unique arthropod families observed revealed a quick increase followed by an initial plateau until ~ 250 total visitors were logged. After this plateau, which coincided with the early flowering period where proportions of all flowers were equal, the number of unique families observed steadily increased until the end of the survey as more male flowers dominated the canopy (**Figure S22**).

Floral Ontogeny and Time of Day

Overall, more visitors were observed in male stage flowers (n=519, 62%) than female or immature stage flowers (n=239, 28%; n=84, 10% respectively; **Figure S23**). This trend held true for all arthropod orders and families observed, except for Araneae which were found much more frequently on immature flowers (**Figures S24, S25**). Few drosophilids and few dipterans overall were observed on immature flowers, while no nitidulid beetles at all were found on immature flowers.

The total number of arthropods observed was more evenly distributed across the three observation times than was seen over flower stages (09:00 h, n=297; 14:00 h, n=206; 19:00 h, n=339), with slight increases in observations during the morning and evening hours over a minor afternoon lull (**Figure S26**). The ratio of observations on male:female:immature flowers was largely consistent over the three observation times (**Figure S27**). Dipterans tended to follow a crepuscular habit (activity at morning and evening), while coleopterans were more likely to be seen in the evening at the 19:00 h time period (**Figure S28**).

Glischrochilus reproductive status and egg viability survey

During our visitor survey in 2019, a total of 20 *Glischrochilus* beetles (15 female, 5 potentially-female) were captured from *A. triloba* flowers and assessed for their mating status and ability to produce viable larvae. Since *Glischrochilus quadrisignatus* individuals were impossible to sex without dissection, we monitored all 5 *G. quadrisignatus* individuals for egg laying despite the possibility that some or all of these captured individuals could be male. Of the 20 beetles captured in flowers, over half (12/20; 60%) were assessed to be gravid as noted by the presence of eggs oviposited in their rearing cups (**Table 3**). Nearly all (11/13; 85%) *Glischrochilus fasciatus* females arrived at flowers gravid. One of two *Glischrochilus sanguinolentus* females arrived at flowers gravid, but this sample was too small to evaluate trends. No *G. quadrisignatus* individuals oviposited in their rearing cups. This result could indicate that all *G. quadrisignatus* individuals collected were male, or if some collected were female – none of those females were gravid. Rearing conditions were likely not a factor in the absence of oviposition from these beetles because we have observed trap collected and/or breeding populations of *G. quadrisignatus* ovipositing freely in the same rearing conditions. For all gravid females, laid eggs were observed to hatch into viable larvae.

Table 3.

Results from an egg viability survey conducted on Glischrochilus beetles captured in A. triloba flowers during the months of May and June 2019.

Date Captured	Time Captured	Capture Site	Beetle Species	Sex	Gravid?	Eggs Viable?
20-May-2019	2pm	male flower	*Glischrochilus fasciatus*	female	no	n/a
21-May-2019	2pm	female flower	*Glischrochilus fasciatus*	female	yes	yes
22-May-2019	2pm	male flower	*Glischrochilus fasciatus*	female	yes	yes
22-May-2019	7pm	female flower	*Glischrochilus fasciatus*	female	yes	yes
23-May-2019	2pm	male flower	*Glischrochilus fasciatus*	female	yes	yes
23-May-2019	7pm	female flower	*Glischrochilus fasciatus*	female	yes	yes
23-May-2019	7pm	male flower	*Glischrochilus fasciatus*	female	yes	yes
23-May-2019	7pm	male flower	*Glischrochilus fasciatus*	female	no	n/a
25-May-2019	9am	male flower	*Glischrochilus fasciatus*	female	yes	yes
25-May-2019	2pm	female flower	*Glischrochilus fasciatus*	female	yes	yes
25-May-2019	7pm	female flower	*Glischrochilus fasciatus*	female	yes	yes
30-May-2019	2pm	male flower	*Glischrochilus fasciatus*	female	yes	yes
6-Jun-2019	9am	male flower	*Glischrochilus fasciatus*	female	yes	yes
25-May-2019	9am	male flower	*Glischrochilus quadrisignatus*	unknown	no	n/a
25-May-2019	7pm	female flower	*Glischrochilus quadrisignatus*	unknown	no	n/a
25-May-2019	7pm	male flower	*Glischrochilus quadrisignatus*	unknown	no	n/a
26-May-2019	2pm	male flower	*Glischrochilus quadrisignatus*	unknown	no	n/a
30-May-2019	7pm	male flower	*Glischrochilus quadrisignatus*	unknown	no	n/a
23-May-2019	7pm	female flower	*Glischrochilus sanguinolentus*	female	yes	yes
23-May-2019	7pm	male flower	*Glischrochilus sanguinolentus*	female	no	n/a

DISCUSSION

Of the many examples of mimicry and deception in flowering plants, those that use brood-site mimicry/deception as a reproductive strategy are perhaps the most underappreciated, despite being the most geographically and phylogenetically widespread of all mimicry types[16]. The fundamental characteristic that links these floral phenotypes is the olfactory mimicry of microbial metabolism that occurs when organic substrates succumb to decay/decomposition. Despite this obvious connotation, no studies to date have systematically investigated whether microbes may contribute to the deceptive phenotype, although the hypothesis has been discussed for decades[38]. This work represents the first study to explicitly culture, sequence and identify the microbes from such flowers, as well as to evaluate whether microbial residence on flowers contributes fundamentally to floral phenotype and pollinator attraction. The insights generated from this work provide a framework for future studies to include microbiological perspectives into the integrated studies of floral chemistry and pollinator behavior in brood-site mimicking flowers.

The Dynamic Microbiome of Asimina triloba

Despite a growing interest in flower-associated microbes and their potential roles in shaping plant-pollinator interactions[20,21], the flower remains an understudied environment for microbial community ecology and the potential effects that floral microbes may have on plant fitness, or in influencing the evolution of floral signals and phenotype[91]. Most studies of floral microbes to date have focused on how microbes may chemically alter floral rewards[92-94], or how microbes may affect the behavior of established pollinators of conventional nectar-rewarding flowers[22-24].

In contrast to these studies, we sought to understand how microbes associated with a brood-site mimicking flower might fundamentally shape its microbial-associated chemical phenotype and attraction of its saprophilic pollinators.

Our model floral species, *Asimina triloba*, is unusual in that its flowers do not present traditional nectar rewards, with epifloral microbes presenting a potential source of the observed floral phenotype, rather than detracting from it. Our results indicate that fermentative bacteria and fungi are resident on *A. triloba* flowers, and that increased abundance of these microbes corresponds with higher fermentative volatile production from flowers. Out of the thousands of OTUs sequenced from flowers, we identified 78 OTUs (48 fungal, 30 bacterial) whose abundances were correlated with floral volatile production. An analysis of fermentation pathway content revealed a significantly higher incidence of fermentation pathways encoded by the genomes of volatile-associated OTUs (VAO) in comparison with OTUs not associated with volatile production.

An important feature of *A. triloba* flowers is the increase in fermentative volatile production as flowers proceed through a protogynous (female to male) development. This generates the prediction that if microbes contribute to floral scent, we should see differences in volatile-associated microbial community composition and microbial biomass as flowers age. Indeed, bacterial biomass increased significantly as flowers matured (**Figure 7**), which is consistent with previous reports of floral microbes increasing in incidence and abundance over time[95,96]. Likewise, we also showed that greater fermentation volatile production in male vs female flowers was correlated with a taxonomic shift in volatile-associated bacteria over floral ontogeny (**Figure 8**). Female flowers were dominated by *Alphaproteobacteria,* which is a predominant entity in the phyllosphere[97], while male flowers were dominated by *Bacilli* and

Gammaproteobacteria, which are more closely associated with insects[98,99]. This result is congruent with the abundance of evidence that insects do vector microbes to and among flowers[100,101], and also tracks with our observations of greater insect visitation to male stage pawpaw flowers in our formal visitor survey. Two of the most abundant volatile-associated microbes linked to male flowers, classified as members of the genus *Pectobacterium,* were associated with increased production of ethyl acetate, acetic acid, and 2,3-butanediol in our microbial cultures and in the headspace of *A. triloba* flowers. Members of the genus *Pectobacterium* are well known for their ability to cause soft rot in plant tissues and, along with the genus *Dickeya* (which are also found on *A. triloba* flowers), can secrete high levels of 2,3-butanediol during plant degradation[85]. Given that *Pectobacterium* is highly abundant in male stage flowers when floral rot is at its peak, and is also correlated with volatile production in male flowers and in culture, we must consider these OTUs leading candidates for the decay seen in *A. triloba* nectary tissue.

Contrary to our expectations, we did not observe a 'core'[102] microbiome associated with *A. triloba* flowers. Rather, it appears that conditions in *A. triloba* flowers select for a varying set of microorganisms interannually, with functional redundancy in fermentative metabolism. Interestingly, several bacteria and fungi associated with volatile production in pawpaw flowers are members of genera which include phytopathogens (*Pectobacterium*, *Dickeya*, *Pseudomonas*, *Monilinia*, *Ramularia,* etc.). This raises questions about the risks/rewards associated with flowers evolving to use commensal microbes rather than a core set of symbionts (like tall fescue and its fungal endophyte *Neotyphodium coenophialum*)[103,104] to enhance functions like pollination or plant fitness. Because flowers of *A. triloba* utilize decaying floral tissue as part of a functional phenotype (e.g. a pollinator reward), it may be too risky for flowers/plant individuals to engage

in long-term associations with microbes capable of floral pathogenicity. Since acquiring pathogens that contribute to decay is likely adaptive as a profitable reward for saprophagous pollinators, perhaps the quick senescence/abscission of *A. triloba* flowers after they reach male maturity is an adequate protection against systemic disease, and an acceptable middle ground between foregoing rotting tissues and symbiosis with pathogens. In addition, the decay in *A. triloba* petals is restricted to the carbohydrate-rich regions of the inner-petal nectariferous tissues which are free of the anthocyanin pigmentation that is present in the rest of the corolla tissue. Inner-petal nectariferous tissues are shared throughout the Annonaceae and serve multiple functions, including as a source of (non-decaying) food rewards to beetles in non-fermenting *Asimina* species[105]. Since anthocyanins have reported antimicrobial properties[106], they may function to contain the rewarding decay only to the unprotected nectary tissues, allowing flowers to maintain structural integrity until floral ontogeny is complete.

Like other brood-site associated floral phenotypes, *A. triloba* attracts floral visitors that are not usually associated with flowers. Rather, their saprophilic visitors are more commonly associated with decaying substrates such as rotting fruit or carrion, and thus have the potential to vector potentially pathogenic microbes from these substrates to flowers. Under normal circumstances, it may not be desirable for a flower to interact with potential pathogens, however since the phenotype of *A. triloba* includes nectary decay and fermentation volatiles, arthropod-vectored microbes might play a critical role in assembling the fermenting phenotype. Indeed, a large proportion of bacterial and fungal OTUs sequenced from flowers were also present in the arthropod microbiome. Strikingly, 90% of bacterial, and 70% of fungal VAOs were present in the arthropod microbiome, and male flowers were significantly more likely than female flowers to harbor insect-borne microbes. In addition, arthropods were more likely to harbor

Gammaproteobacteria and *Bacilli* than *Alphaproteobacteria* and *Bacteroidia,* which is in agreement with the taxonomic shift in bacterial OTUs observed over floral ontogeny (**Figure 8**). Taken together, these data suggest that arthropod-borne microbes do contribute to the *A. triloba* microbiome and volatile production, and may be key sources of microbes for the decay seen in *A. triloba* nectary tissues.

Given that *A. triloba* does not maintain a core set of symbionts and that insect-vectored microbes are a significant source of volatile production in flowers, the best interpretation of our data for how microbes enhance volatile production in pawpaw flowers is as follows: the initial low emission of floral volatiles is produced by a combination of plant metabolism and resident VAOs. Immature and female flowers that open early in the blooming period are likely visited infrequently. However, as flowers mature and time progresses, microbial biomass (and thus volatile production) increases on flowers for bacteria specifically, while the biomass of fungi either decreases or remains constant. Saprotrophic/pathotrophic microorganisms that colonize flowers contribute to the rot observed in inner petals which, combined with plant-produced volatiles, further increases fermentative volatile production. Insect visitors attracted to the increased fermentative odor arrive at flowers carrying bacteria which specialize in fermentation and that do not otherwise thrive in the phyllosphere. The vectored bacteria enhance volatile production even more, which increases subsequent visitation to male flowers. As male flowers become populated, insect-associated fermentative microbes are then vectored to newly mature female flowers when insects leave male flowers and then visit female flowers by mistake. This feed-forward sequence continues until the blooming period ends.

Volatile associated fungal OTUs may contribute in a similar capacity, but the ecology governing their assembly is less defined in our data set. Fungal populations were more stochastic than bacteria and tended to be present in higher abundance on immature flowers, which don't produce fermentation volatiles. However, particular fungal VAOs, like the *Saccharomycetaceae*, were consistent in the arthropod microbiome (particularly *Drosophila* and Coleoptera) interannually, which could suggest that fly and beetle visitors may play a specific role in shaping fungal populations that contribute to volatile production in flowers.

Taken together, our results indicate that a subset of epifloral fermentative microbes are associated with volatile production and tissue decay in *A. triloba* flowers, and that their assembly and dynamism (particularly of bacteria) is consistent with the increased volatile production and insect visitation seen in male stage flowers. Several bacteria in the genus *Pectobacterium* known for soft rot are candidate OTUs for the initiation of nectary tissue rot that begins in female stage flowers. Though fungi are more stochastic and evidence for their function as significant volatile producers is less clear, they still may play critical metabolic roles upstream of volatile production. Most fungal VAOs were classified as saprotrophic/pathotrophic, and several key fungal OTUS capable of plant cell wall degradation were central nodes in the co-occurrence network, suggesting that their presence may be crucial in assembling the microbial-derived phenotype. Lastly, it appears insect (not arachnid) visitation is important for vectoring VAOs to flowers, and may be an essential source of fermentative microbes to low volatile producing female flowers early in the blooming period.

Floral Visitation to Asimina triloba Flowers

Asimina triloba flowers were shown to attract a dynamic and diverse visitor spectrum based on the observations of 842 arthropods (782 insects, 60 arachnids) in flowers, which included species from 28 arthropod families and 10 orders, over the course of the 18-day survey. Our results revealed patterns of visitation that changed throughout the blooming season, as well as trends in visitation based on time of observation and floral stage.

Based on the yeast-like floral scent of *A. triloba*, as well as the microbial decay within the petal nectariferous tissues, one might expect the flowers of *A. triloba* to be pollinated by saprotrophic flies or beetles and for the flowers to ecologically specialize on a small set of pollinators that maximize their reproductive effort[15]. We were surprised then to observe such a diverse visitor spectrum to *A. triloba* flowers in our region. Indeed, this result was especially surprising given that previous studies have recorded very low rates of insect visitation to *A. triloba* flowers. For example, Willson & Schemske (1980)[36] found no insect visitation to flowers on several pollinator observation bouts from their field sites in Illinois. Only upon systematic flower observations from stem to stem did they find evidence of visitation, with the visitor spectrum consisting mainly of large-bodied flies (e.g. Muscidae, Sarcophagidae) in few numbers. It is also common for pawpaw growers to hang roadkill in their pawpaw patches to increase large-bodied dipteran visitation and fruit set[110], which underscores the significant pollinator limitation that appears common across the *A. triloba* range[36]. Nevertheless, our observations document a diverse, abundant, and dynamic visitor spectrum in a riparian woodlot at the northern limit of the *A. triloba* range.

In a departure from the observations of Wilsson & Schemske (1980)[36], the most common visitors to pawpaw flowers in our range were drosophilid flies (30% of total), ants (7% of total), sap beetles (Nitidulidae; 6% of total), an unidentified hemipteran species (14% of total), and interestingly arachnids (7% of total). A majority of visitors were observed early in the *A. triloba* blooming period (2-3 days after the first female flower) when the ratio of male to female flowers in the canopy was approximately equal. Our rarefaction results (**Figure S22**) showed an early plateau after the initial high attraction of visitors during the early blooming period, followed by a steadily rising increase in the number of families observed over the rest of the pawpaw blooming season. This result makes sense in light of how quickly the abiotic and biotic environment at our field site in New York state changes at this time of year. As Price et al. (2005)[111] show, the visitor spectrum to flowers commonly changes within a blooming season as well as from year to year. At our field site, the bloom of *A. triloba* commences before any leaves are out in the canopy, and by the time that the last flowers are blooming the canopy has completely filled in, including with foliage from *A. triloba* trees. Weather conditions are also quite dynamic during this period, with the blooming period spanning the transition from spring-like weather with colder and wetter conditions, to summer-like weather characteristic of drier conditions with more warmth and sun. Despite the non-asymptotic rarefaction results, we are confident that we surveyed the visitor spectrum at depth (having observed flowers over the whole blooming period), and we suspect that these results are largely artifacts of transient floral visitors moving through a dynamic ecological environment.

Male flowers were most attractive to all insect families and orders totaling 68% of visits vs. 28% of visits to female flowers. This and previous work[38] has shown that male flowers produce different volatile bouquets from females in both quality and quantity (however with

significant volatile overlap), and we have observed that male flowers provide greater amounts of rewards, including pollen, more nectar secretions/exudates, and more pronounced decaying tissue for saprotrophic visitors. Considering this, visits to female flowers may be a consequence of intersexual mimicry between dichogamous flowers[112], which may exist as a way to attract visitors to the less rewarding sex (females) to ensure pollination in a pollinator limited system.

Despite male flowers being more attractive to all insects, we observed that arachnids in our study largely preferred immature green flowers over both mature sexes. We were initially intrigued by the hypothesis that spiders may "seed" immature flowers with volatile producing microbes that could then serve to attract insects that the spiders could use for prey. We found that most microbes transferred to flowers from arthropods that were associated with fermentative volatile production were not vectored by arachnids, but rather by dipteran and coleopteran visitors (**Figure 10**). Given this result, we can reject the hypothesis that spiders seed flowers with microbes for their own benefit, however spiders may still use the flowers as sites to stalk prey. Given that immature flowers are the floral stage least visited by insects, this hypothesis may also be tenuous. Though we have not observed direct predation by spiders in flowers, we have observed spiders on sexually mature flowers, including spiders in the family Thomisidae which are known to use flowers as predation sites[113]. Despite this, and considering our lack of observed predation on flowers, we believe the most likely scenario to be that spiders use the flowers as shelter from the rain or cold temperatures associated with the early blooming period, however more observation of spiders in general are needed to confirm or reject these hypotheses.

Asimina triloba is an interesting case study for flower generalization/specialization in that it represents a temperate species in an otherwise pantropical plant family (Annonaceae), which is known for its highly specialized pollination syndromes (see Goodrich & Raguso 2009 and

references therein)[39,114]. Based on its temperate habitat, and apparent pollinator limitation we might expect *A. triloba* to adopt a generalized pollination strategy to ensure pollination under dynamic abiotic and ecological conditons[115]. However, despite the generalized visitor spectrum observed at flowers, most visitors appeared to be either transient, or poor pollen vectors due to unsuitable size (e.g. Drosophilidae) or behavioral characteristics like limited mobility (e.g. Formicidae, Araneae). As Ollerton et al. (2007)[116] suggest, and demonstrated convincingly by Fishbein & Venable (1996)[117], the degree of specialization attributed to flowers should refer to the number of effective pollinators that engage with the flowers, rather than all visitors, some of which may be low quality in terms of pollen export/deposition. In our study, the only consistent visitors to be observed with copious pollen, and a size that matched the floral architecture, were sap beetles of the genus *Glischrochilus* (Nitidulidae). *Glischrochilus* beetles were observed eating decaying nectariferous tissue from the inner petals of male stage flowers and were also observed mating inside the cup-shaped chamber around the flowers' androgynoecium. The mating posture that the beetles adopted resulted in both partners brushing against the floral stamens and consequently acquiring enough pollen to cover each insect body. Though *Glischrochilus* beetles appear to be the most suited pollinators for *A. triloba* flowers in our region, we cannot exclude that transient visitors of appreciable size (e.g. calyptrate or dryomyzid flies) might move some pollen in the system. Indeed, sarcophagid flies appear to play a more critical pollination role in pawpaw populations in Delaware/Pennsylvania, having been observed much more frequently than *Glischrochilus* beetles in those populations in addition to having been photographed covered in pollen (Kate Goodrich personal communication). Taken together, these data suggest that *A. triloba* flowers attract a generalized set of floral visitors using a stereotyped fermentative floral scent but may be more ecologically specialized based on our

observations of insect size, floral architecture, and pollen carriage among insect visitors. However, it should be noted that our pollinator observations appear unique compared with other surveys of *A. triloba,* which may indicate a greater degree of generalization across *A. triloba's* large geographic range.

Insights from Electroantennography

The yeast-like floral scent of *A. triloba* consists of a complex blend of short-chain alcohols, acids, and esters typical of microbial fermentation. In addition, the headspace also contains monoterpene, sesquiterpene, and irregular terpenoid compounds typical of plant vegetation, as well as amino acid derived aldoximes and nitriles[38]. Using electroantennography, we confirmed that the antennae of *Glischrochilus fasciatus* beetles were able to detect all compounds we tested for EAG responses, including fermentation associated volatiles (ethanol, ethyl acetate, 2,3-butandione, isobutyl alcohol, 3-methyl-1-butanol, acetoin, acetic acid, 2,3-butanediol) and putative floral volatiles (linalool, (*E*)-β-caryophyllene), all of which are found in *A. triloba* headspace. Beetles responded with the strongest magnitudes to acetoin and 3-methyl-1-butanol (**Figure 11**), sometimes on the order of 2X-5X more than other compounds, but interestingly only when presented at the 1 M concentration. Indeed, at lower concentrations, responses to acetoin and 3-methyl-1-butanol were roughly equal to all other compounds tested. Ethanol elicited the weakest response from beetle antennae and required at least a 1 M concentration for activation. Ethyl acetate and (*E*)-β-caryophyllene required the lowest concentrations (0.01M) to elicit responses from beetle antennae relative to all other volatiles tested. Taken together, these results suggest that *G. fasciatus* beetles have an olfactory system which is capable of responding to a broad and complex blend of volatile compounds from diverse chemical classes, and from

related but distinct emission sources including microbial and floral-associated volatiles. Responding to a broad mixture of fermentation and plant associated volatiles is likely adaptive for a generalist insect that must locate profitable food/brood sites that are ephemeral and widely dispersed in the environment. *Glischrochilus* beetles appear to be true generalists in this sense, having been documented using a plethora of fermenting resources for food/brood sites including oak wilt mats[118,119], fermenting hardwood sap[120,121], compost piles (personal observation), and various overripe fruits or damaged crops such as strawberries[122], raspberries, tomatoes, and sweet corn[123,124].

However, as Stensmyr et al. (2001)[125] caution, determining whether a generalist insect has broad antennal receptivity depends on the number of stimuli screened, and also the selectivity of single olfactory receptor neurons (ORN) to each individual stimulus. In their study, Stensmyr et al. concluded that the African fruit chafer *Pachnoda marginata* (Coleoptera: Scarabaeidae) exhibited a highly selective olfactory system because only 59 total compounds (out of many hundreds screened) elicited responses from ORNs. Further, of the 28 ORN classes identified, 11 of these classes responded exclusively to single odorants. In our study, 10 out of 10 compounds from *A. triloba* headspace elicited significant responses from *G. fasciatus* whole antennae. It is possible that, like polyphagous chafer beetles, *G. fasciatus* beetles may have highly tuned olfactory systems for the detection of ephemeral food/brood sites, however such testing is beyond the scope of this work as we have confirmed that the major volatiles emitted from *A. triloba* flowers are detected by its primary pollinator.

The ability of beetle antennae to detect all the compounds we tested might also suggest that mixtures of volatiles are important to *G. fasciatus* for food/brood site location, rather than single compounds. Several studies aimed at developing chemical lures for *Glischrochilus* beetles

have shown that mixtures are often required for beetle attraction. Lin and Phelan (1991)[126] found that a seven-component blend of ethyl acetate, acetaldehyde, ethanol, 1-propanol, 3-methyl-1-butanol, 2-methyl-1-butanol, and 2-methyl-1-propanol (isobutyl alcohol) was equally effective as baker's yeast inoculated whole wheat bread dough or inoculated banana at trapping both *G. fasciatus* and *G. quadrisignatus* beetles. Experimental removal of compounds from the seven-component blend revealed that the simplest blend with equal attraction to the positive controls for both species was a four-component blend containing ethyl acetate, acetaldehyde, ethanol, and 2-methyl-1-butanol. For *G. fasciatus*, removal of either ethyl acetate or ethanol from the blend resulted in loss of attraction. Similarly, ethyl acetate, ethanol, and acetaldehyde were essential for *G. quadrisignatus* attraction. Other studies have explored whether single[127] or paired compounds[128] could be as effective as food baits for *Glischrochilus* lures. Alm et al. (1985)[127] found that butyl acetate was effective as a single component lure for *G. quadrisignatus* when compared to food bait controls, but not for *G. fasciatus* which was only attracted to the odor blends of inoculated whole wheat bread dough, fresh corn, or banana. As far as we know, *G. fasciatus* has not been shown to be attracted to single compounds, and this is consistent with our work showing antennal receptivity to multiple plant and fermentation associated volatiles.

One intriguing aspect about the aforementioned studies exploring *Glischrochilus* host choice and chemical attraction is, in almost all cases, the attractive substrates in these studies were either naturally occurring overripe fruits or fermenting plant matter, baker's yeast inoculated foods for bait, or chemical blends comprising fermentation compounds. All of these substrates are associated with some form of microbial fermentation. *Glischrochilus* beetle adults and larvae have also been reported as myceto- or saprophagous[118,129], and several species of sap beetles (Nitidulidae) have been shown to be attracted to volatile compounds emitted exclusively

from the fungal causative agent of oak wilt disease, *Ceratocystis fagacearum*[130]. Of the 10 volatiles we tested for EAG responses, 8 are more closely associated with microbial metabolism[131-133] (ethyl acetate, ethanol, 2,3-butandione, isobutyl alcohol, 3-methyl-1-butanol, acetoin, acetic acid, 2,3-butanediol) than with flowers, and 5 of 8 (ethyl acetate, ethanol, isobutyl alcohol, 3-methyl-1-butanol, acetoin) show overlap with the compounds tested as lures for sap beetles (including *Glischrochilus*) in several studies by Lin and Phelan[126,134,135]. Given this pattern of EAG receptivity, it is possible that *G. fasciatus* beetles have olfactory systems tuned to recognize fermentation volatiles as reliable cues for profitable food/brood sites, but also plant-dedicated olfactory receptivity that could function to receive contextual information for host finding in a noisy olfactory environment. In this respect, both linalool and (*E*)-β-caryophyllene are ubiquitous plant volatiles[136] and have been recorded as components of the headspace of fruits[137,138], however it should be noted that microorganisms have also been shown to be capable of producing both compounds[139]. Nevertheless, our analyses of volatiles collected from plated *A. triloba* microbial communities detected linalool in 1 of 90 plated replicates, and only 5 of 90 replicates showed the presence of (*E*)-β-caryophyllene, all of which were in trace amounts. Thus, we can be confident in the case of *A. triloba* flowers that these were plant associated compounds.

One surprising result from our EAG experiments was the large activation threshold required to elicit responses from *G. fasciatus* antennae. Most compounds required a dose of at least 0.1M-1.0M to elicit a significant response, and EAG amplitude was dose-dependent for all compounds. This raises questions to how beetles find profitable food/brood sites in a complex environment. Given that *G. fasciatus* environments must be awash in microbial volatiles from non-profitable substrates all at various stages of decomposition in the carbon cycle, an olfactory system that only activates when the signal-to-noise ratio is large may prevent wasted host

searching from an optimal foraging perspective. Indeed, it has been shown for several nitidulid species[128,140] that higher concentrations of attractive volatiles result in higher trap catches, indicating that concentration of stimuli matter to beetles. We also showed in our trap bioassays that sterilized *A. triloba* flowers (i.e. with lower concentrations of fermentation volatiles) were less attractive to *Glischrochilus* beetles than sham-sterilized *A. triloba* flowers, and this attraction was restored with the addition of *A. triloba* floral microbes back into traps with sterilized flowers. Therefore, it appears that large concentrations of fermentation volatiles emitted from *G. fasciatus* food/brood sites may be honest signals that convey information about the abundance of microorganisms present to host-searching myceto- or saprophagous beetles. This has interesting implications for the pollination of *A. triloba* flowers. The spring bloom of *A. triloba* patches often results in thousands of mature flowers open simultaneously, all emitting fermentation volatiles. In this scenario, *A. triloba* may exploit the beetles' sensory biases for highly concentrated host volatiles by providing a putative supernormal[141] floral advertisement that is irresistible to the beetles. Since patches of *A. triloba* are largely clonal[37], this could represent a shared reproductive investment by each individual ramet to ensure pollination is achieved for a species that is largely pollinator-limited[36].

In total, we have shown that *Glischrochilus* beetles are capable of detecting both fermentation and plant associated volatiles found in the headspace of *A. triloba* flowers, and that a majority of these detected compounds overlap with a set of fermentation compounds that have been shown to be attractive to *Glischrochilus* beetles in both field and laboratory experiments. We hypothesize that *Glischrochilus* beetles orient toward reliable microbial cues that honestly indicate microbial abundance/presence, and that requiring large concentrations of stimuli for antennal activation is adaptive for beetles searching for maximally profitable food/brood sites in

a noisy olfactory environment. Further, we posit that *A. triloba* flowers provide a sensory trap based on the beetles' bias for concentrated odor to ensure pollination.

The Influence of Microbial Volatiles on Pollinator Attraction

Insights from our microbiome analyses revealed that *A. triloba* flowers were colonized by fermentative bacteria and fungi, and that increases in relative abundances of these microbes on flowers were associated with increased production of fermentative floral volatiles, and corresponded with a shift in floral scent composition from blends with a vegetative character to those dominated by fermentative compounds over floral ontogeny. In the next step, our EAG experiments revealed that *G. fasciatus* beetles were able to detect fermentation volatiles present in *A. triloba* flowers. This result served as a proof of concept to show that the microbial production of fermentative floral volatiles in *A. triloba* was within the beetles' olfactory vocabulary, and therefore had the potential to influence pollinator behavior.

Using trap bioassays in the field we confirmed that microbial volatiles emitted from pawpaw flowers do affect pollinator attraction, thereby supporting our hypothesis of microbial-mediated pollinator attraction in *A. triloba.* Furthermore, our results show that only the primary pollinators of *A. triloba* flowers (beetles of the genus *Glischrochilus* in our region of NY) were influenced by microbial volatiles, with our treatments leaving the generalized, non-pollinating visitor spectrum associated with the flowers largely unaffected.

We designed our trap bioassays with influence from Koch's postulates and Douglas & Dobson (2013)[44] as a classic microbial removal and reintroduction experiment to test whether microbial volatiles specifically were attractive to floral visitors. Experiment 1 tested whether sterilized flowers were less attractive to visitors than sham-sterilized flowers. We were aware

from the start that the sterilization procedure would not eliminate all microbes from flowers[142] (also see microbiome data), therefore this treatment functioned as a microbial (and volatile) reduction rather than removal. Next, we conducted experiment 2, testing whether sterilized flowers with floral microbes (and their volatiles) reintroduced via plating were more attractive than sterilized flowers with empty media plates. Given that the two experiments were linked conceptually, we made the conservative choice to interpret our results as significant only if the same response variable tested was significant across both experiments 1 & 2. In addition, we were aware that Steenhuisen et al. (2013)[143] found that trap color visual cues had a significant effect on Cetoniine beetle capture, and that color alone could be sufficient to attract beetles to empty traps. Therefore, all our experiments also included an empty trap to control for visitor attraction via visual cues. We did not catch a single insect in our control traps over the entirety of our experiments and thus can be confident that olfactory cues alone explain our results.

Over the entire trapping experiment, we captured 508 arthropods representing 29 families and 61 species, which supports our natural history observations and results from our formal visitor survey that pawpaw flowers attract a wide assemblage of arthropods via its fermentative floral scent. It is interesting to us then, that only *Glischrochilus* beetles were significantly affected by our treatments in both experiments. We suspect that concentration of volatiles could play a role in this partitioning. Given that *G. fasciatus* beetles require large concentrations of several fermentation volatiles to elicit significant responses from antennae, relatively small changes in fermentation volatile emittance due to floral sterilization could drop the concentration threshold below the beetles' receptivity or behavioral preference, while simultaneously remaining attractive to other visitor guilds. Indeed, van der Niet et al. (2011)[144] showed that quantity of oligosulfide volatiles alone was sufficient to partition insect assemblages to carrion

bait. In their study, they found that carcasses that produced small amounts of carrion volatiles were more attractive to sarcophagid flies than the same carcass types emitting greater amounts of volatiles, which attracted significantly more muscid and calliphorid flies. They observed similar selective attraction of sarcophagids to the sympatric carrion mimicking orchid *Satyrium pumilum* and posited that the low volatile release of the flowers was responsible for this effect, as was seen with the carcasses. Stökl et al. (2010)[40] also showed a dose-dependent influence on drosophilid attraction to a synthetic blend of fermentation volatiles based on the scent of the yeast-mimicking Solomon's lily *Arum palaestinum*. Intriguingly, the doses required for antennal activation and attraction in their study were orders of magnitude lower for drosophilids than for the activation of *G. fasciatus* beetle antennae in our study. This alone could explain why drosophilid and total arthropod abundance (51% of which were drosophilids) was not significantly affected by floral sterilization, as even a reduction of microbial volatiles may not have dropped the concentration below the drosophilids' activation thresholds or behavioral preferences.

This olfactory threshold hypothesis is especially interesting to consider in light of *A. triloba* floral evolution. One hypothesis for why a flower might employ microbes for floral advertisement is that it could drive an evolutionary shift to a more effective pollinator class, for example see Shuttleworth and Johnson (2010)[145]. Drosophilid flies do not appear to be efficient pollinators for *A. triloba* as their small size prevents them from unintentionally making contact with the flowers' stamens, which sit at the center of a cup-shaped chamber formed by the flowers' inner petals with a diameter much larger than the body of a drosophilid fly. In addition, their behavior (especially of males) doesn't always result in flies entering the flowers' open chamber. Instead, groups of males are often observed on the outer petals of the flowers

performing courtship rituals[146] to attract females. By providing a more concentrated fermentative floral scent, as well as providing a food/brood-site reward with microbial decay of its tissues, pawpaw flowers in our region may be selectively attracting *Glischrochilus* beetles which spend much more time inside the flower chamber eating decaying tissue and mating. The floral architecture is sized such that when beetles feed or assume a mating posture they become covered in pollen. Therefore, the size of the chamber may also function as a floral filter[147] to match beetle size and restrict low-efficiency pollinators that are attracted to pawpaw's stereotyped fermentative scent via microbial decay. Another example of this can be seen in the maroon colored relative *A. triloba, Asimina parviflora.* Flowers of *A. parviflora* attract flies and beetles with their yeasty floral scent but are pollinated mainly by drosophilids, which are small enough to access the androgynoecium via the ~3 mm aperture formed by the inner petals[148]. This combination of fermentative floral scent and size-limiting aperture has been invoked as an explanation for pollinator specificity in the orchid *Gastrodea similis*, thought to mimic rotting fruit in Reunion Island[149]. In this species, a large guild of saprophilic drosophilid flies is selectively attracted by specific compounds/ratios in the floral scent of *G. similis* and is further winnowed by the small floral aperture, through which only the pollinator (*Scaptodrosophila bangi)* can enter. Similar trait combinations appear to be at play in pollinator diversification in the genus *Asimina*.

The results of experiment 2 were largely the same as experiment 1 with one difference being that total arthropod abundance and *Drosophila* abundance were also significantly increased by the reintroduction of microbes, in addition to increased *Glischrochilus* abundance. It is possible that the concentration of volatiles emitting from microbial plates may exceed that which was reduced from flowers via sterilization, though GC-MS peaks from plated microbes

show similar peak areas as whole flowers. However, traps were left in the field for 7 hours each day, and abiotic factors such as temperature may have affected microbial growth while the plates were in the traps. Conducting bioassays *in situ* often comes with tradeoffs, including a lack of the strict control and reproducibility provided by laboratory assays. Thus, even though we observed greater attraction of *Glischrochilus* beetles over both treatments in experiment 2 compared with experiment 1, we are encouraged that the ratio of *Glischrochilus* beetle capture between treatments (sterile vs. sham-sterile; sterile vs. sterile + microbes) was consistent across both experiments (1:3.2 for both).

Experiment 3 tested whether microbes alone were attractive to visitors. For both treatments (empty agar plates vs. inoculated media plates), too few arthropods were captured to evaluate trends. This could suggest that volatiles from floral microbes alone, either qualitatively or quantitatively, were not sufficient to attract visitors. However, we find this scenario unlikely as microbial plates with diverse types of inocula have been shown to be attractive, at least to drosophilids, in our region[133]. However, one possible difference is that this study[133] did not examine attractiveness of diverse community plates as we did. Another possibility could be, in terms of *Glischrochilu*s attraction specifically, that contextual cues from plant-produced floral volatiles were absent from the microbial plates. We know that *G. fasciatus* beetle antennae are receptive to the plant volatiles linalool and (*E*)-β-caryophyllene, and that typical food/brood sites for *Glischrochilus* beetles consist of fermenting plant matter that also release contextual plant volatiles in addition to microbial fermentation products. In this respect, plant contextual volatiles may be crucial for accurate host finding for *G. fasciatus* beetles. Dedicated behavioral experiments with addition and removal of plant contextual volatiles are needed to confirm this possibility. A more likely scenario may be that seasonal effects or depletion of individuals in the

immediate vicinity from our own capture and removal may have contributed to our low capture results. *Glischrochilus* beetles in particular show seasonality in their behavior, as well as preferences for certain habitat types[150,41]. We have also noticed local depletion of *Glischrochilus* beetles from our own trapping efforts unrelated to this study (observational only), a phenomenon that is of concern when abundance of visitors is monitored over time[151].

In conclusion, our combined results from experiments 1 & 2 confirm that microbial volatiles emitted from flowers do affect visitor attraction, primarily targeting the main pollinators of pawpaw flowers in our region of New York. Rather than through an exploitation of a phylogenetic sensory bias specific to *Glischrochilus* beetles, we hypothesize that increased concentration of fermentation volatiles via floral decay, combined with a morphological floral filter, contributes to a selective usage of *Glischrochilus* beetles as primary pollinators of *A. triloba* flowers.

What's in a phenotype? Thoughts on mimicry, deception, and conventional floral signaling in Asimina triloba

In flowers, mimicry is described as an adaptive resemblance of a flower (via advergent evolution) to a particular model, such that operators that usually interact with the model interact with the flower by mistake[16]. In a pollination context, the term deception is more related to floral reward status, than whether mimicry is being employed as a floral advertisement strategy. Rewarding flowers provide their advertised rewards, while deceptive flowers withhold their advertised rewards. Both mimicry and deception can involve false advertisement and withholding of specific rewards, but deception is different from mimicry in that advergent evolution to resemble a *specific* model need not be present. This is exemplified in the many

instances of flowers using generalized food deception (GFD) as a reproductive strategy. GFD flowers exploit the innate (or learned) biases of operators (pollinators) by producing abstract or stereotyped signals typical of food-rewarding flowers, without strict adherence to the signals of particular model species[152,153]. Exploitation of perceptual biases (EPB) in these scenarios may function by producing signals that elicit involuntary responses from operators, which is thought to release the emitter from the evolutionary constraints of strict model adherence[154,155]. We suspect that EPB is at work in pawpaw floral signaling, as the operators (*Glischrochilus* beetles) appear to use a combination of generic microbial fermentation volatiles, which are not representative of one particular fermenting resource, to find their typical food/brood sites. The myriad of food/brood resources documented to be used by *Glischrochilus* beetles[119,121-124] supports this hypothesis. However, EPB likely underlies the evolution of all animal-pollinated floral advertisement and says nothing about reward status, nor whether mimicry is operational in the system[5,156].

Considering these points, an important question is raised: do pawpaw flowers really 'play dead' (i.e. do they use some form of mimicry or deception for floral advertisement to exploit saprophagous insects for pollination)? Or, in contrast, is *being* dead a more accurate description of their strategy (i.e. advertising and providing fermenting plant matter as a reward to saprophagous insects as a pollination mutualism)? As Renner (2006)[157] has comprehensively reviewed, the answers to these types of questions are complicated because determining whether a flower is rewardless may depend on many variables including, ecological context, floral sex, type of pollinator(s), sex of pollinators, and pollinator physiological or cognitive state, among others. To complicate matters further, we have shown that microbes in pawpaw flowers are not neutral parties, therefore third-party chemical information must also be considered. In an effort

to answer this question, we provide a theoretical framework (**Figure 13)** to consider and discuss the possibilities below.

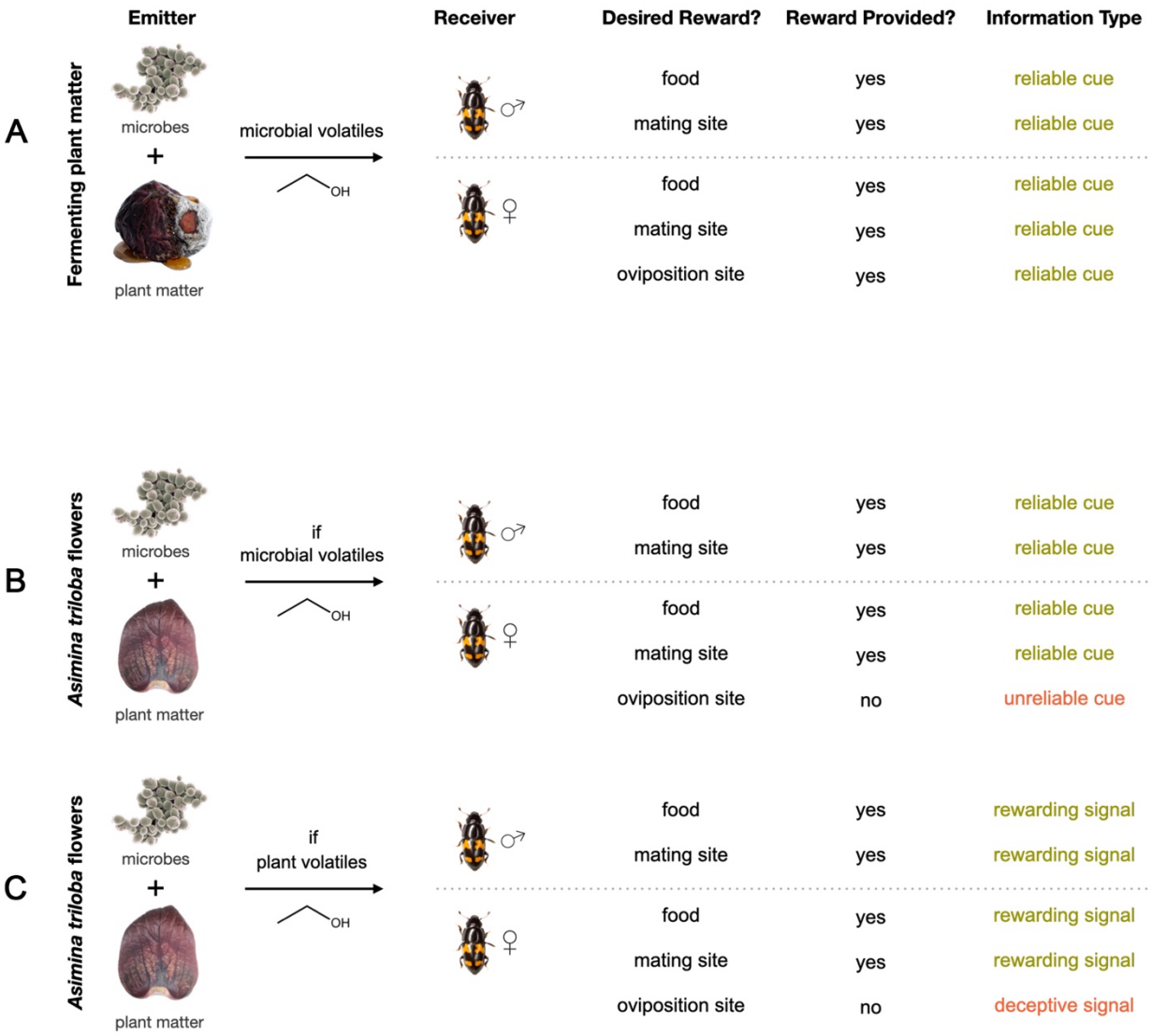

Figure 13. Signal theory comparing volatile information received by *Glischrochilus* beetles when the emitter is a conventional beetle food/brood-site (**A**), or *Asimina triloba* flowers (**B, C**). Male and female beetles differ in their desired rewards in that only females search for oviposition sites. Beetles usually locate their conventional food/brood-sites by following volatile cues produced by the microbial decomposition of organic matter (**A**). In this scenario, both male and female beetles receive all possible advertised rewards, thus making the microbial cues reliable. *A. triloba* flowers are functionally similar to conventional beetle food/brood-sites with the floral phenotype consisting of decaying plant matter plus microbial-produced volatiles emitted. If beetles are using microbial-produced volatiles to engage with *A. triloba* flowers (**B**), all advertised rewards are received by beetles except by females looking for an oviposition-site. In this one scenario, the microbial cues are unreliable. If beetles are using plant-produced volatiles to engage with *A. triloba* flowers (**C**), again all advertised rewards are received except by females searching for an oviposition-site. Only in this last scenario can the floral phenotype be considered 'deceptive'.

At its base level, a rotting fruit (i.e. the putative model; **Figure 13A**) consists of organic plant matter plus its associated fermentative microbes, which metabolize the carbohydrates in the fruit for energy[158]. This results in the release of fermentation volatile cues that *Glischrochilus* beetles use for host location. When searching for a host, the aggregate of desired rewards is likely food (microbes, decaying plant mater, or both), a mating opportunity, or in the case of female beetles only, an oviposition site. *Glischrochilus* beetles of both sexes have been documented receiving all of these reward types from fermenting substrates[159], therefore we can classify the microbial volatiles emitted from these substrates as 'reliable cues' that the beetles use to find all their desired rewards.

If we consider pawpaw flowers (the putative mimic or deceiver; **Figure 13B**), they also consist of organic plant matter that is decayed by fermentative microbes. We have shown that microbial volatiles emitted from fully functional flowers affect beetle attraction, and we have consistently observed male and female beetles feeding and mating within the flowers. We have not observed oviposition nor the presence of eggs within the flowers, however a dedicated survey is needed to be sure. We also do not know whether larval development could be supported in flowers. However, flowers abscise from trees rapidly and become part of the forest floor detritus. It is possible that eggs laid in attached flowers could be supported by additional resources in the leaf litter after they have fallen, as *Glischrochilus* beetles have been reported to use soil and leaves as sites for larval development[159]. Another possibility may be that beetles oviposit in fallen flowers (similar to the system described by Sakai 2002)[160], obtaining leftover pollen in the process, but extensive surveys of fallen flowers are needed to confirm or reject this hypothesis. We have confirmed that most *Glischrochilus* beetles arrive at flowers gravid (see **Table 3**), which provides circumstantial evidence that females may be using microbial cues

emitted from flowers to locate oviposition sites. Therefore, we can classify the microbial volatiles emitted as reliable cues for all advertised rewards except in the case of female beetles specifically searching for oviposition sites. In this one case the classification becomes an 'unreliable cue', and not a 'deceptive signal', since the microbial metabolic byproducts are received by beetles presumably inadvertently.

Since the employment of microbes to produce fermentation volatiles is not mutually exclusive with flowers also producing fermentation volatiles via their own metabolism, we must consider a scenario where plant-produced fermentative compounds are attractive to beetles (**Figure 13C**). The advertised and provided rewards are the same as the previous scenario, with only oviposition sites not provided to female beetles. Therefore, if beetles are engaging with plant-produced fermentation compounds, the information received by beetles would be considered rewarding signals if the floral phenotype evolved to track the beetles' sensory preferences[161]. Only in the case of female beetles searching for oviposition sites would the floral phenotype be considered deceptive.

What, then, does this mean in terms of conventional floral signaling? Conventional floral signaling is usually described in terms of a mutualism, in which floral signals have evolved to exploit the preferences and sensory abilities of pollinators in ways that maximize reproduction for the plant[162,163]. In return, pollinators are rewarded, usually with nectar or pollen as food, but many other rewards have been documented (e.g. oils, nest materials, nursery sites, etc.)[164]. Does the microbial decay and fermentative advertisement in pawpaw flowers represent an undocumented type of reward that is provided as part of a mutualistic partnership? Perhaps part of the answer lies in whether female beetles do oviposit in flowers, or whether beetles of both sexes find the flowers as profitable resources in terms of all the desired rewards discussed. If

beetle fitness is negatively affected by floral visitation, certainly the system would be exploitative (possibly EPB) rather than mutualistic, even if rewards are provided[155]. Another consideration is that the beetles do not use the flowers singularly for their food/brood site niches. In this vein, Goodrich & Jürgens (2017)[155], make a case for a rewarding mimicry system termed nondeceitful abstract homotypy (NAH) in which operators may find rewards and fitness benefits from interacting with flowers, while being attracted by abstract signals that are meaningful to operators in unrelated contexts. We would favor this description, but because mimicry implies some form of pretense and pawpaw flowers in most cases provide what they advertise, this classification isn't quite suitable. However, it may be the appropriate term if beetles are engaging with strictly plant-produced fermentation volatiles and find pawpaw flowers rewarding.

Semantics aside, these topics raise many questions and suggest avenues for future work. How do pawpaw flowers coordinate the rot in their nectary tissue? Is programmed cell death initiated in those tissues to allow saprotrophic microbes to proliferate? Or are those tissue left undefended (e.g. with no anthocyanin pigments) so that they are vulnerable to pathotrophic microorganisms? What role has the employment of microbes played in the evolution of derived, maroon brood-site associated floral phenotypes in the genus *Asimina*, from the ancestral condition demonstrated in the white, sweet-smelling flowers of sister taxa[165]? Do microbes represent a symbiotic bridge across low-fitness valleys in a theoretical fitness landscape, allowing a shift to a new pollinator class? Of the many brood-site associated phenotypes worldwide[14,15], how many are harnessing microbes as signal producers? Further exploration in this system promises to shed light on these and many other questions.

REFERENCES

1. Stebbins GL. 1970. Adaptive Radiation of reproductive characteristics in angiosperms, I: Pollination mechanisms. *Annual Review of Ecology and Systematics*. 1(1): 307-326
2. Crepet WL. 1984. Advanced (constant) insect pollination mechanisms – pattern of evolution and implications vis-à-vis angiosperm diversity. *Annals of the Missouri Botanical Garden*. 71: 607-630
3. Vamosi JC, Vamosi SM. 2010. Key innovations within a geographical context in flowering plants: towards resolving Darwin's abominable mystery. *Ecology Letters.* 13: 1270-1279
4. Whittall JB, Hodges SA. 2007. Pollinator shifts drive increasingly long nectar spurs in columbine flowers. *Nature*. 447: 706-712
5. Schiestl FP, Johnson SD. 2013. Pollinator-mediated evolution of floral signals. *Trends in Ecology and Evolution.* 28(5): 307-315
6. Dodd ME, Silvertown J, Chase MW. 1999. Phylogenetic analysis of trait evolution and species diversity variation among angiosperm families. *Evolution.* 53(3): 732-744
7. Dellinger AS, Chartier M, Fernández-Fernández D, Penneys DS, Alvear M, Almeda F, Michelangeli FA, Staedler Y, Armbruster WS, Schönenberger J. 2019. Beyond buzz-pollination - departures from an adaptive plateau lead to new pollination syndromes. *New Phytologist*. 221(2): 1136-1149
8. Endress PK. 2012. The immense diversity of floral monosymmetry and asymmetry across angiosperms. *The Botanical Review*. 78(4): 345-397

9. Jesson, LK, Kang J, Wagner SL, Barrett SC, Dengler NG. 2003. The development of enantiostyly. *American Journal of Botany*. 90(2): 183-195
10. Whittal JB, Hodges SA. 2007. Pollinator shifts drive increasingly long nectar spurs in columbine flowers. *Nature.* 447: 706-709
11. Smith SD. 2015. Pleiotropy and the evolution of floral integration. *New Phytologist.* 209(1): 80-85
12. Davis CC, Endress PK, Baum DA. 2008. The evolution of floral gigantism. *Current Opinion in Plant Biology.* 11(1): 49-57
13. Davis CC, Latvis M, Nickrent DL, Wurdack KJ, Baum DA. Floral gigantism in Rafflesiaceae. *Science*. 315(5820): 1812
14. Jürgens A, Wee SL, Shuttleworth A, Johnson SD. 2013. Chemical mimicry of insect oviposition sites: a global analysis of convergence in angiosperms. *Ecology Letters*. 16(9): 1157-1167
15. Urru I, Stensmyr MC, Hansson BS. 2011. Pollination by brood-site deception. *Phytochemistry*. 72: 1655-1666
16. Johnson SD, Schiestl FP. 2016. Floral Mimicry. Oxford (UK): Oxford University Press
17. Brodie BS, Babcock T, Gries R, Benn A, Gries G. 2016. Acquired smell? Mature females of the common green bottle fly shift semiochemical preferences from feces feeding sites to carrion oviposition sites. *Journal of Chemical Ecology*. 42(1): 40-50
18. Recinos-Aguilar YM, García-García MD, Malo EA, Cruz-López L, Cruz-Esteban S, Rojas JC. 2020. The Succession of Flies of Forensic Importance Is Influenced by Volatiles Organic Compounds Emitted During the First Hours of Decomposition of Chicken Remains. *Journal of Medical Entomology*. 57(5): 1411-1420

19. Chen G, Ma XK, Jürgens A, Lu J, Liu EX, Sun WB, Cai XH. 2015. Mimicking livor mortis: a well-known but unsubstantiated color profile in sapromyiophily. *Journal of Chemical Ecology*. 41(9): 808-815
20. Aleklett K, Hart M, Shade A. 2014. The microbial ecology of flowers: an emerging frontier in phyllosphere research. *Botany*. 92(2014): 253-266
21. Vannette RL. 2020. The Floral Microbiome: Plant, Pollinator, and Microbial Perspectives. *Annual Review of Ecology, Evolution, and Systematics*. 51(1): 363-386
22. Schaeffer RN, Irwin RE. 2014. Yeasts in nectar enhance male fitness in a montane perennial herb. *Ecology.* 95(7): 1792-1798
23. Herrera CM, Pozo MI, Medrano M. 2013. Yeasts in nectar of an early-blooming herb: sought by bumble bees, detrimental to plant fecundity. *Ecology.* 94(2): 273-279
24. Vannette RL, Gauthier MPL, Fukami T. 2013. Nectar bacteria, but not yeast, weaken a plant-pollinator mutualism. *Proceeding of the Royal Society B*. 280(1752): 20122601
25. Good AP, Gauthier MPL, Vannette RL, Fukami T. 2014. Honey bees avoid nectar colonized by three bacterial species, but not by a yeast species, isolated from the bee gut. *PLoS ONE.* 9(1): e86494. https://doi.org/10.1371/journal.pone.0086494
26. Rering CC, Beck JJ, Hall GW, McCartney MM, Vannette RL. 2017. Nectar-inhabiting microorganisms influence nectar volatile composition and attractiveness to a generalist pollinator. *New Phytologist.* 220(3): 750-759
27. Golonka AM, Johnson BO, Freeman J, Hinson DW. 2014. Impact of nectarivorous yeasts on *Silene caroliniana*'s scent. *Eastern Biologist.* 2014(3): 1-26
28. de Vega C, Albaladejo RG, Guzmán B, Steenhuisen SL, Johnson SD, Herrera CM, Lachance MA. 2017. Flowers as a reservoir of yeast diversity: description of

Wickerhamiella nectarea f.a. sp. nov., and *Wickerhamiella natalensis* f.a. sp. nov. from South African flowers and pollinators, and transfer of related *Candida* species to the genus *Wickerhamiella* as new combinations. *FEMS Yeast Research.* 17(5): August 2017, fox054.

29. Steenhuisen SL, Raguso RA, Jürgens A, Johnson SD. 2010. Variation in scent emission among floral parts and inflorescence developmental stages in beetle-pollinated Protea species (Proteaceae). *South African Journal of Botany*. 76(4): 779-787
30. Johnson SD, Jürgens A. 2010. Convergent evolution of carrion and faecal scent mimicry in fly-pollinated angiosperm flowers and a stinkhorn fungus. *South African Journal of Botany.* 76(4): 796-807
31. Stensmyr MC, Urru I, Collu I, Celander M, Hansson BS, Angioy AM. 2002. Rotting smell of dead-horse arum florets. *Nature*. 420: 625-626
32. Seymour RS, Gibernau M, Ito K. 2003. Thermogenesis and respiration of inflorescences of the dead horse arum *Heliodiceros muscivorus*, a pseudo-thermoregulatory aroid associated with fly pollination. *Functional Ecology.* 17(6): 886-894
33. Policha T, Grimaldi DA, Manobanda R, Troya A, Ludden A, Dentinger BTM, Roy BA. 2019. Dracula orchids exploit guilds of fungus visiting flies: new perspectives on a mushroom mimic. *Ecological Entomology*. 44(4): 457-470
34. Xi Z, Bradley RK, Wurdack KJ, Wong KM, Sugumaran M, Bomblies K, Rest JS, Davis CC. 2010. Horizontal transfer of expressed genes in a parasitic flowering plant. *BMC Genomics.* 13(1): 227

35. Barthlott W, Szarynski J, Vlek P, Lobin W, Korotkova N. 2008. A torch in the rainforest: Thermogenesis of the Titan arum (*Amorphophallus titanium*). *Plant Biology.* 11(4): 499-505

36. Willson MF, Schemske DW. 1980. Pollinator limitation, fruit production, and floral display in Pawpaw (*Asimina triloba). Bulletin of the Torrey Botanical Club*. 107(3): 401-408

37. Kral R. 1960. A revision of *Asimina* and *Deeringothamnus* (Annonaceae). *Brittonia*. 12(4): 233-278

38. Goodrich KR, Zjhra ML, Ley CA, Raguso RA. 2006. When flowers smell fermented: the chemistry and ontogeny of yeasty floral scent in pawpaw (*Asimina triloba*: Annonaceae). *International Journal of Plant Science*. 167(1): 33-46

39. Goodrich KR, Raguso RA. 2009. The olfactory component of floral display in *Asimina* and *Deeringothamnus* (Annonaceae). *New Phytologist*. 183(2): 457-469

40. Stökl J, Strutz A, Dafni A, Svatos A, Doubsky J, Knaden M, Sachse S, Hansson BS, Stensmyr MC. 2010. A deceptive pollination system targeting drosophilids through olfactory mimicry of yeast. *Current Biology*. 20(20): 1846-1852.

41. Blackmer JL, Phelan PL. 1995. Ecological analyses of Nitidulidae: seasonal occurrence, host choice and habitat preference. *Journal of Applied Entomology*. 119(1-5): 321-329

42. Skalbeck TC. 1976. The distribution of Nitidulidae in deciduous forests of North America[dissertation]. St. Paul: University of Minnesota

43. Sakai S, Kato M, Nagamasu H. 2000. *Artocarpus* (Moraceae)-gall midge pollination mutualism mediated by a male-flower parasitic fungus. *American Journal of Botany*. 87(3): 440-445

44. Douglas AE, Dobson AJ. 2013. New synthesis: animal communication mediated by microbes: fact or fantasy?. *Journal of Chemical Ecology.* 39:1149

45. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. *Applied and Environmental Microbiology*. 79:5112–5120

46. Koechli C, Campbell AN, Pepe-ranney C, Buckley DH. 2019. Assessing fungal contributions to cellulose degradation in soil by using high-throughput stable isotope probing. *Soil Biology and Biochemistry.* 130:150–158

47. Zhang J, Kobert K, Flouri T, Stamatakis A. 2014. PEAR: A fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics*. 30:614–620

48. Edgar RC. Search and clustering orders of magnitude faster than BLAST. 2010. *Bioinformatics*. 26:2460–2461

49. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, *et al.* 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*. 75:7537–7541

50. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, *et al.* The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. 2013. *Nucleic Acids Research*. 41:590–596

51. Bengtsson-Palme J, Ryberg M, Hartmann M, Branco S, Wang Z, *et al.* 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of

fungi and other eukaryotes for analysis of environmental sequencing data. *Methods in Ecology and Evolution*. 4:914–919

52. Palmer JM, Jusino MA, Banik MT, Lindner DL. 2018. Non-biological synthetic spike-in controls and the AMPtk software pipeline improve mycobiome data. *PeerJ*;2018. Epub ahead of print. DOI: 10.7717/peerj.4925
53. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. 2016. VSEARCH: A versatile open source tool for metagenomics. *PeerJ*. 2016:1–22
54. Nilsson RH, Larsson KH, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, *et al.* 2019. The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research*. 47:D259–D264
55. Costello M, Fleharty M, Abreu J, Farjoun Y, Ferriera S, *et al.* 2018. Characterization and remediation of sample index swaps by non-redundant dual indexing on massively parallel sequencing platforms. *BMC Genomics*. 2018:1–10
56. Farouni R, Djambazian H, Ferri LE, Ragoussis J, Najafabadi HS. 2020. Model-based analysis of sample index hopping reveals its widespread artifacts in multiplexed single-cell RNA-sequencing. *Nature Communications*. 11:1–8
57. Kim HY. 2017. Statistical notes for clinical researchers : Chi-squared test and Fisher ' s exact test. *Restorative Dentistry & Endodontics.* 42(2):152-155
58. Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, *et al.* 2016. FUNGuild : An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*. 20:241–248
59. De Caceres M, Legendre P. 2009. Associations between species and groups of sites: indices and statistical inference. Ecology, URL

http://sites.google.com/site/miqueldecaceres/. *Ecology*. 90:3566–3574

60. Douglas GM, Maffei VJ, Zaneveld J, Yurgel SN, Brown JR, *et al.*2020. PICRUSt2: An improved and customizable approach for metagenome inference 2. *bioRxiv* 2020:1–16
61. Caspi R, Altman T, Billington R, Dreher K, Foerster H, *et al.* 2014. The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of Pathway / Genome Databases. 42:459–471.
62. Liu H, Roeder K, Wasserman L. 2010. Stability approach to regularization selection (StARS) for high dimensional graphical models. *Advances in Neural Information Processing Systems.* 24(2):1432-1440
63. Kurtz ZD, Müller CL, Miraldi ER, Littman DR, Blaser MJ, *et al.* 2015. Sparse and Compositionally Robust Inference of Microbial Ecological Networks. *PLoS Computational Biology*. 11:1–25
64. Csardi G, Nepusz T. 2006. The igraph software package for complex network research. *InterJournal*. http://igraph.org
65. Bousquet Y. 1990. Beetles associated with stored products in Canada: an identification guide. Research Branch, Agriculture Canada, Publication 1837, Ontario (Canada)
66. Peng C, Williams RN. 1990. Multiple-species rearing diet for sap beetles (Coleoptera: Nitidulidae). *Annals of the Entomological Society of America.* 83(6): 1155-1157
67. Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*. 67(1): 1-48. doi:10.18637/jss.v067.i01.
68. R core team. 2021. R: a language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria.* URL https://www.R-project.org
69. Lenth R. 2019. emmeans: Estimated Marginal Means, aka Least-Squares

Means. R package version 1.3.3. https://CRAN.R-project.org/package=emmeans

70. Schindelin, J, Arganda-Carreras I, Frise E et al. 2012. Fiji: an open-source platform for biological-image analysis. *Nature Methods.* 9(7): 676-682
71. Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods.* 9(7): 671-675
72. Harrison XA. 2014. Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ.* 2:e616 https://doi.org/10.7717/peerj.616
73. Smith B, Wilson J. 1996. A Consumer's Guide to Evenness Indices. *Oikos.* 76(1): 70-82. doi:10.2307/3545749
74. Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)*. 57(1): 289-300.
75. Chen Y, Zhang W, Yi H, Wang B, Xiao J, *et al.* 2020. Microbial community composition and its role in volatile compound formation during the spontaneous fermentation of ice wine made from Vidal grapes. *Process Biochemistry*. 92:365–377
76. Li AH, Yuan FX, Groenewald M, Bensch K, Yurkov AM, *et al.* 2020. Diversity and phylogeny of basidiomycetous yeasts from plant leaves and soil: Proposal of two new orders, three new families, eight new genera and one hundred and seven new species. *Studies in Mycology*. 96:17–140
77. Liu D, Legras J-L, Zhang P, Chen D, Howell K. 2021. Diversity and dynamics of fungi during spontaneous fermentations and association with unique aroma profiles in wine. *International Journal of Food Microbiology*. 338:108983

78. Merín MG, Mendoza LM, Morata de Ambrosini VI. 2014. Pectinolytic yeasts from viticultural and enological environments: Novel finding of *Filobasidium capsuligenum* producing pectinases. *Journal of Basic Microbiology*. 54:835–842

79. Handel S, Wang T, Yurkov AM, König H. 2016. *Sugiyamaella mastotermitis* sp. nov. and *Papiliotrema odontotermitis* f.a., sp. nov. from the gut of the termites *Mastotermes darwiniensis* and Odontotermes obesus. *International Journal of Systematic and Evolutionary Microbiology*. 66:4600–4608

80. Leite RSR, Bocchini DA, Martins EDS, Silva D, Gomes E, *et al.* 2007. Production of cellulolytic and hemicellulolytic enzymes from *Aureobasidium pulluans* on solid state fermentation. *Applied Biochemistry and Biotechnology*. 137:281–288

81. Molnárová J, Vadkertiová R, Stratilová E. 2014. Extracellular enzymatic activities and physiological profiles of yeasts colonizing fruit trees. *Journal of Basic Microbiology*. 54:74–84

82. Ghimire N, Han SR, Kim B, Park H, Lee JH, *et al.* 2020. Comparative Genomic Study of Polar Lichen-Associated *Hymenobacter* sp. PAMC 26554 and *Hymenobacter* sp. PAMC 26628 Reveals the Presence of Polysaccharide-Degrading Ability Based on Habitat. *Current Microbiology*. 77:2940–2952

83. Buczolits S, Busse H. 2015. *Hymenobacter*. *Bergey's Manual of Systematics of Archaea and Bacteria*. 1–11

84. Nabhan S, De Boer SH, Maiss E, Wydra K. 2013. *Pectobacterium aroidearum* sp. nov., a soft rot pathogen with preference for monocotyledonous plants. *International Journal of Systematic and Evolutinary Microbiology.* 63(7): 2520–2525

85. Effantin G, Rivasseau C, Gromova M, Bligny R, Hugouvieux-Cotte-Pattat N. 2011. Massive production of butanediol during plant infection by phytopathogenic bacteria of the genera *Dickeya* and *Pectobacterium*. *Molecular Microbiology.* 82:988–997

86. Prior R, Feige A, Begerow D. 2017. Antagonistic activity of the phyllosphere fungal community. *Sydowia*. 69:183–198

87. Boiocchi F, Porcellato D, Limonta L, Picozzi C, Vigentini I, *et al.* 2017. Insect frass in stored cereal products as a potential source of Lactobacillus sanfranciscensis for sourdough ecosystem. *Journal of Applied Microbiology.*123(4): 944–955

88. Molina JJ, Harrison MD, Brewer JW. 1974. Transmission of *Eriwinia carotovora* var. atroseptica by *Drosophila melanogaster* - acquisition and transmission of the bacterium. *American Potato Journal.* 51:245–250

89. Welte CU, de Graaf RM, van den Bosch TJM, Op den Camp HJM, van Dam NM, *et al.* 2016. Plasmids from the gut microbiome of cabbage root fly larvae encode SaxA that catalyses the conversion of the plant toxin 2-phenylethyl isothiocyanate. *Environmental Microbiology*. 18(5):1379–1390

90. Rossmann S, Dees W, Perminow J, Meadow R, Brurberg B. 2018. Soft Rot *Enterobacteriaceae* Are Carried by a Large Range of Insect Species in Potato Fields. *Applied and Environmental Microbiology*. 84(12):1–11

91. Rebolleda-Gómez M. Forrester NJ, Russell AL, Wei N, Fetters AM, Stephens JD, Ashman TL. 2019. Gazing into the anthosphere: considering how microbes influence floral evolution. *New Phytologist.* 224(3):1012-1020

92. Schaeffer RN, Vannette RL, Irwin RE. 2005. Nectar yeasts in *Delphinium nuttallianum* (Ranunculaceae) and their effects on nectar quality. *Fungal Ecology.* 18:100-106

93. Herrera CM, García IM, Pérez R. 2008. Invisible floral larcenies: microbial communities degrade floral nectar of bumble bee-pollinated plants. *Ecology*. 89(9):2369-2376

94. Vannette RL, Fukami T. 2018. Contrasting effects of yeast and bacteria on floral nectar traits. *Annals of Botany.* 121:1343-1349

95. von Arx M, Moore A, Davidowitz G, Arnold AE. 2019. Diversity and distribution of microbial communities in floral nectar of two night-blooming plants of the Sonoran Desert. *PLoS ONE*: 14:e0225309

96. Morris MM, Frixione NJ, Burkert AC, Dinsdale EA, Vanette RL. 2020. Microbial abundance, composition, and function in nectar are shaped by flower visitor identity. *FEMS Microbiology Ecology.* 96(3):fiaa003

97. Vorholt JA. 2012. Microbial life in the phyllosphere. *Nature Reviews Microbiology*. 10:828–840

98. Engel P, Moran NA. 2013. The gut microbiota of insects - diversity in structure and function. *FEMS Microbiol Reviews*. 37:699–735

99. Yun JH, Roh SW, Whon TW, Jung MJ, Kim MS, *et al.* 2014. Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. *Applied and Environmental Microbiology*. 80:5254–5264.

100. Lachance MA, Starmer WT, Rosa CA, Bowles JM, Barker JSF, Janzen DH. 2001. Biogeography of the yeasts of ephemeral flowers and their insects. *FEMS Yeast Research.* 1(1):1-8

101. de Vega C. Herrera CM. 2013. Microorganisms transported by ants induce changes in floral nectar composition of an ant-pollinated plant. *American Journal of Botany.* 100(4):792-800

102. Shade A, Handelsman J. 2011. Beyond the Venn diagram: the hunt for a core microbiome. *Environmental Microbiology.* 14(1):4-12

103. Clay K, Holah J. 1999. Fungal endophyte symbiosis and plant diversity in successional fields. *Science*. 285(5434):1742-1744

104. Rudgers JA, Holah J, Orr SP, Clay K. 2007. Forest succession suppressed by an introduced plant-fungal symbiosis. *Ecology*. 88(1):18-25

105. Norman EM, Clayton D. 1986. Reproductive biology of two Florida pawpaws: *Asimina obovata* and *A. pygmaea* (Annonaceae). *Bulletin of the Torrey Botanical Club*. 113(1):16-22

106. Lev-Yadun S, Gould KS. 2008. Anthocyanins. New York (NY): Springer. Role of Anthocyanins in Plant Defense; p. 22-28

107. Schmidt SK, Nemergut DR, Darcy J, Lynch R. 2014. Do bacterial and fungal communities assemble differently during primary succession? *Molecular Ecology*. 23(2):254–258

108. Wang J, Liu G, Zhang C, Wang G, Fang L, *et al.* 2019. Higher temporal turnover of soil fungi than bacteria during long-term secondary succession in a semiarid abandoned farmland. *Soil and Tillage Research*. 194:104305

109. Hilber-Bodmer M, Schmid M, Ahrens CH, Freimoser FM. 2017. Competition assays and physiological experiments of soil and phyllosphere yeasts identify *Candida subhashii* as a novel antagonist of filamentous fungi. *BMC Microbiology*. 17:1–15

110. Moore A. 2015. Pawpaw: in search of America's forgotten fruit. VT (USA): Chelsea Green Publishing

111. Price MV, Waser NM, Irwin RE, Campbell DR, Brody AK. 2005. Temporal and spatial variation in pollination of a montane herb: a seven-year study. *Ecology.* 86(8):2106-2116

112. Ashman TL. 2009. Sniffing out patterns of sexual dimorphism in floral scent. *Functional Ecology.* 23(5):852-862

113. Heiling AM, Herberstein ME, Chittka L. Crab-spiders manipulate flower signals. *Nature*. 421:334

114. Gottsberger G. 2012. How diverse are Annonaceae with regard to pollination?. *Botanical Journal of the Linnean Society*. 169(1):245-261

115. Waser NM, Chittka L, Price MV, Williams NM, Ollerton J. 1996. Generalization in pollination systems, and why it matters. *Ecology.* 77(4):1043-1060

116. Ollerton J, Killick A, Lamborn E, Watts S, Whiston M. 2007. Multiple meanings and modes: on the many ways to be a generalist flower. *Taxon*. 56(3):717-728

117. Fishbein M, Venable DL. 1996. Diversity and temporal change in the effective pollinators of Asclepias tuberosa. *Ecology*. *77*(4):1061-1073

118. Juzwik J, French DW. 1983. *Caratocystis fagacearum* and *C. piceae* on the surfaces of free-flying and fungus-mat-inhabiting nitidulids. *Phytopathology*. 73:1164-1168

119. Juzwik J, Cease KR. 2001. Predominant nitidulid species (Coleoptera: Nitidulidae) associated with spring oak wilt mats in Minnesota. *Canadian Journal of Forest Research*. 31: 635–643

120. Vogt GB. 1950. Occurrence and records of Nitidulidae. *The Coleopterist's Bulletin*. 4(6):81-91

121. Majika CB. 2010. Insects attracted to maple sap: observations from Prince Edward Island, Canada. *Zookeys*. 51:73-83

122. Myers L. 2004. Sap Beetles (of Florida), Nitidulidae (Insecta: Coleoptera: Nitidulidae). University of Florida-IFAS, Cooperative Extension Service, EENY-256.

123. Foott WH, Timmins PR. 1970. Importance of field corn as a reproductive site for *Glischrochilus quadrisignatus* (Say) (Coleoptera: Nitidulidae). *Proceedings of the Entomological Society of Ontario*. 101:73-75

124. Foott, WH, Hybsky JE. 1986. Capture of *Glischrochilus quadrisignatus* (Coleoptera: Nitidulidae) in bait traps, 1970-74. *The Canadian Entomologist.* 108:837-839

125. Stensmyr MC, Larsson MC, Bice S, Hansson BS. 2001. Detection of fruit- and flower-emitted volatiles by olfactory receptor neurons in the polyphagous fruit chafer *Pachnoda marginata* (Coleoptera: Cetoniinae). *Journal of Comparative Physiology A.* 187:509-519

126. Lin H, Phelan PL. 1991. Identification of food volatiles attractive to *Glischrochilus quadrisignatus* and *Glischrochilus fasciatus* (Coleoptera: Nitidulidae). *Journal of Chemical Ecology*. 17(12):2469-2480

127. Alm SR, Hall FR, Ladd Jr. TL, Williams RN. 1985. A chemical attractant for *Glischrochilus quadrisignatus* (Coleoptera: Nitidulidae). *Journal of Economic Entomology.* 78:839-845

128. Alm SR, Hall FR, McGovern TP, Williams RN. 1986. Attraction of *Glischrochilus quadrisignatus* (Coleoptera: Nitidulidae) to semiochemicals: butyl acetate and propyl propionate. *Journal of Economic Entomology.* 79:654-658

129. Lee MH, Lee S, Leschen RAB, Lee S. 2020. Evolution of feeding habits of sap beetles (Coleoptera: Nitidulidae) and placement of Calonecrinae. *Systematic Entomology.* 45(4):911-923

130. Lin H, Phelan PL. 1992. Comparison of volatiles from beetle-transmitted *Ceratocystis fagacearum* and four non-insect-dependent fungi. *Journal of Chemical Ecology.* 18:1623-1632

131. Becher PG, Flick G, Rozpedowska E, Schmidt A, Hagman A, Lebreton S, Larsson MC, Hansson BS, Piskur J, Witzgall P, Bengtsson M. 2012. Yeast, not fruit volatiles mediate *Drosophila melanogaster* attraction, oviposition and development. *Functional Ecology.* 26:822–828

132. Arguello JR, Sellanes C, Lou YR, Raguso RA. 2013. Can yeast (*S. cerevisiae*) metabolic volatiles provide polymorphic signaling? *PLoS ONE* 8(8): e70219. https://doi.org/10.1371/journal.pone.0070219

133. Bueno E, Martin KR, Raguso RA, Mcmullen II JG, Hesler SP, Loeb GM, Douglas AE. 2020. Response of wild spotted wing Drosophila (*Drosophila suzukii*) to microbial volatiles. *Journal of Chemical Ecology.* 46:688–698

134. Lin H, Phelan PL. 1991. Identification of food volatiles attractive to *Carpophilus lugubris* (Coleoptera: Nitidulidae). *Journal of Chemical Ecology*. 17:1273-1286

135. Phelan PL, Lin H. 1991. Chemical characterization of fruit and fungal volatiles attractive to the dried fruit beetle, *Carpophilus hemipterus* (L.) (Coleoptera: Nitidulidae) *Journal of Chemical Ecology*. 17:1253-1272

136. Knudsen JT, Eriksson R, Gershenzon J, Ståhl B. 2006. Diversity and distribution of floral scent. *The Botanical Review*. 72(1):1-120

137. Bernreuther A, Schreierc P. 1991. Multidimensional gas chromatography/mass spectrometry: A powerful tool for the direct chiral evaluation of aroma compounds in

plant tissues. II. Linalool in essential oils and fruits. *Phytochemical Analysis.* 2(4):167-170

138. Lalel HJD, Singh Z, Tan SC. 2003. Aroma volatiles production during fruit ripening of 'Kensington Pride' mango. *Postharvest Biology and Technology*. 27(3):323-336

139. Schulz S, Dickschat JS. 2007. Bacterial volatiles: the smell of small organisms. *Natural Product Reports.* 24:814-842

140. Stuhl CJ. 2021. Small hive beetle (Coleoptera: Nitidulidae) attraction to a blend of fruit volatiles. *Florida Entomologist*.104(3):153-157

141. Vereecken NJ, Schiestl FP. 2008. The evolution of imperfect floral mimicry. *PNAS.* 105(21):7484-7488

142. Turner TR, James EK, Poole PS. 2013. The plant microbiome. *Genome Biology*. 14:209

143. Steenhuisen SL, Jürgens A, Johnson SD. Effects of volatile compounds emitted by *Protea* species (Proteaceae) on antennal electrophysiological responses and attraction of cetoniine beetles. *Journal of Chemical Ecology.* 39:438-446

144. van der Niet T, Hansen DM, Johnson SD. 2011. Carrion mimicry in a South African orchid: flowers attract a narrow subset of the fly assemblage on animal carcasses. *Annals of Botany*. 107(6):981-992

145. Shuttleworth A, Johnson SD. 2010. The missing stink: sulphur compounds can mediate a shift between fly and wasp pollination systems. *Proceedings of the Royal Society B*. 277:2811-2819

146. Spieth HT. 1974. Courtship behavior in *Drosophila*. *Annual Review of Entomology.* 19:385-405

147. Johnson SD, Hargreaves AL, Brown M. 2006. Dark, bitter tasting nectar functions as a filter of flower visitors in a bird-pollinated plant. *Ecology*. 87(11):2709-2716

148. Norman EM, Rice K, Cochran S. 1992. Reproductive biology of *Asimina parviflora* (Annonaceae). *Bulletin of the Torrey Botanical Club*. 19(1):1-5

149. Martos F, Cariou ML, Pailler T, Fournel J, Bytebier B, Johnson SD. 2015. Chemical and morphological filters in a specialized floral mimicry system. *New phytologist*. 207(1):225-234

150. Dowd PF, Nelsen TC. 1994. Seasonal variation of sap beetle (Coleoptera: Nitidulidae) populations in central Illinois cornfield-oak woodland habitat and potential influence of weather patterns. *Environmental Entomology*. 23(5):1215-1223

151. van Herk WG, Vernon RS. 2020. Local depletion of click beetle populations by pheromone traps is weather and species dependent. *Environmental Entomology*. 49(2):449-460

152. Jersáková J, Johnson SD, Jürgens A. 2009. Plant-Environment Interactions, Signaling and Communication in Plants. Berlin Heidlberg: Springer-Verlag. Deceptive behaviour in plants II. Food deception by plants: from generalized systems to specialized floral mimicry; p. 223-246

153. Jersáková J, Johnson SD, Kindlmann P. 2006. Mechanism and evolution of deceptive pollination in orchids. *Biological Reviews*. 81:219-235

154. Schaefer HM, Ruxton GD. 2009 Deception in plants: mimicry or perceptual exploitation? *Trends in Ecology & Evolution.* 24(12):676-685

155. Goodrich KR, Jürgens A. 2017. Pollination systems involving floral mimicry of fruit: aspects of their ecology and evolution. *New Phytologist.* 217(1):74-81

156. Schiestl FP, Johnson SD, Raguso RA. 2010. Floral evolution as a figment of the imagination of pollinators. *Trends in Ecology & Evolution.* 25(7):382-383

157. Renner SS. 2006. Plant-Pollinator Interactions: From Specialization to Generalization. Chicago (IL): University of Chicago Press. Rewardless Flowers in the Angiosperms and the Role of Insect Cognition in Their Evolution; p. 123-144

158. Janzen DH. 1977. Why fruits rot, seeds mold, and meat spoils. *The American Naturalist.* 111(980):691-713

159. McCoy CE, Brindley TA. 1961. Biology of the four-spotted fungus beetle, *Glischrochilus q. quadrisignatus* and its effect on european corn borer populations. *Journal of Economic Entomology*. 54(4):713-117

160. Sakai S. *Aristolochia* spp. (Aristolochiaceae) pollinated by flies breeding on decomposing flowers in Panama. *American Journal of Botany.* 89(3):527-534

161. Schaefer HM, Ruxton GD. 2011. Plant-Animal Communication. Oxford (UK): Oxford University Press

162. Dobson HEM. 1994. Insect-Plant Interactions Volume V. Boca Raton (FL): CRC press. Floral Volatiles in Insect Biology; p. 47-81

163. Wright GA, Schiestl FP. The evolution of floral scent: the influence of olfactory learning by insect pollinators on the honest signalling of floral rewards. *Functional Ecology.* 23(5):841-851

164. Simpson BB, Neff JL. 1981. Floral rewards: Alternatives to pollen and nectar. *Annals of the Missouri Botanical Garden.* 68(2):301-322

165. Mercer E, Griffin B, Steele J. 2015. Phylogenetic relationships of *Asimina* and *Deeringothamnus* (Annonaceae) based on morphology, floral scent chemistry, and Inter-Simple Sequence Repeat data. *Journal of the Torrey Botanical Society*. 143(1):58-68

APPENDIX

Figure S1. Floral visitor exclusion experiment bagging procedure. Branches were either (**A**) bagged completely or (**B**) sham-bagged with a 15 cm hole cut into the bag allowing access to floral visitors (biotic microbial vectors). (**C**) Terminal ends of branches were bagged at random with up to 8 floral buds contained in each bag. (**D**) Flowers were left to develop in bags until sexual maturity and then harvested into sterile containers for GC-MS and microbiome analyses.

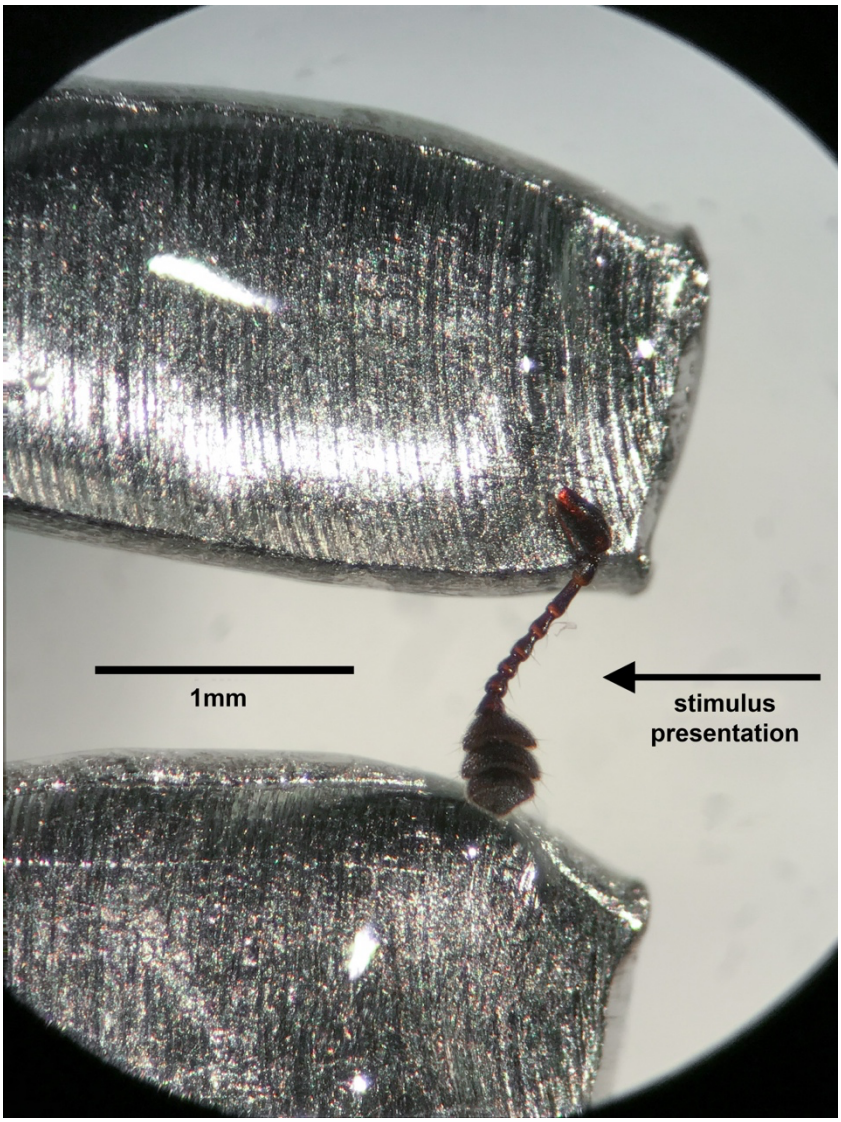

Figure S2. Gel electrode setup for EAG recordings from *Glischrochilus fasciatus* antennae.

Figure S3. Homemade trap design for insect bioassays. The trap consists of a 1000 ml clear deli cup modified to use a commercial funnel trap opening found in Rescue! brand pest control traps. Shown here is a trap baited with a microbial agar plate, five female stage *A. triloba* flowers, and a screen between the two stimuli to prevent insects from reaching the microbes for easy capture and cleanup, while also allowing microbial volatiles to emanate from the trap.

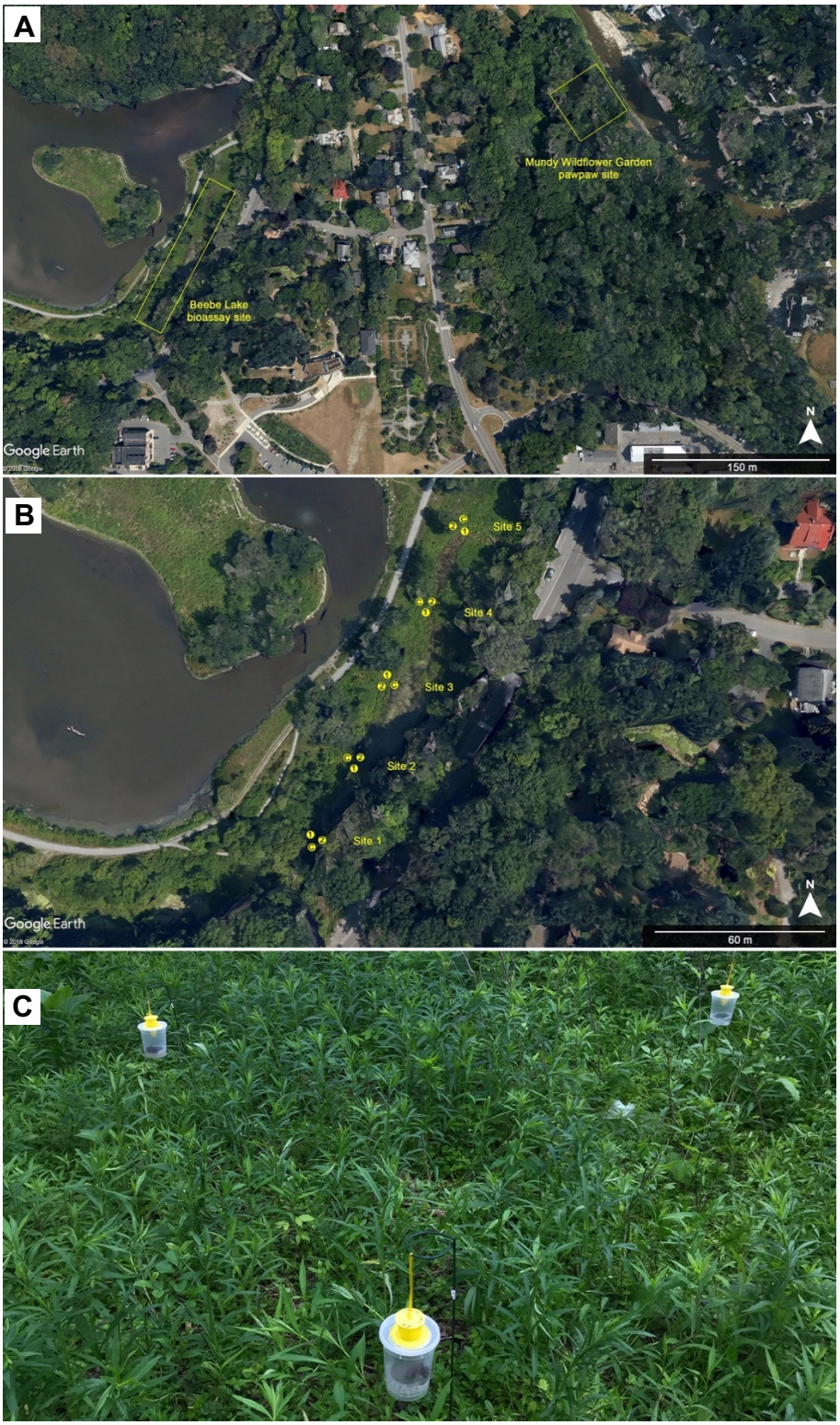

Figure S4. Insect Bioassay and *A. triloba* field experiment sites on Cornell University campus. (**A**) The Beebe Lake bioassay site and Mundy Garden *A. triloba* field site separated by 350m, each in a riparian/mesic woodland habitat. (**B**) Bioassay design at the Beebe Lake site showing trap arrangement in equilateral triangles at five sites along a 120m transect. (**C**) Three-choice triangle trap arrangement.

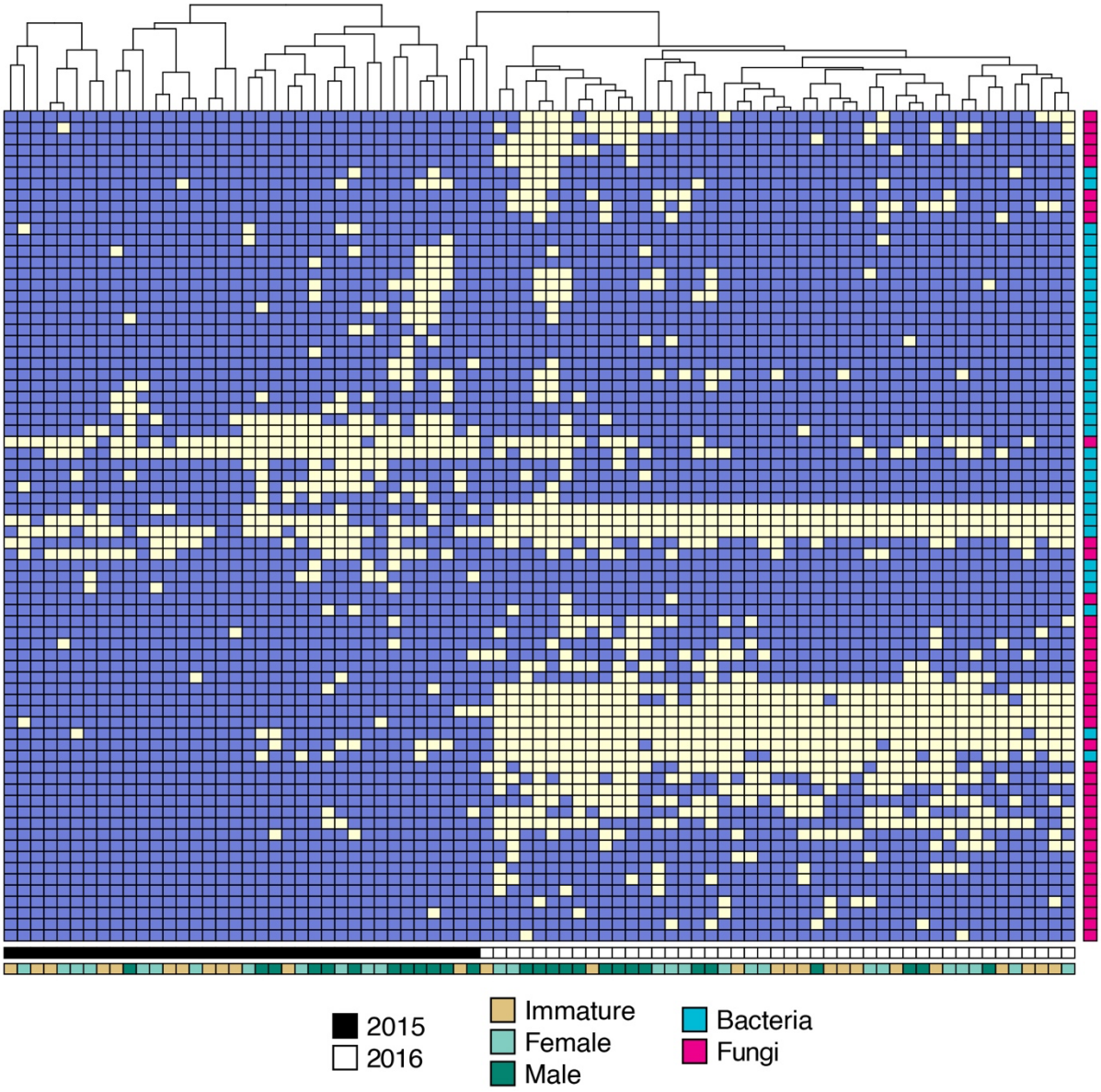

Figure S5. A heat map clustered by Bray-Curtis dissimilarity of volatile-associated OTUs present on flowers *in vivo.* Each small square represents one OTU; yellow boxes represent presence of a particular OTU, while blue boxes represent absences. The patterns of presence/absence differed strongly from year-to-year and by developmental stage.

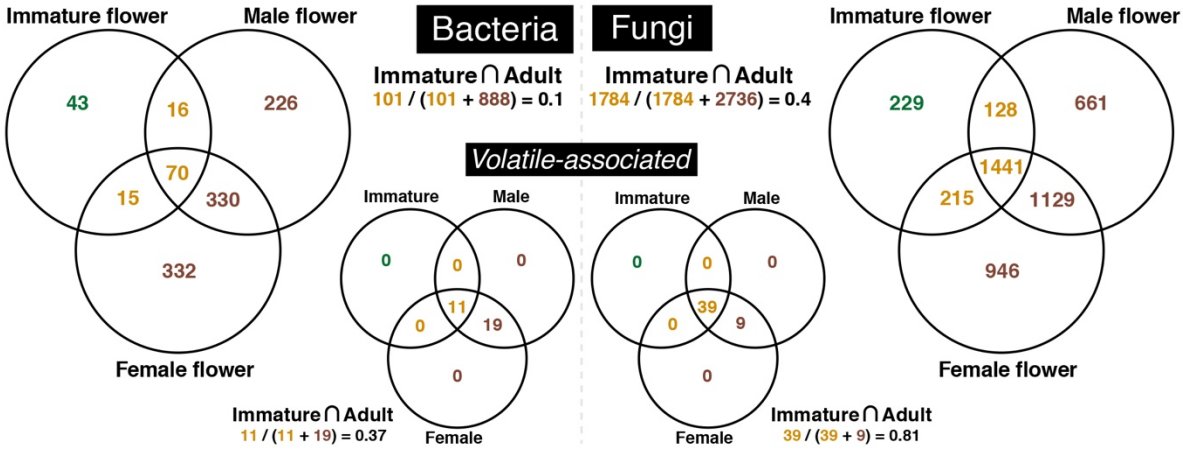

Figure S6. Venn diagrams showing overlap of OTUs among immature, female and male flowers for whole and volatile associated microbial communities.

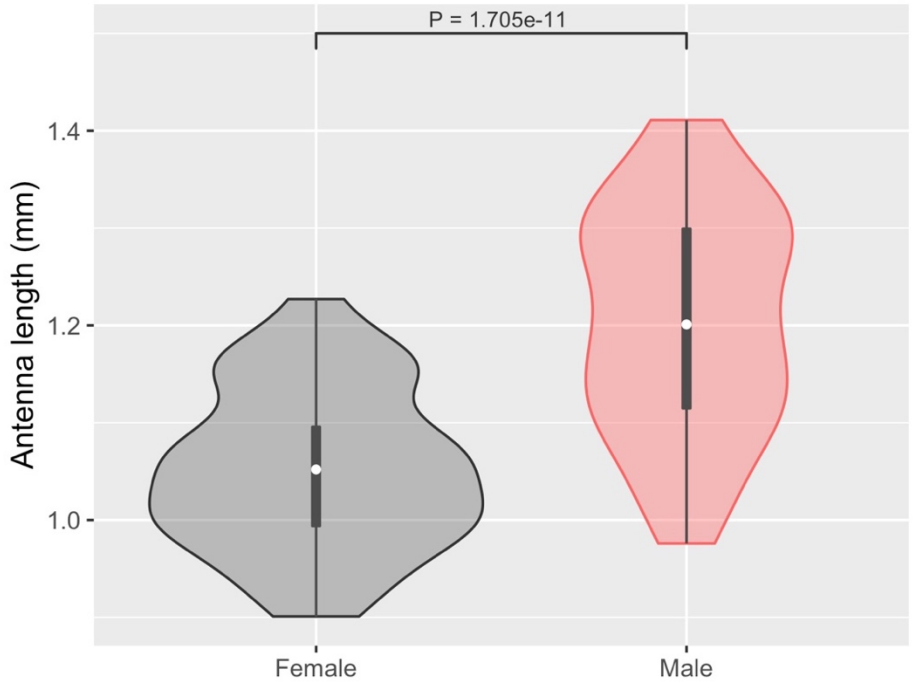

Figure S7. Violin plots depicting the sexual dimorphism in antennal length between female and male *G. fasciatus* beetles. A *p*-value is given to indicate significant differences between means (Welch's t-test). White dots within boxplots indicate the median antennal length for each sex.

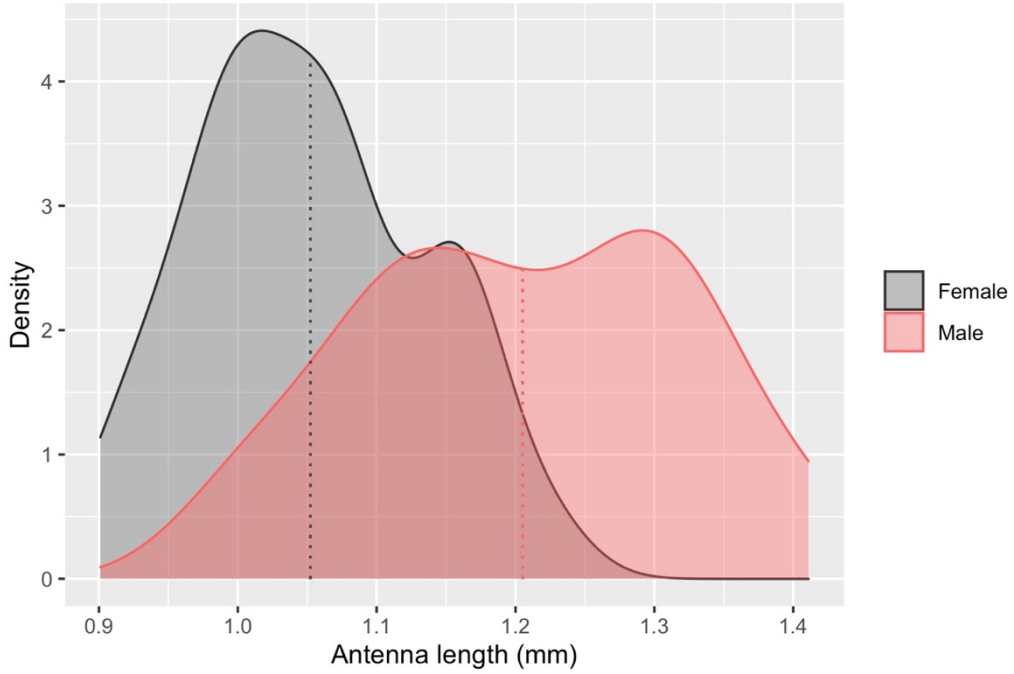

Figure S8. A kernel density plot depicting the sexual dimorphism in antennal length between female and male *G. fasciatus* beetles. Mean antennal length for each sex is indicated by a vertical dotted line.

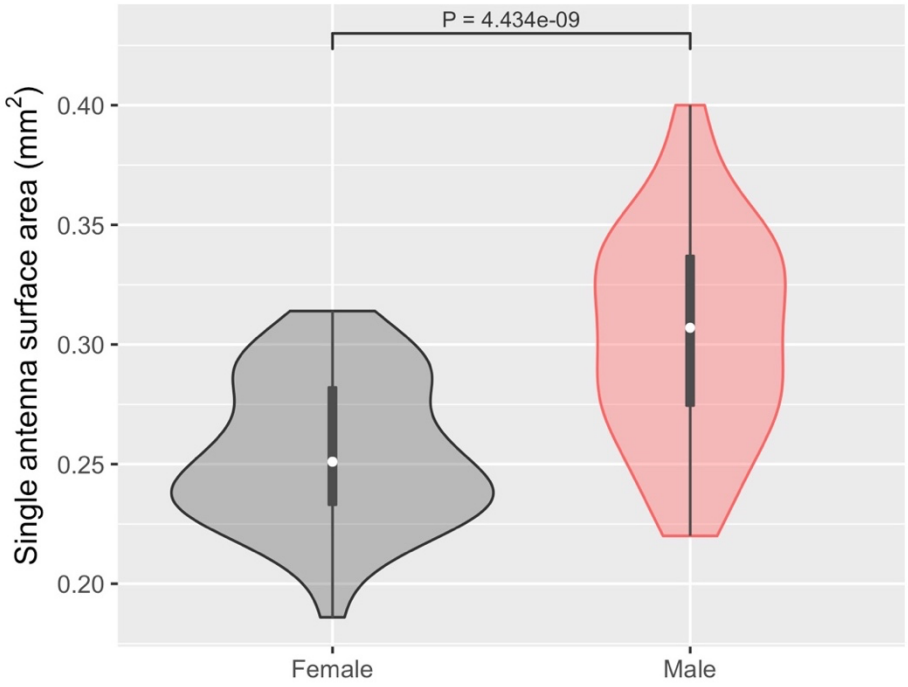

Figure S9. Violin plots depicting the sexual dimorphism in antennal surface area between female and male *G. fasciatus* beetles. A *p*-value is given to indicate significant differences between means (Welch's t-test). White dots within boxplots indicate the median antennal surface area for each sex.

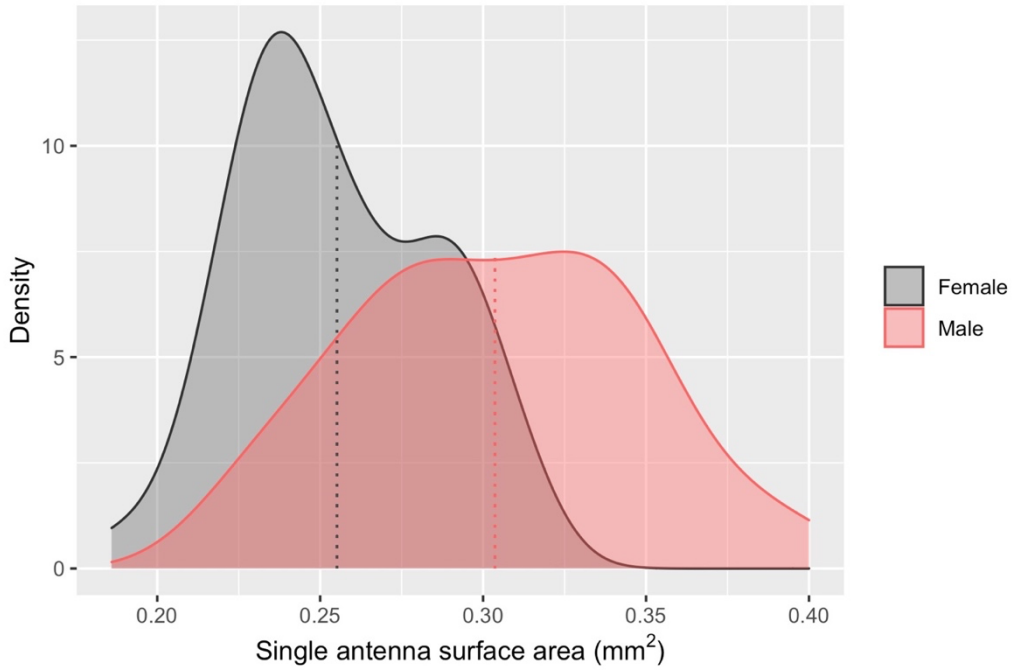

Figure S10. A kernel density plot depicting the sexual dimorphism in antennal surface area between female and male *G. fasciatus* beetles. Mean antennal length for each sex is indicated by a vertical dotted line.

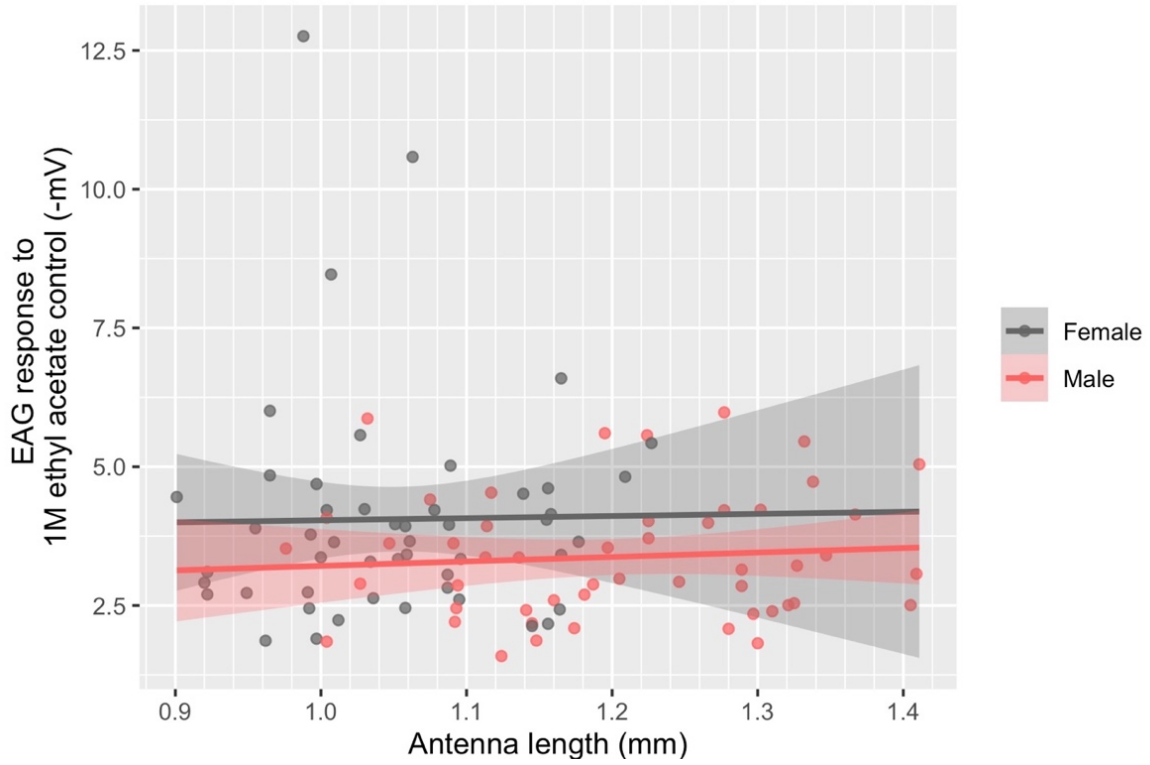

Figure S11. EAG response (-mV) to a 1M Ethyl acetate reference stimulus as a function of antennal length (single linear regression, one for each sex). Transparent bands represent 0.95 confidence intervals of values as predicted by each linear regression. Antennal length was not a significant predictor or EAG response for either sex (female, $R^2 = 0.0002$, $F_{1, 48} = 0.0115$, $p = 0.9151$; male, $R^2 = 0.0068$, $F_{1, 48} = 0.3280$, $p = 0.5695$).

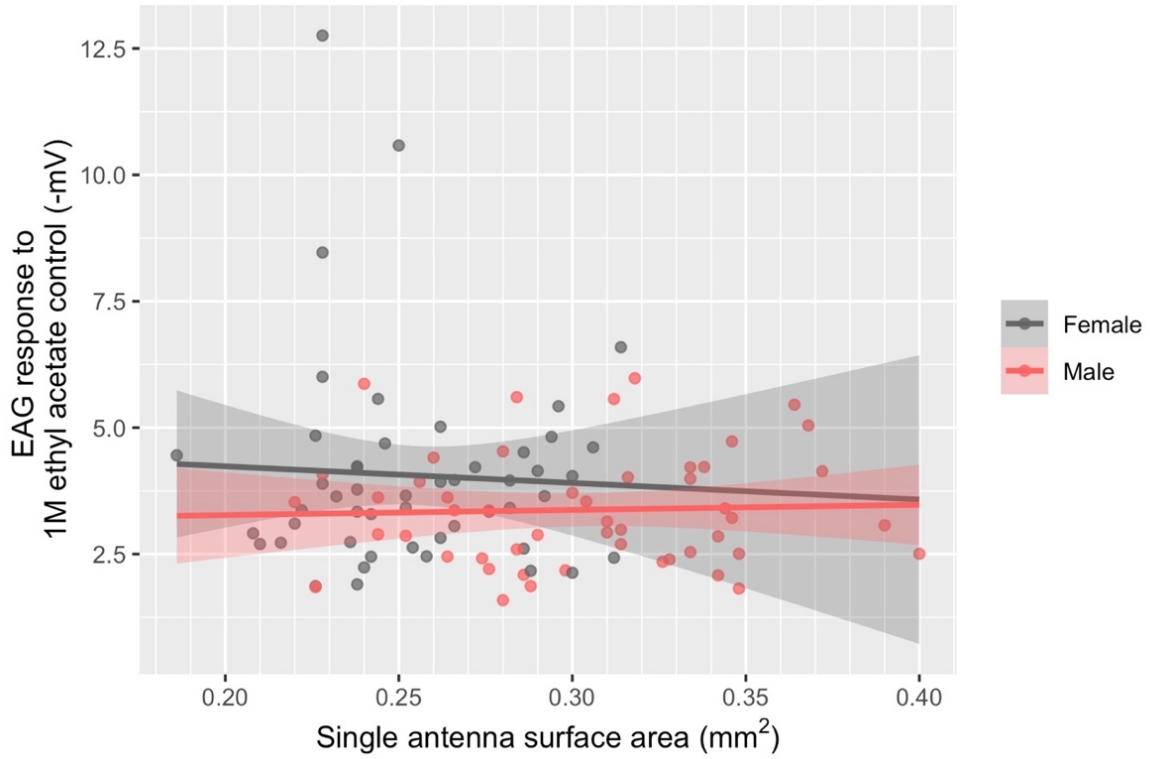

Figure S12. EAG response (-mV) to a 1M Ethyl acetate reference stimulus as a function of antennal surface area (single linear regression, one for each sex). Transparent bands represent 0.95 confidence intervals of values as predicted by each linear regression. Antennal surface area was not a significant predictor or EAG response for either sex (female, $R^2 = 0.0024$, $F_{1, 48} = 0.1174$, $p = 0.7334$; male, $R^2 = 0.0015$, $F_{1, 48} = 0.0742$, $p = 0.7864$).

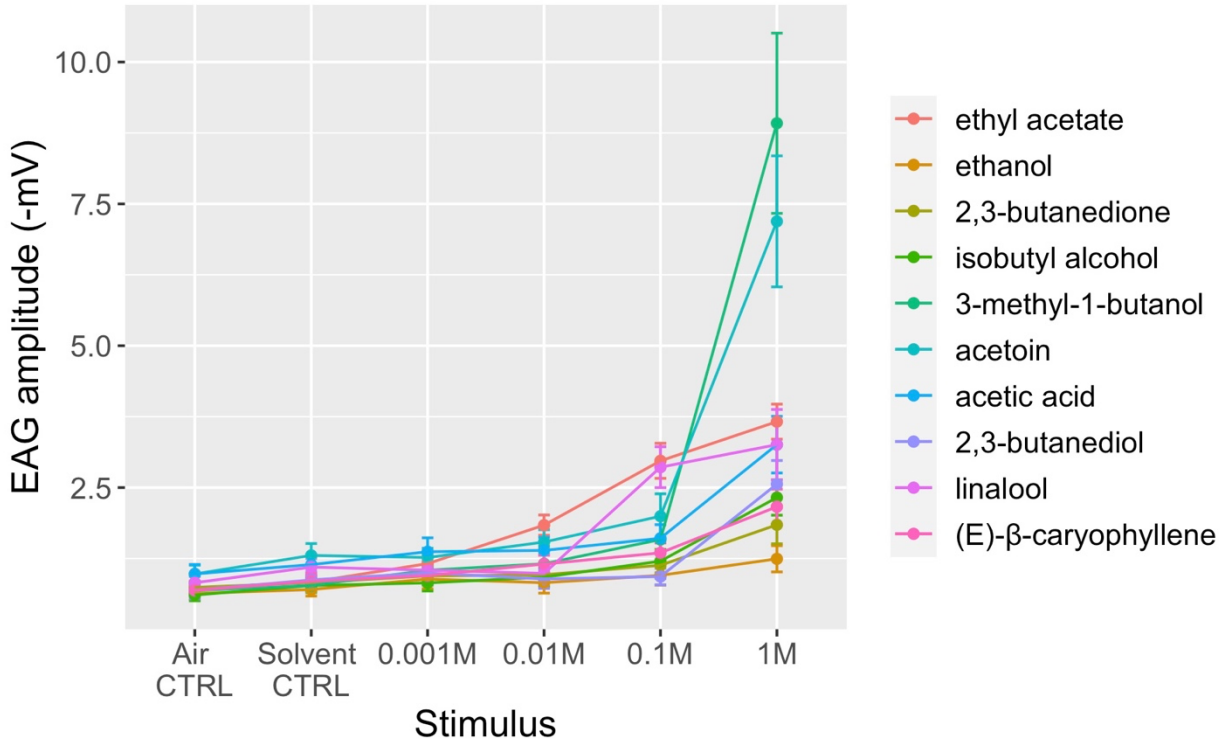

Figure S13. Mean ± SE uncorrected electroantennographic responses from *G. fasciatus* beetle antennae to serial dilutions of ten volatile compounds found in the headspace of *A. triloba* flowers. Mean values for each stimulus represent pooled data from male and female beetles (n=10 beetles per chemical) because sex was not a significant predictor of -mV response ($F_{1, 80} = 1.84$, $p = 0.18$). Significant pairwise differences between stimuli are not shown here for clarity; see **Figure 3** for these comparisons.

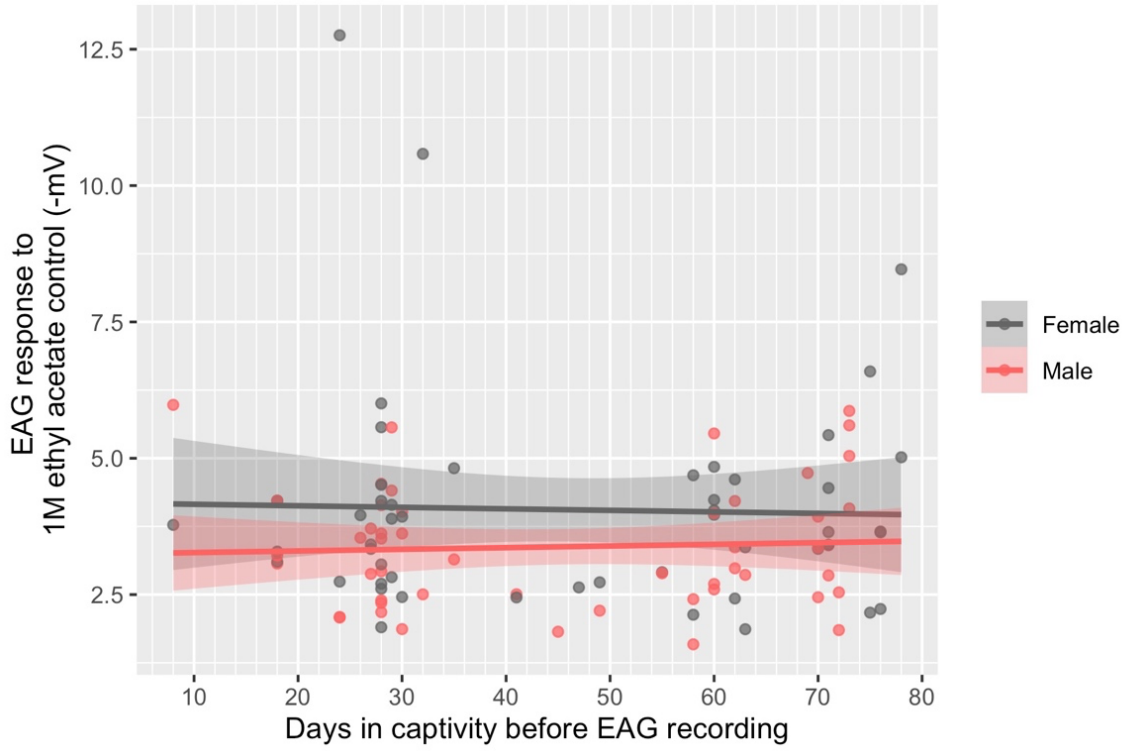

Figure S14. EAG response (-mV) to a 1M Ethyl acetate reference stimulus as a function of days spent in captivity (single linear regression, one per sex). Transparent bands represent 0.95 confidence intervals of values as predicted by each linear regression. Days in captivity before EAG recording was not a significant predictor of EAG response for either sex (female, $R^2 = 0.0009$, $F_{1, 48} = 0.0409$, $p = 0.8406$; male, $R^2 = 0.0029$, $F_{1, 48} = 0.1414$, $p = 0.7085$).

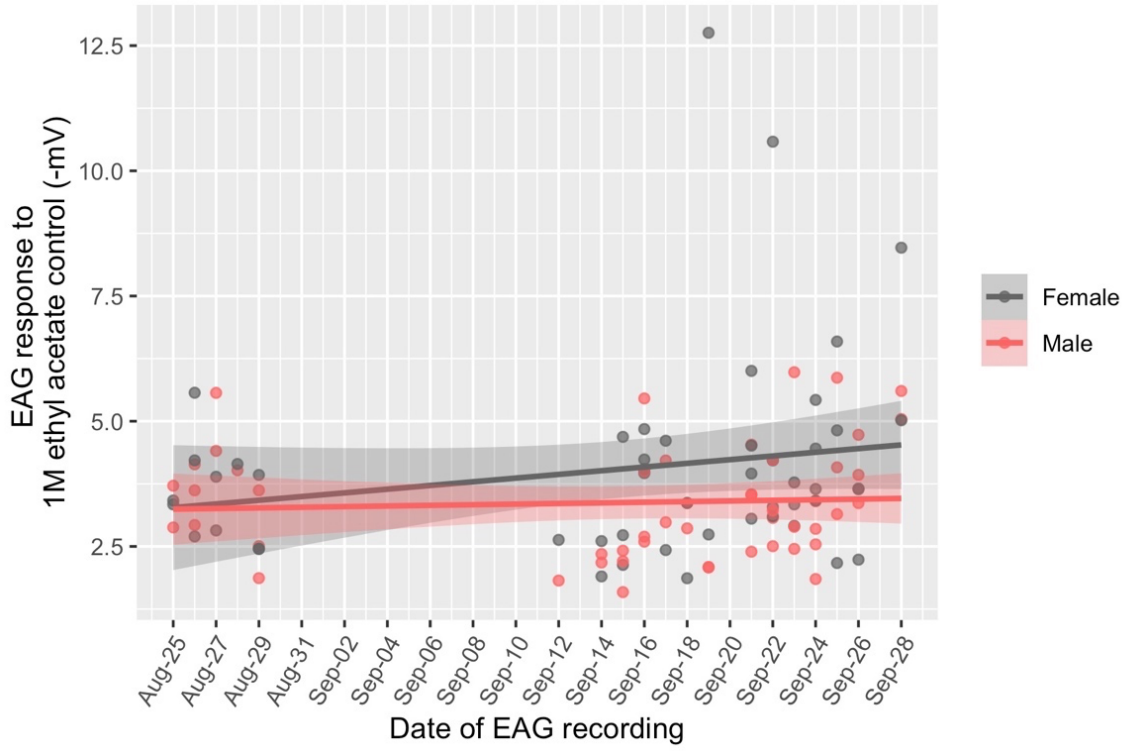

Figure S15. EAG response (-mV) to a 1M Ethyl acetate reference stimulus as a function of EAG recording date (single linear regression, one per sex). Transparent bands represent 0.95 confidence intervals of values as predicted by each linear regression. The date of EAG recording was not a significant predictor or EAG response for either sex (female, $R^2 = 0.0400$, $F_{1, 48} = 2.000$, $p = 0.1638$; male, $R^2 = 0.0037$, $F_{1, 48} = 0.1792$, $p = 0.6739$).

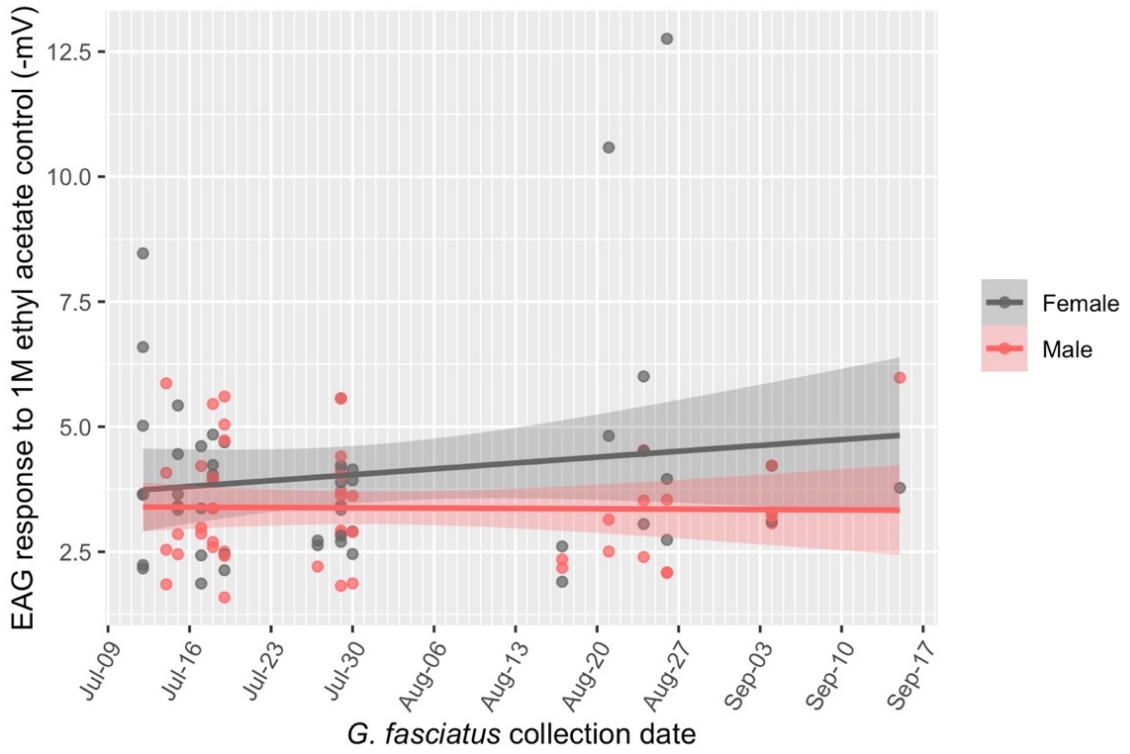

Figure S16. EAG response (-mV) to a 1M Ethyl acetate reference stimulus as a function of beetle collection date (single linear regression, one per sex). Transparent bands represent 0.95 confidence intervals of values as predicted by each linear regression. Beetle collection date was not a significant predictor or EAG response for either sex (female, $R^2 = 0.0234$, $F_{1, 48} = 1.150$, $p = 0.2889$; male, $R^2 = 0.0002$, $F_{1, 48} = 0.0108$, $p = 0.9177$).

Table S1.

Number of arthropods captured across all experiments and treatments at the Beebe Lake bioassay site in May & June, 2019.

		Experiment 1		Experiment 2		Experiment 3	
Family	Binomial	sham-sterile flowers	sterile flowers	sterile flowers with media plate	sterile flowers with microbe plate	media plate	microbe plate
Drosophilidae (258)	*Drosophila affinis*	38	33	21	42	0	1
	Drosophila sp. obscura group	29	23	9	11	0	4
	Drosophila falleni	10	4	1	1	0	0
	Drosophila putrida	6	1	0	1	0	0
	Drosophila robusta	5	3	0	0	0	0
	Drosophila athabasca	1	2	0	0	0	0
	Microdrosophila quadrata	1	1	0	1	0	0
	Drosophila algonquin	0	2	0	0	0	0
	Chymomyza amoena	1	0	0	0	0	0
	Drosophila immigrans	0	0	0	0	1	0
	Drosophila sp. funebris group	0	0	0	1	0	0
	Drosophila sp. melanica group	0	1	0	0	0	0
	Drosophila quinaria	0	0	0	1	0	0
	Drosophila recens	1	0	0	0	0	0
	Drosophila sp.	1	0	0	0	0	0
Nitidulidae (87)	*Glischrochilus quadrisignatus*	13	3	6	25	0	0
	Glischrochilus fasciatus	3	2	4	7	0	0
	Cryptarcha ampla	4	4	0	2	0	0
	Cryptarcha concinna	2	0	4	3	0	0
	Colopterus truncatus	2	1	0	0	0	0
	Carpophilus brachypterus	0	0	1	0	0	0
	Carpophilus sayi	1	0	0	0	0	0
Dryomyzidae (75)	*Dryomyza anilis*	21	20	13	21	0	0
Curculionidae (16)	*Xyleborini sp.*	5	6	5	0	0	0
Muscidae (13)	*Muscidae sp. 1*	0	1	0	3	0	0
	Muscidae sp. 2	1	0	2	3	0	0
	Muscidae sp. 3	0	1	0	0	0	0
	Mydaea sp.	1	1	0	0	0	0
Chironomidae (10)	*Chironomidae sp. 1*	1	8	0	0	0	0
	Chironomidae sp. 2	1	0	0	0	0	0
Anisopodidae (8)	*Sylvicola alternatus*	1	1	2	1	0	0
	Sylvicola fenestralis	0	0	0	1	0	0
	Sylvicola fuscatus	0	1	0	0	0	0

	Sylvicola punctatus	0	0	0	1	0	0
Sciomyzidae (7)	*Dictya sp.*	0	0	0	2	3	0
	Sepedon gracilicornis	1	1	0	0	0	0
Anthomyiidae (4)	*Anthomyiidae sp. 1*	0	1	0	1	0	0
	Anthomyiidae sp. 2	1	0	0	1	0	0
Mycetophilidae (4)	*Neoempheria illustris*	1	3	0	0	0	0
Chloropidae (3)	*Chloropidae sp.*	0	0	1	0	2	0
Cerambycidae (2)	*Cyrtophorus verrucosus*	1	0	0	0	0	0
	Grammoptera haematites	0	1	0	0	0	0
Phoridae (2)	*Phoridae sp. 1*	0	0	0	1	0	0
	Phoridae sp. 2	0	0	0	0	0	1
Salticidae (2)	*Phidippus audax*	0	0	0	0	2	0
Sarcophagidae (2)	*Ravinia sp.*	0	1	0	0	0	0
	Sarcophagidae sp.	1	0	0	0	0	0
Ulidiidae (2)	*Chaetopsis fulvifrons*	1	0	0	0	1	0
Anyphaenidae	*Anyphaena sp.*	1	0	0	0	0	0
Aphidae	*Aphidae sp.*	0	0	0	0	0	1
Araneae #2	*Araneae sp. 2*	0	0	0	0	1	0
Araneae #3	*Araneae sp. 3*	0	0	0	0	0	1
Brentidae	*Trichapion rostrum*	0	0	0	0	0	1
Caliphoridae	*Phormia regina*	0	1	0	0	0	0
Coleoptera #2	*Coleoptera sp. 2*	0	0	0	0	1	0
Formicidae	*Prenolepis impairs*	1	0	0	0	0	0
Psocidae	*Psocidae sp.*	0	1	0	0	0	0
Scarabaeidae	*Euphoria inda*	0	0	0	1	0	0
Sciaridae	*Sciaridae sp.*	0	0	0	1	0	0
Syrphidae	*Ferdinandea buccata*	0	0	0	1	0	0
Throcidae	*Throcidae sp.*	0	0	1	0	0	0

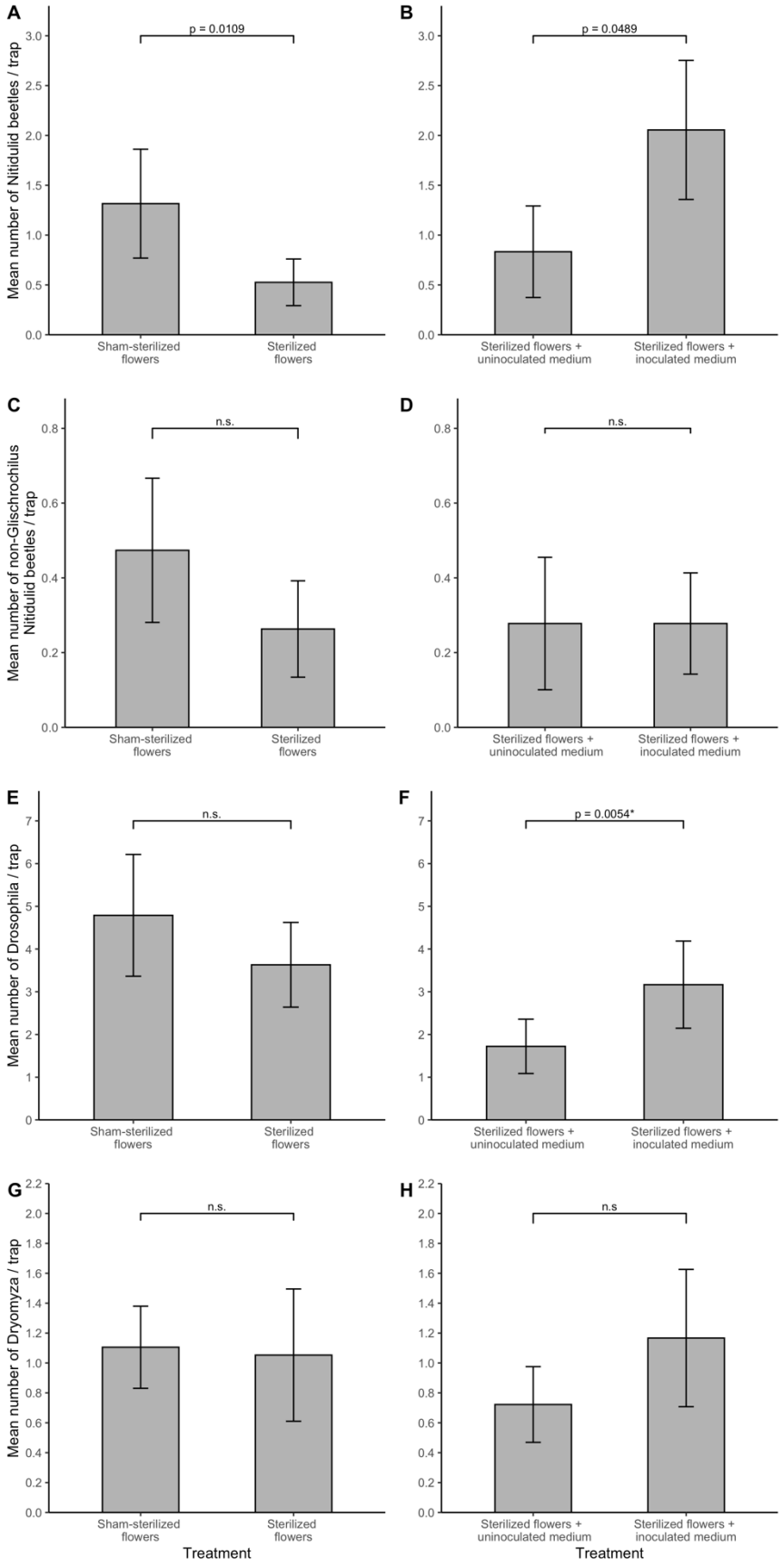
A
p = 0.0109
Mean number of Nitidulid beetles / trap
Sham-sterilized flowers
Sterilized flowers
B
p = 0.0489
Sterilized flowers + uninoculated medium
Sterilized flowers + inoculated medium
C
n.s.
Mean number of non-Glischrochilus Nitidulid beetles / trap
D
E
Mean number of Drosophila / trap
F
p = 0.0054*
G
Mean number of Dryomyza / trap
H
n.s
Treatment

Figure S17. The effect of flower sterilization (**A**, **C, E, G**; experiment 1) or experimental addition of floral microbes to sterile flowers (**B**, **D, F, H**; experiment 2) on the mean (± SE) number of total nitidulid beetles (**A**, **B**), non-*Glischrochilus* nitidulid beetles (**C**, **D**), *Drosophila* individuals (**E**, **F**), or *Dryomyza* individuals (**G**, **H**) captured in cup-funnel traps. Traps baited with sham-sterile flowers vs. traps baited with sterile flowers were tested in one experiment, while traps baited with sterile flowers + uninoculated agar medium vs. traps baited with sterile flowers + floral microbe-inoculated medium were tested in a separate experiment. Negative control traps did not capture any insects over the course of the entire experiment and have been excluded from analyses and figures. *p*-values are given to indicate significant differences between means (GLMM). Asterisks indicate *p*-values robust to FDR multiple comparisons correction (**Table 2**); n.s. = not significant.

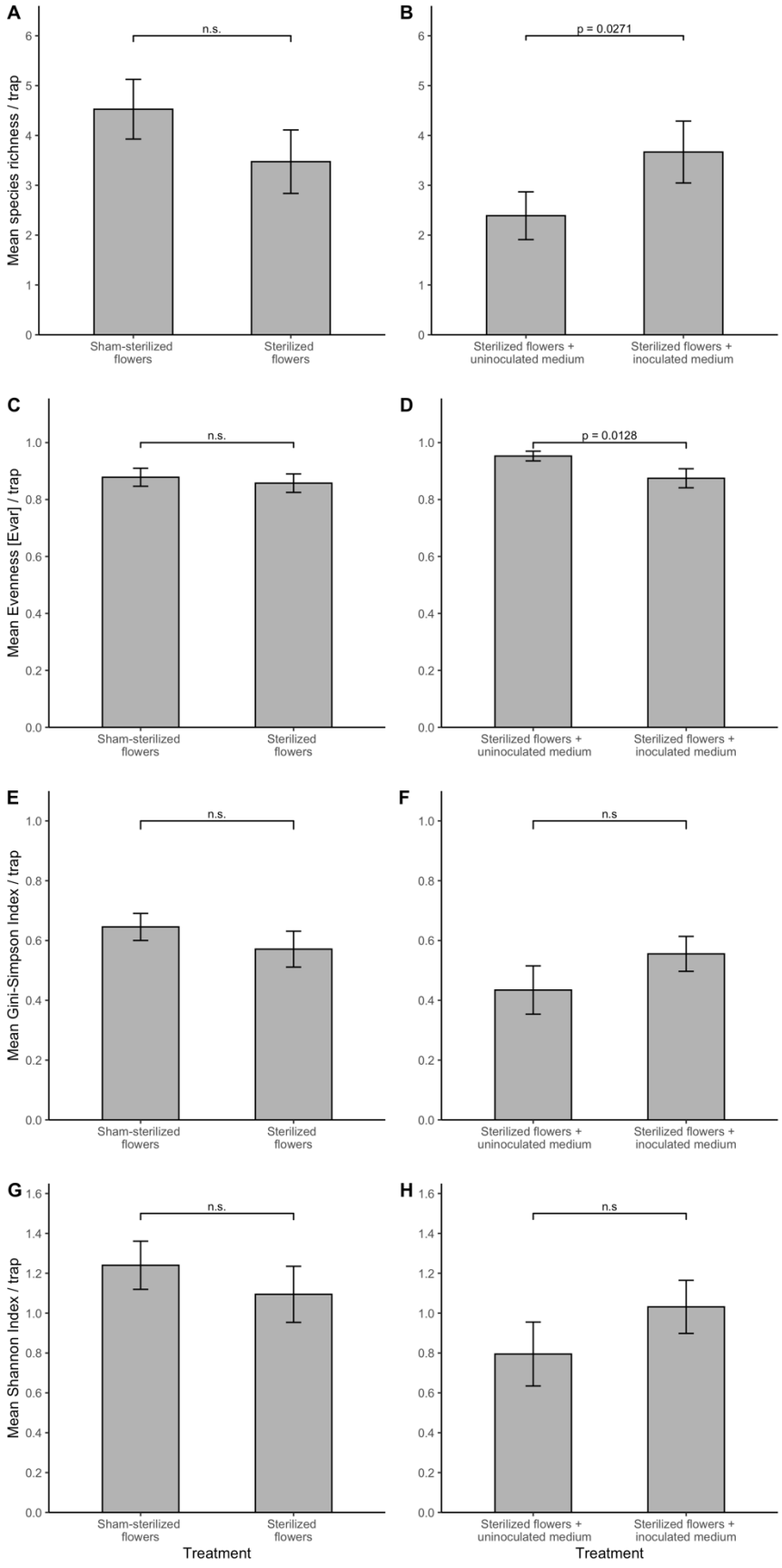
A
B
C
D
E
F
G
H
n.s.
p = 0.0271
p = 0.0128
n.s
Mean species richness / trap
Mean Evenness [Evar] / trap
Mean Gini-Simpson Index / trap
Mean Shannon Index / trap
Sham-sterilized flowers
Sterilized flowers
Sterilized flowers + uninoculated medium
Sterilized flowers + inoculated medium
Treatment

Figure S18. The effect of flower sterilization (**A**, **C, E, G**; experiment 1) or experimental addition of floral microbes to sterile flowers (**B**, **D, F, H**; experiment 2) on the mean (± SE) species richness (**A**, **B**), species evenness [E_{var}] (**C**, **D**), Gini-Simpson index (**E**, **F**), or Shannon index (**G**, **H**) of trap capture. Traps baited with sham-sterile flowers vs. traps baited with sterile flowers were tested in one experiment, while traps baited with sterile flowers + uninoculated agar medium vs. traps baited with sterile flowers + floral microbe-inoculated medium were tested in a separate experiment. Negative control traps did not capture any insects over the course of the entire experiment and have been excluded from analyses and figures. *p*-values are given to indicate significant differences between means (LMM). No significant *p*-values were robust to FDR multiple comparisons correction (**Table 2**); n.s. = not significant.

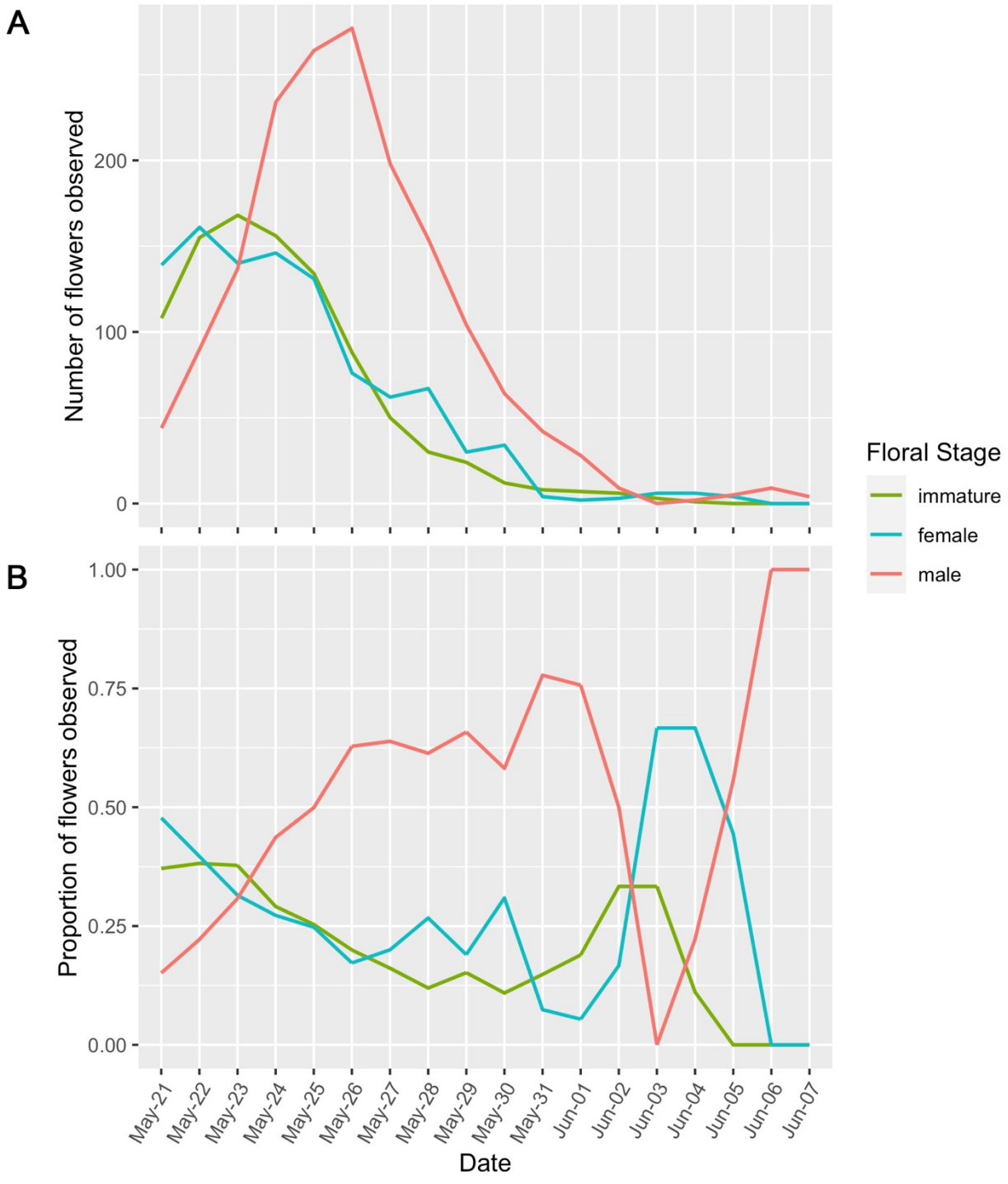

Figure S19. The number (**A**) or proportion (**B**) of *A. triloba* flowers of each floral ontogenic stage observed over the course of the 2019 visitor survey. The same 12 individual *A. triloba* trees were surveyed each day, and up to 10 flowers of each ontogenic stage were surveyed per tree.

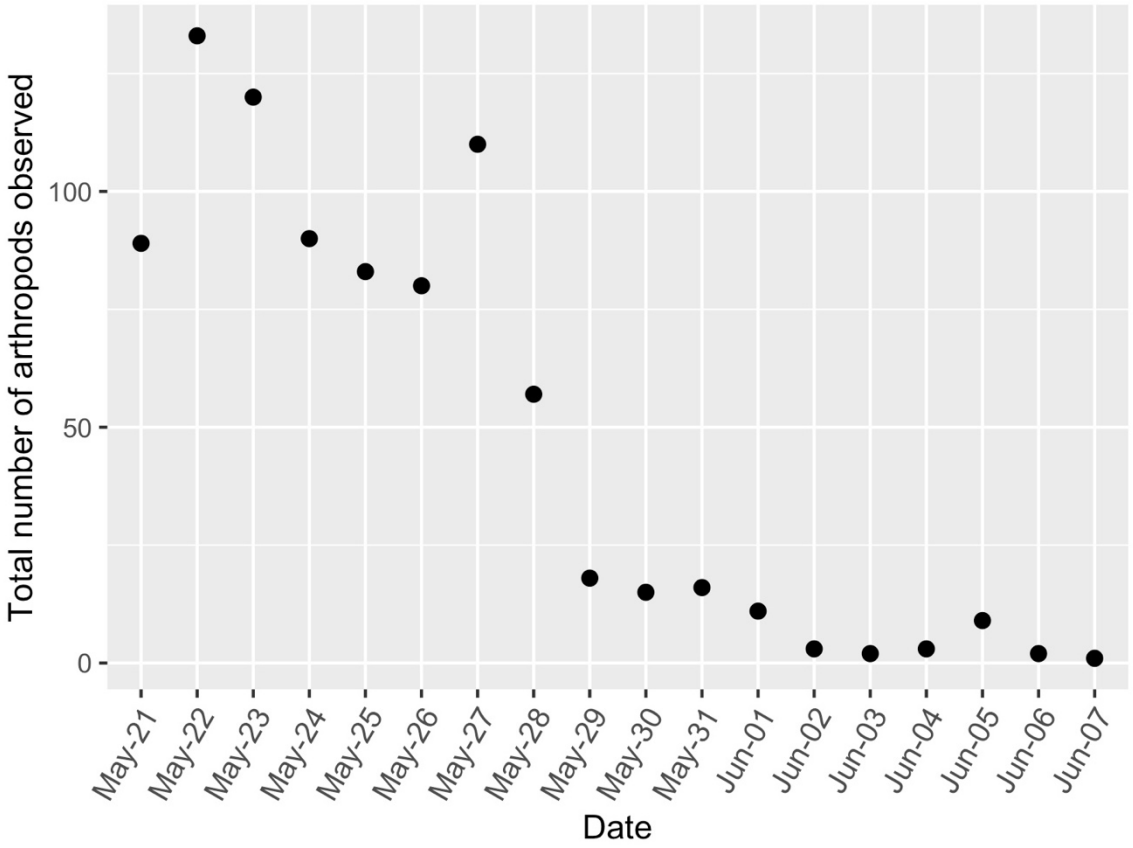

Figure S20. Total number of arthropods observed in all flower types and over all times of day during the visitor survey in May and June of 2019.

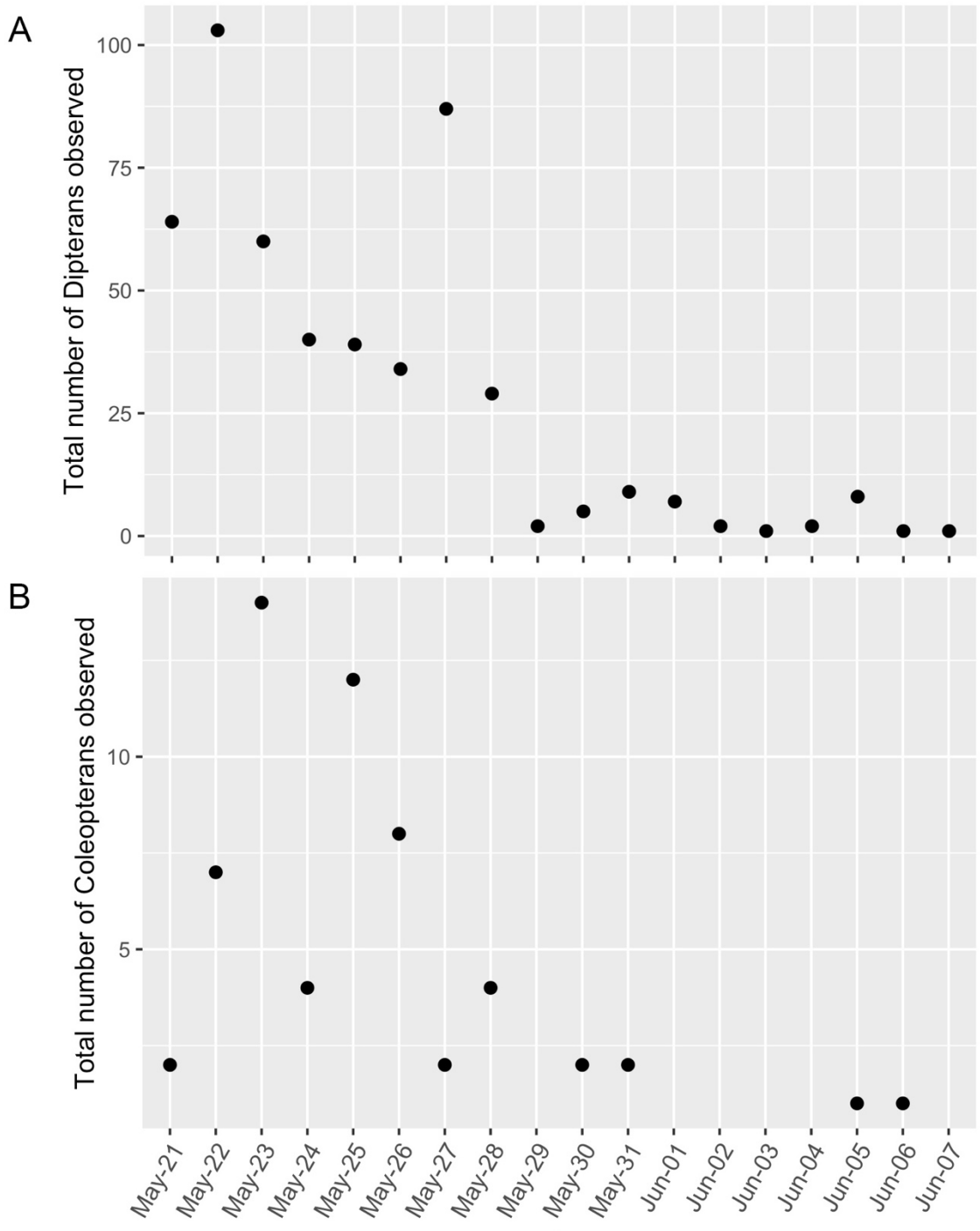

Figure S21. Total number of Diptera (**A**) or Coleoptera (**B**) observed in all flower types and over all times of day during the visitor survey in May and June of 2019.

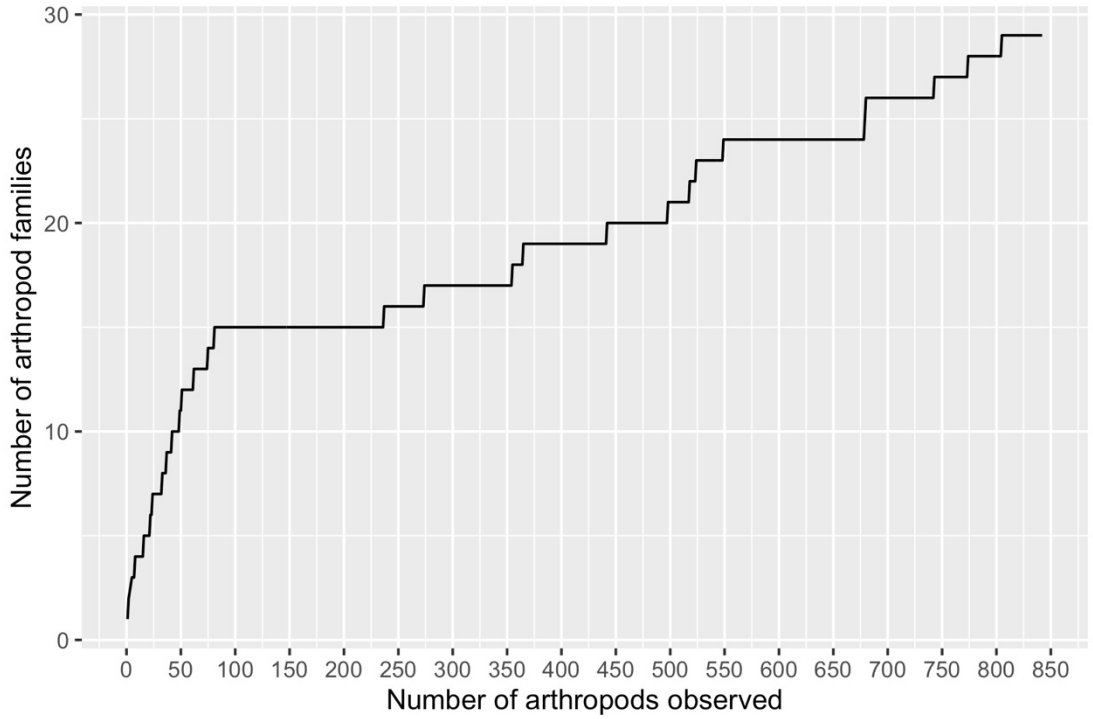

Figure S22. Rarefaction curve for the number of arthropod families observed as a function of number of arthropods observed in *A. triloba* flowers during the visitor survey.

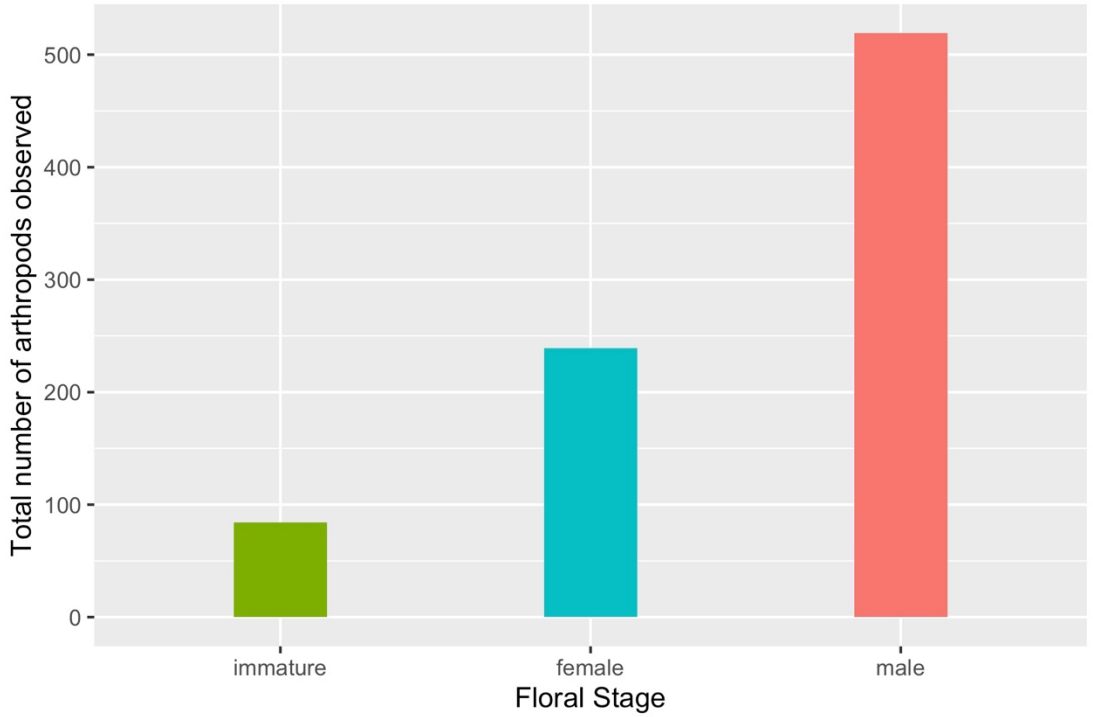

Figure S23. The cumulative number of arthropods observed in immature, female, and male stage flowers over the course of the *A. triloba* visitor survey.

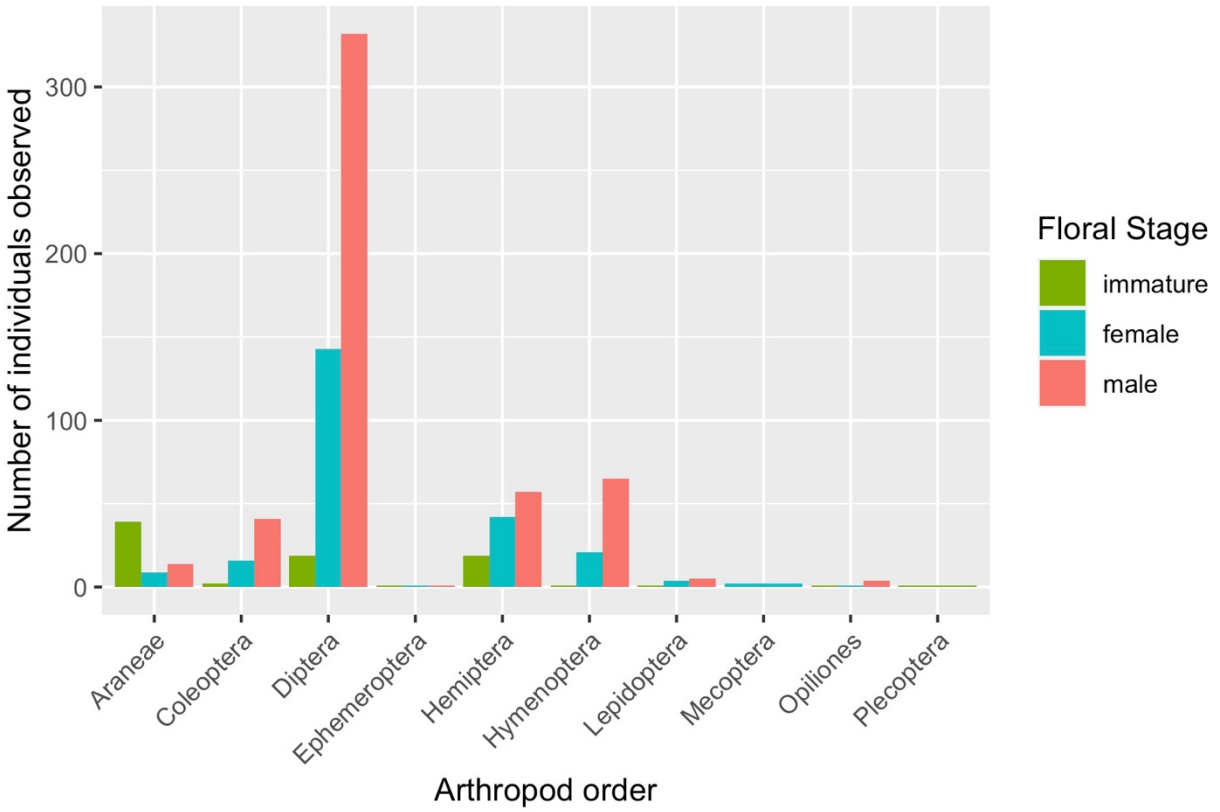

Figure S24. The cumulative number of arthropods observed in immature, female, and male stage flowers over the course of the *A. triloba* visitor survey; organized by arthropod order.

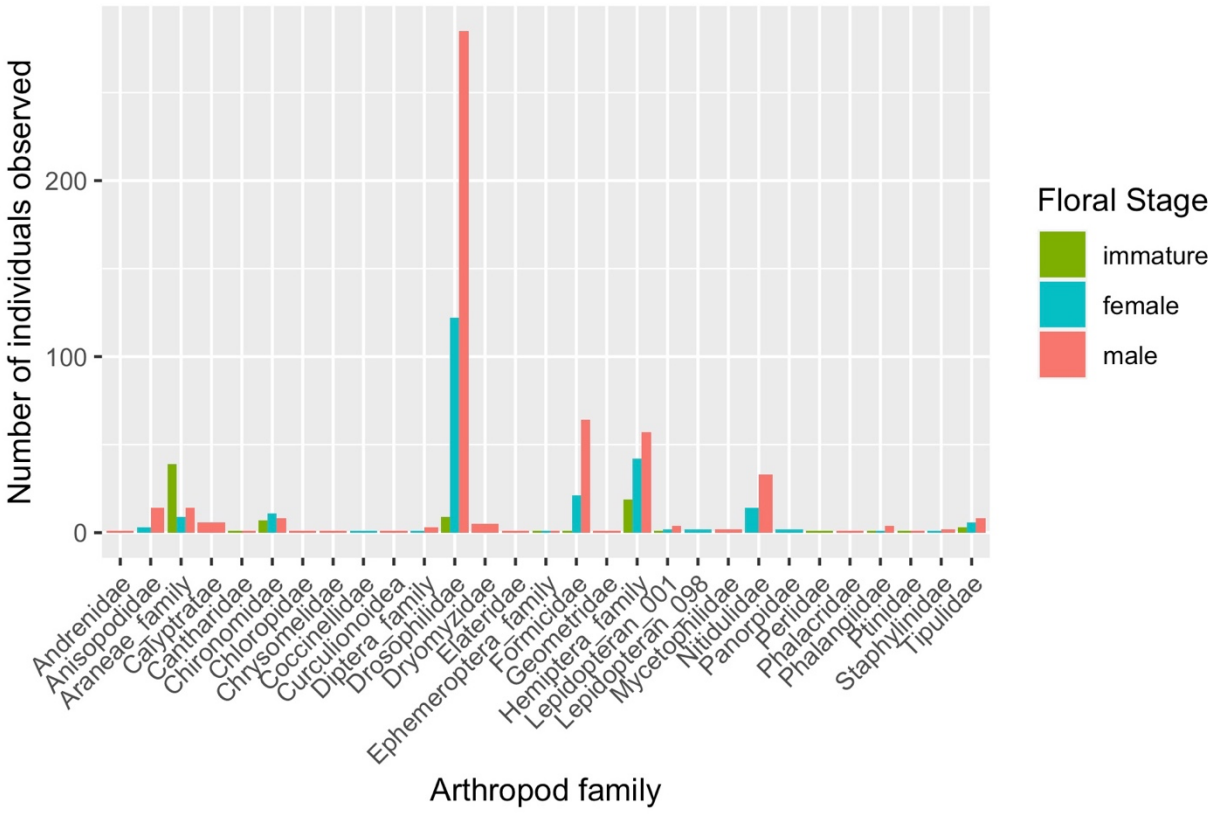

Figure S25. The cumulative number of arthropods observed in immature, female, and male stage flowers over the course of the *A. triloba* visitor survey; organized by arthropod family.

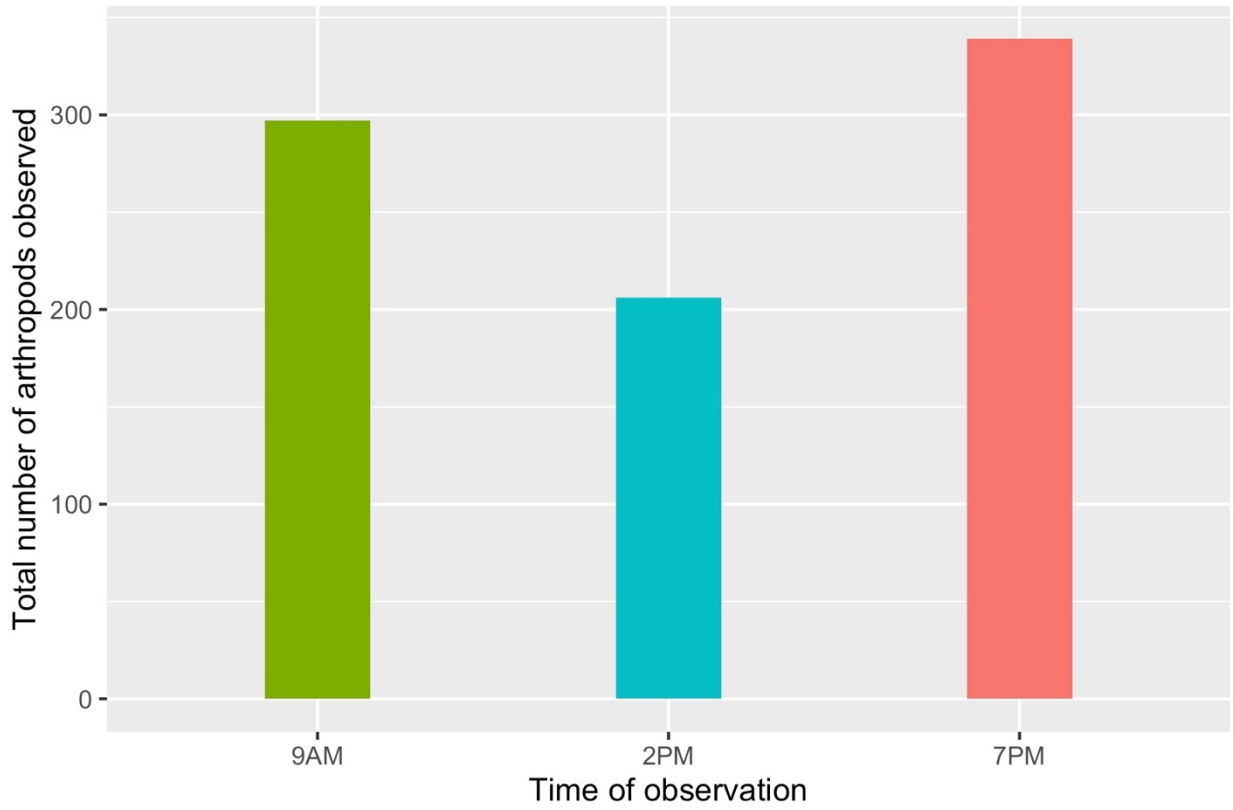

Figure S26. The cumulative number of arthropods observed in all flower stages at 09:00h, 14:00h, and 19:00h over the course of the *A. triloba* visitor survey.

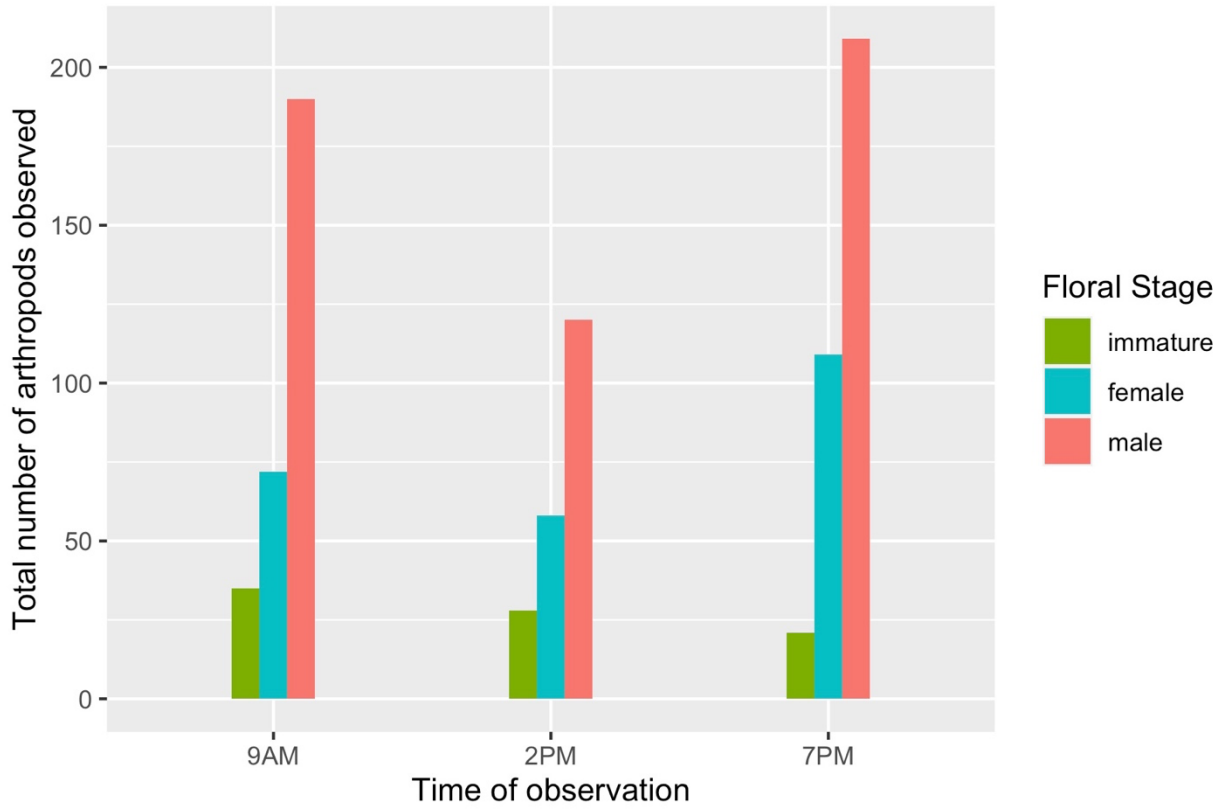

Figure S27. The cumulative number of arthropods observed immature, female, and male stage flowers at 09:00h, 14:00h, and 19:00h over the course of the *A. triloba* visitor survey.

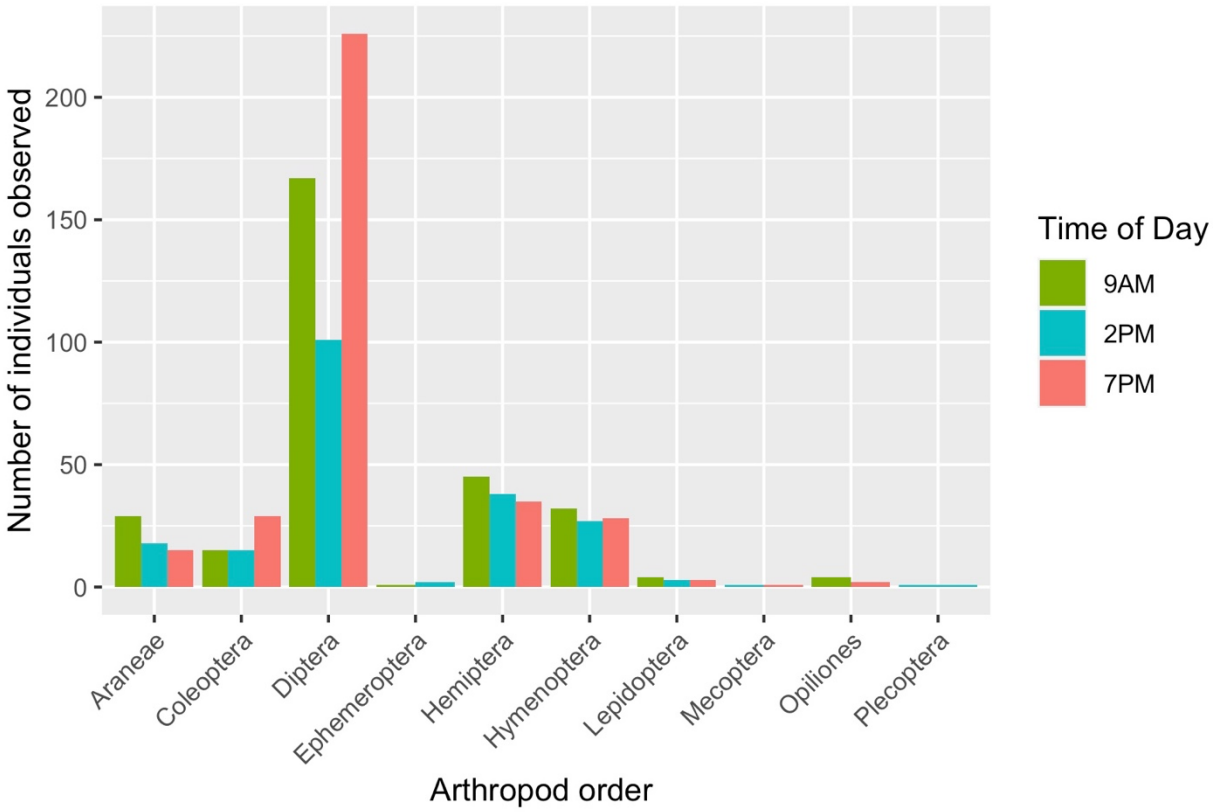

Figure S28. The cumulative number of arthropods observed in flowers of all stages at 09:00, 14:00, or 19:00 over the course of the entire visitor survey; organized by arthropod order.