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Perspective

The Floral Microbiome and Its Management in Agroecosystems: A Perspective

Emily C. Burgess and Robert N. Schaeffer*

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ABSTRACT: Disease management is critical to ensuring healthy crop yields and is often targeted at flowers because of their susceptibility to pathogens and direct link to reproduction. Many disease management strategies are unsustainable however because of the potential for pathogens to evolve resistance, or nontarget effects on beneficial insects. Manipulating the floral microbiome holds some promise as a sustainable alternative to chemical means of disease control. In this perspective, we discuss the current state of research concerning floral microbiome assembly and management in agroecosystems as well as future directions aimed at improving the sustainability of disease control and insect-mediated ecosystem services.

KEYWORDS: *anthosphere, biological control, insect-mediated ecosystem services, microbiome, plant pathogens*

■ INTRODUCTION

Flowers harbor a diversity of microbes including archaea, bacteria, fungi, protists, and viruses (Table 1).[1] Collectively, the floral microbiome has potential to be an important mediator of plant reproduction.[1,2] As such, there is growing interest in taking an expanded, community-level perspective in studying the floral microbiome, not only for the potential to improve disease management[3] but also our understanding of the extended floral phenotype, the role these communities play in pre- and postpollination processes, and the health of beneficial insects that rely on floral rewards.[2,4,5] In this perspective, we briefly review the current state of research on floral microbiome assembly and function in agroecosystems, as well as future avenues for research that could advance microbiome management and resulting outcomes for both disease control and insect-mediated ecosystem services. In particular, we strive to note both advances and gaps with respect to major research priorities identified for the management of plant-associated microbiomes in agroecosystems.[6]

■ FLORAL MICROBIOME ASSEMBLY IN AGROECOSYSTEMS

Floral microbiome assembly and structure can be affected by a number of factors within agroecosystems (Figure 1). Though microbes can be detected in or on floral tissues preanthesis, their incidence and abundance have been found to be typically low.[1] Only upon anthesis do we tend to observe a rapid increase in both microbial incidence and abundance,[7] with microbes arriving to floral tissues via the aid of air-, water-, or insect-mediated dispersal.[1] More recent evidence though also suggests an important role for within-plant emigration, as soil microbes have been found to colonize floral tissues via surface, vessel, and/or gas convection movement.[8,9] Notably, floral taxa identified to date appear to be a subset of those found in the phyllo- or rhizo-sphere[8,10] and are likely adapted to the stresses experienced in this ephemeral environment including desiccation, UV radiation, variable nutrient availability, and secondary metabolites,[1,11,12] among other factors.

Importantly, the pool of microbes potentially able to disperse to host floral tissues within an agroecosystem can be shaped by both landscape- and local-level processes. At the landscape-scale, compositional and configurational variation in land cover surrounding a focal crop has potential to affect both the makeup of the species pool and the spatial and temporal flow of microbes.[13] For example, in a recent landscape-level survey of pear flowers,[14] bacterial and fungal community beta-diversity were found to be affected by the type of land cover surrounding orchards, with pear cultivation and natural forest affecting bacterial and fungal community composition, respectively. This and other work suggest an important role for neighboring vegetation in defining the pool of microbial species that can emigrate to flowers during bloom.[15] Furthermore, for microbes that depend on insects for dispersal, the impact of land cover on vector population dynamics and movement will likely cascade to affect floral microbiome assembly, though this remains largely untested beyond managed pollinators within crop fields.[16]

More locally, whether microbes can establish and proliferate postdispersal can depend strongly on the floral environment. Host- and genotypic-level traits [e.g., volatile organic compounds (VOCs)], the presence of agrochemicals, and priority effects exerted by pre-established microbes [microbial biological control agent (mBCAs) and/or others that are naturally occurring] can all shape the floral environment and

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Table 1. Examples of Flower-Inhabiting Microbes That Cause Disease or Contribute to Disease Suppression in Flowering Crops

microbial group	type	examples	host	ref
bacteria	beneficial	*Bacillus amyloliquefaciens, B. subtilis, Pantoea agglomerans, Pseudomonas fluorescens*	Numerous flowering crops	48
	pathogenic	*Erwinia amylovora, E. tracheiphila, Pseudomonas syringae* pv *actinidiae*	*Malus x domestica, Pyrus communis, Curcubita* spp.*, Actinidia deliciosa*	28, 63, 64
fungi	beneficial	*Aureobasidium pullulans, Beuveria bassiana, Clonostachys rosea, Trichoderma atriviride, T. harzianum*	Numerous flowering crops	41, 65
	pathogenic	*Botrytis cinerea, Colletotrichum acutatum, Monilinia fructicola, M. laxa, M. vaccinii-corymbosi*	Numerous flowering crops, *Prunus* spp.	16, 66−68
viruses	beneficial	Phages ΦEa1337−26, ΦEa2345−6, and Y2	*Erwinia amylovora*	69, 70
	pathogenic	*Blueberry shock ilarvius* (BlShV), *Prunus necrotic ringspot virus* (PNRSV), *Raspberry bushy dwarf virus* (RBDV)	*Vaccinium* spp., *Prunus* spp., *Rubus* spp.	71−73

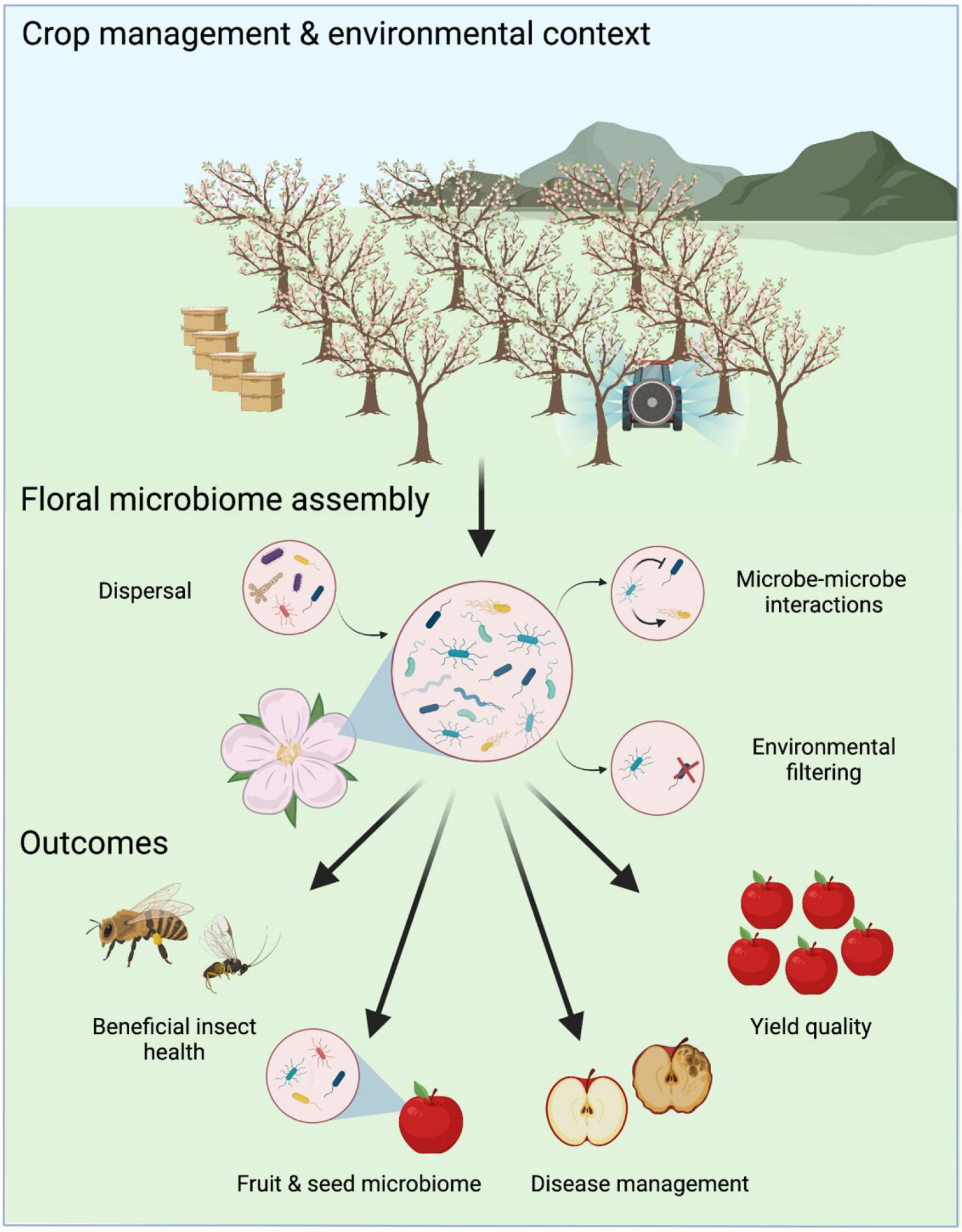

Figure 1. Conceptual overview of floral microbiome assembly and function in agroecosystems. Figure created with BioRender.com.

its suitability for microbe establishment.[10,17−21] Regarding VOCs, many compounds of floral origin have been studied extensively for their antimicrobial effects *in vitro*;[22] however, *in vivo* volatile function with respect to microbial (beneficial to

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pathogenic) establishment and the mediation of intra- and interkingdom interactions in the anthosphere is only recently gaining attention.[17,23−26] For example, terpene and benzenoid VOCs were recently found to be associated with patterns of floral bacterial and fungal diversity across different cultivars of strawberry.[17] Continued research in this area is likely to yield significant dividends for improved understanding of anthosphere assembly, plant-microbe-insect interactions, as well as the role of plant and microbial VOCs in disease control.

Disease management practices employed during bloom can also significantly alter the floral microbiome. While bloom-time application of antibiotics, chemicals, and/or mBCAs can often yield a desired reduction in pathogen occurrence and abundance, nontarget effects on other floral inhabitants can also be observed. More specifically, in the limited studies conducted to date, disease management, including the identity of suppressive agent(s) used, can affect the abundance, diversity, and composition of bacteria and fungi that colonize floral tissues.[7,14,18,19,27] Whether there are consequences for microbiome function, including contributions to disease resistance and host physiology and development remains unclear.

DISEASE MANAGEMENT AND THE FLORAL MICROBIOME

In many cropping systems, management efforts are often targeted at floral tissues, given their susceptibility to plant pathogens and direct link to reproduction. Indeed, floral tissues are primary sites of infection for numerous plant pathogenic bacteria, fungi, and viruses (Table 1). Upon infection, these pathogens can potentially trigger development of disease and loss of reproductive structures. A prime example is the Gram-negative bacterium *Erwinia amylovora*, the causal agent of fire blight in apple and pear, which colonizes floral stigmas and can invade hosts via the hypanthium.[28,29] Furthermore, additional losses can stem from pathogen spread throughout the host, or through latent infections with disease development and symptoms only manifesting postharvest.[30] Thus, the bloom window for flowering crops can be a period of intense disease management, for both prevention of pathogen establishment and mitigation of potential losses at different stages of the produce supply chain.

When it comes to disease management, growers can leverage biological, chemical, and/or cultural control methods, among others. Unfortunately, many chemicals employed for use against flower-invading pathogens have proven to be unsustainable. Major plant pathogens, including *E. amylovora, Monilinia* spp. (brown rot of stone fruits), and *Botrytis cinerea* (gray mold) have evolved resistance to many of the bacteri- and fungi-cidal agents commonly used by producers in their respective pathosystems.[31,32] This is perhaps not surprising, given overreliance on a limited number of single-target-site chemical agents. For instance, six single-target-site fungicides make up approximately 77% of global markets.[33] Likewise, the few antibiotics used in agricultural systems, such as streptomycin, also exhibit a limited number of modes of action.[34] Though regions exist where some of these diseases have yet to evolve resistance, it is grown increasingly clear that one of the best strategies for avoiding the evolution of resistance in plant pathogens is limiting use of chemical agents and diversifying control strategies.[34]

Beyond costs associated with evolved resistance, chemical agents applied during bloom can also pose a significant threat to beneficial insects that confer pest control or pollination services. Natural enemies and pollinators that rely on floral rewards (i.e., nectar and pollen) to fuel both energetic demands and reproduction risk exposure to chemical agents through multiple routes, including larval ingestion, adult ingestion, direct contact, or transovarial transmission.[35] Alone, chemical exposure can potentially disrupt host interactions with beneficial microbial associates, impair cognition, increase susceptibility to pathogen infection, or even cause death.[36] Additionally, evidence for synergism and increased toxicity among active and/or additive ingredients is rapidly growing,[37,38] spurring further demand for sustainable approaches to disease control that minimize nontarget effects.

Microbial-based solutions to disease management have been hailed as a promising alternative for the sustainable control of plant pathogens including those that invade floral tissues.[39] To our knowledge, some of the earliest work on this idea can be traced back to Dr. Kenneth Parker (New York Agricultural Experiment Station) and his investigation on the potential for microbial biocontrol of *E. amylovora* in apple and pear flowers a near century ago.[40] In recent decades, significant progress has been made on this front, particularly for fire blight management, where mBCAs are now commercially available and adopted by many apple and pear producers for use during bloom.[41]

Microbial biocontrol agents applied to flowers for disease management can be sourced from different environments, including the antho-, phyllo-, or rhizo-sphere. While the flower environment is a prime target for bioprospecting potential mBCAs, as observed recently in Crowley-Gall et al. and almond for brown rot control,[42] notable commercial agents like the basidiomycete fungus *Aureobasidium pullulans* (Blossom Protect, Westbridge Agricultural Products) and Gram-positive bacterium *Bacillus subtilis* (Serenade OPTI, Bayer Crop Science) were originally sourced from leaves and soil, respectively. When applied during bloom, mBCAs can potentially achieve control of floral pathogens through diverse modes of action including mBCA-pathogen competition, hyperparasitism, antibiosis (e.g., production of antimicrobial compounds), and/or mBCA-priming of the host's immune system and associated defenses.[43] With respect to competitive exclusion, mBCAs can prevent pathogen establishment when applied early in bloom, as such timing allows for mBCA preemption of both colonization sites and critical resources for growth.[44] This has been observed for *Pseudomonas fluorescens* (Pfa506) and *E. amylovora*,[45] as well as other mBCA-pathogen interactions. The secretion of antimicrobial compounds by biocontrol agents is also a common mode of action and frequently the suppressive mechanism of *Pseudomonas* spp., *Pantoea spp.*, and *Bacillus* spp. used as mBCAs.[46] The other noted modes of action however can also be critical. For instance, priming of the host's immune system and associated defenses likely occurs following treatment with Blossom Protect, as the mBCAs (*A. pullulans* strains CF10 and CF40) confer protection against fire blight in apple and pear despite sustained growth of epiphytic *E. amylovora* populations.[1,47] Further research is needed however to confirm this exact mechanism of suppression.

Though there has been considerable progress in mBCA development and adoption, challenges remain. First, solitary mBCA performance in floral disease suppression can be highly variable,[41] or even substantially inferior to antibiotics or other synthetic products applied.[48] Numerous factors can contribute

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to this variability including the timing and frequency of mBCA application during the bloom period, physiological state of the treated host, cultivar identity, as well as local growing conditions. Thus, considerable effort has been expended on development of integrated approaches that leverage both biological and chemical treatments for improved disease suppression and sustainability.[41] Here, mBCA compatibility with antibiotic or nonantibiotic chemical treatments (e.g., lime sulfur) is critical, and can potentially be achieved through careful evaluation of the sequencing of material applications.[41] Moreover, there is growing interest in taking an expanded, community-level perspective on microbe−microbe interactions within the anthosphere, and calls have been made for development of mBCA consortia, which could include "helper strains" to encourage mBCA establishment and performance.[49] For instance, the floral microbiome naturally plays a role in mediating pathogen establishment, and there is some evidence to suggest that more complex microbial communities can enhance disease resistance.[50,51] Given variable performance of solitary mBCAs, it is likely that complex interactions among microbiome constituents can contribute to such observed outcomes; however, this requires more thorough investigation. As next-generation sequencing continues to be increasingly embraced as a tool for identifying members of the floral microbiome,[1] combining it with culture-based approaches and chemical analyses could allow researchers to identify novel mBCAs or microbial consortia for improved control, as well as the mechanisms involved.

Second, many floral colonists, including mBCAs, end up residing in fruit and seed tissues as they develop.[49,52] Thus, assembly patterns during the bloom window are likely to strongly dictate microbiome structure and function at later development stages. As example, *A. pullulans*, an important mBCA used for fire blight control during bloom, can also be found within the fruit microbiome and has been implicated in the russeting of pome fruits, a physiological disorder characterized by brown and corky areas on the fruit skin surface that develop ∼1−4 weeks after full bloom.[53] While this has largely been observed for *A. pullulans* alone, careful dissection of microbial transmission patterns from flower to fruit are largely lacking for many flowering crops. Given their range of potential effects, from continued disease prevention to impaired fruit development and marketability, such information should be an important consideration for candidate mBCA testing, development, and eventual commercialization.

■ POTENTIAL EFFECTS OF FLORAL MICROBES ON BENEFICIAL INSECTS

Numerous insects visit flowering crops to fuel their energetic demands and reproduction. Among those that are beneficial, pollinators and natural enemies are known to rely upon floral attractive traits, including chemosensory cues, to identify rewarding flowers for visitation. Recently, floral microbes have been shown to alter cues and resources important for insect attraction including nectar taste and scent chemistry.[1] With respect to those that colonize floral nectar, impacts include changes in nectar acidity, as well as the composition and concentration of sugars and amino acids. Generally, microbes decrease sucrose concentration in nectar relative to fructose and glucose, decrease amino acid concentrations, and lower nectar pH.[1] The magnitude of these effects however is often species-dependent. This is also true for VOC profiles emitted by nectar-inhabiting microbes *in vitro*,[25] with alcohols, aldehydes, and esters largely detected, but in varying concentrations.

Floral VOCs are an important signal to floral visitors of both nectar availability and quality. As such, it should not be surprising that floral visitors may be sensitive to microbe-induced changes in nectar chemistry. To date, lab-based studies have revealed that honey bees and bumble bees, key pollinators of flowering crops, can respond to variation among microbial species in VOC composition.[23,25] More specifically, electroanntenographic assays on both pollinators show a sensitivity to volatiles 2-ethyl-1-hexanol and 2-phenylethanol, produced by both nectar specialist yeasts and bacteria, albeit in varying amounts.[23,25] Complementary preference assays that include taste cues further reveal a preference for the nectar specialist yeast *Metschnikowia reukaufii* over the common mBCA *A. pullulans*, as well as bacteria *Asaia astilbes* and *Neokomagataea thailandica*.[23,25] Beyond common pollinators, parasitoid natural enemies have also been found to be sensitive yeast and bacterial VOCs.[54,55]

The potential impact of floral microbes on the attraction of floral visitors brings up a number of important considerations. First, there is potential for this sensitivity to translate to effects on the quality of pollination services provided in agroecosystems. Recent work in wildflower systems has revealed that floral microbes can affect plant reproductive outcomes;[2] however, evidence for such an effect in agricultural systems is limited. In a recent study conducted with pear, Colda et al.[56] found that application of a mixture of *M. reukaufii* and *Acinetobacter nectaris* at bloom can increase hoverfly and honey bee visitation to blossoms. Despite this, no effect on fruit set was observed in two consecutive years of treatment. The authors hypothesized however that this lack of an effect was likely due to the hosts not being pollen-limited, and further, that under such conditions, treating flowers with attractive microbes could potentially boost pollination.[56] In another study involving almond, Schaeffer et al.[7] also observed a neutral effect of nectar-inhabiting microbes on pollination, as measured through pollen tube number. In contexts where crop yield is not pollinator limited, or pollinators lack alternate floral choices such potential effects of floral microbes on pollinator foraging may not be a significant concern. However, more research is required, including work on vegetable crops, to draw any clear conclusions regarding potential impacts of floral microbes on pollinator behavior and services rendered. Such potential should also be addressed during the development of new mBCAs or microbial consortia for disease control, as demonstrated recently in Crowley-Gall et al.[42] At minimum, effective mBCA or consortia should ideally not reduce visitor attraction to focal crops. Finally, regarding the pollination process, new evidence demonstrates that microbes can induce pollen germination.[57] Whether changes in pollen-associated microbiota translate to effects on reproductive outcomes in flowering crops remains an open question.

Second, while a number of agricultural products have known impacts on pollinators,[37,38] less is known regarding nontarget effects of mBCAs and other shifts in floral microbiome structure for the health of floral visitors. For registered mBCA products, toxicology tests have been performed to assess lethal effects on honey bee pollinators. While lethal effects for products such as Blossom Protect have not been documented, sublethal effects may still occur. For instance, in a microcolony assay involving the bumble bee *Bombus impatiens*, ingestion of *B. subtilis* led to a reduction in days to oviposition, drone

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(male) production, and days to drone emergence.[58] Interestingly, topical exposure to the same mBCA led to a hormetic response, and increased drone production. In contrast, topical and dietary exposure to the mBCA *Beauveria bassiana* was observed to have no effect. Given the diversity of exposure routes, and high levels of exposure in systems where mBCAs are applied during bloom, especially when acting as entomovectors of these disease treatments,[59] more work concerning sublethal effects in agricultural systems is needed.

Microbial effects on the health of beneficial insects can also stem through modification of nutritional resources such as nectar sugars and amino acids.[5] Microbial colonists of floral nectar, including those purposely applied for disease management during bloom, have potential to alter these metabolites and in turn visitor health. For instance, in a pair of recent studies involving the generalist parasitoid *Aphidius ervi*, adult dietary exposures to yeasts and bacteria were found to increase, decrease, or have a neutral effect on longevity, which is likely to affect the magnitude of pest suppression females can inflict.[54,60] Of note, *A. pullulans* was found to repel adult feeding, and also decreased longevity.[54] In another set of recent studies involving the bumble bee *B. terrestris*, a key pollinator of flowering crops in Europe and other regions where it has been introduced for greenhouse use, nectar bacteria and yeasts were also found to affect pollinator health via reproduction. While effects at the individual level were negligible, dietary exposure at the colony level was found to result in changes in egg laying, brood size, and the production of workers, with effects depending on microbial species identity.[61,62]

PERSPECTIVE

Despite its critical link to reproduction, the floral organ has received considerably less attention than that of other plant compartments (e.g., roots) when characterizing drivers, roles, and mechanisms of microbiome assembly. We believe that improved understanding of the floral microbiome can aid improvement of flowering crops through breeding, disease and pest management, as well as pollination. Many questions (Table 2) remain, however, and continued pursuit of answers has potential to inform microbiome management and the sustainability of flowering production systems.

Table 2. Outstanding Questions Concerning Flower Microbiome Assembly and Function in Agroecosystems

process	question
microbiome assembly	How does land cover impact the available pool of potential floral microbes and microbe vectors?
	Is the flower microbiome more or less sensitive to crop management practices and landscape context than other plant-associated microbiomes?
	How do mBCAs impact fruit and seed microbiomes, and are there implications for fruit and seed quality?
biocontrol	Can microbial consortia be manipulated to provide more robust disease control than single mBCAs or synthetics?
	How compatible are mBCAs with other strategies employed in an integrated pest management system (e.g., SAR-inducers, insecticides, etc.)?
pollination	For flowering crops that face disease and pollination issues during bloom (e.g., pear), can individual mBCAs or consortia be identified that synergize disease suppression and pollinator attraction?
	How do microbes influence pollen-pistil interactions, and ultimately crop fertilization?
beneficial insects	What are the lethal and nonlethal impacts of floral microbes on beneficial insects?

AUTHOR INFORMATION

Corresponding Author

Robert N. Schaeffer − *Department of Biology, Utah State University, Logan, Utah 84322, United States;* orcid.org/0000-0001-7930-6878; Email: robert.schaeffer@usu.edu

Author

Emily C. Burgess − *Department of Biology, Utah State University, Logan, Utah 84322, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jafc.2c02037

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Notes

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REFERENCES

(1) Vannette, R. L. The foral microbiome: Plant, pollinator, and microbial perspectives. *Annu. Rev. Ecol. Evol. Syst.* **2020**, *51* (1), 363−386.

(2) Cullen, N. P.; Fetters, A. M.; Ashman, T.-L. Integrating microbes into pollination. *Curr. Opin. Insect Sci.* **2021**, *44*, 48−54.

(3) Cui, Z.; Huntley, R. B.; Zeng, Q.; Steven, B. Temporal and spatial dynamics in the apple flower microbiome in the presence of the phytopathogen *Erwinia amylovora*. *ISME J.* **2021**, *15* (1), 318−329.

(4) Rebolleda-Gómez, M.; Forrester, N. J.; Russell, A. L.; Wei, N.; Fetters, A. M.; Stephens, J. D.; Ashman, T.-L. Gazing into the anthosphere: Considering how microbes influence floral evolution. *New Phytol.* **2019**, *224* (3), 1012−1020.

(5) Martin, V. N.; Schaeffer, R. N.; Fukami, T. Potential effects of nectar microbes on pollinator health. *Philos. Trans. R. Soc. B Biol. Sci.* **2022**, *377*, 20210155.

(6) Busby, P. E.; Soman, C.; Wagner, M. R.; Friesen, M. L.; Kremer, J.; Bennett, A.; Morsy, M.; Eisen, J. A.; Leach, J. E.; Dangl, J. L. Research priorities for harnessing plant microbiomes in sustainable agriculture. *PLOS Biol.* **2017**, *15* (3), e2001793.

(7) Schaeffer, R. N.; Crowder, D. W.; Illán, J. G.; Beck, J. J.; Fukami, T.; Williams, N. M.; Vannette, R. L. Ecological dynamics of the almond floral microbiome in relation to crop management and pollination. *bioRxiv* **2020**, DOI: 10.1101/2020.11.05.367003.

(8) Massoni, J.; Bortfeld-Miller, M.; Widmer, A.; Vorholt, J. A. Capacity of soil bacteria to reach the phyllosphere and convergence of floral communities despite soil microbiota variation. *Proc. Natl. Acad. Sci. U. S. A.* **2021**, *118* (41), e2100150118 DOI: 10.1073/pnas.2100150118.

(9) Kim, D.-R.; Cho, G.; Jeon, C.-W.; Weller, D. M.; Thomashow, L. S.; Paulitz, T. C.; Kwak, Y.-S. A mutualistic interaction between Streptomyces bacteria, strawberry plants and pollinating bees. *Nat. Commun.* **2019**, *10* (1), 1−10.

(10) Wei, N.; Ashman, T.-L. The effects of host species and sexual dimorphism differ among root, leaf and flower microbiomes of wild strawberries in situ. *Sci. Rep.* **2018**, *8* (1), 5195.
(11) Vorholt, J. A. Microbial life in the phyllosphere. *Nat. Rev. Microbiol.* **2012**, *10* (12), 828−840.
(12) Mueller, T. G.; Francis, J. S.; Vannette, R. L. Nectar compounds impact bacterial and fungal growth and shift community dynamics in a nectar analog. *bioRxiv* **2022**. DOI: 10.1101/2022.03.29.485809
(13) Bell, T.; Tylianakis, J. M. Microbes in the anthropocene: Spillover of agriculturally selected bacteria and their impact on natural ecosystems. *Proc. R. Soc. B Biol. Sci.* **2016**, *283* (1844), 20160896.
(14) Schaeffer, R. N.; Pfeiffer, V. W.; Basu, S.; Brousil, M.; Strohm, C.; DuPont, S. T.; Vannette, R. L.; Crowder, D. W. Orchard management and landscape context mediate the pear floral microbiome. *Appl. Environ. Microbiol.* **2021**, *87* (15), e00048.
(15) Lindow, S. E.; Andersen, G. L. Influence of immigration on epiphytic bacterial populations on navel orange leaves. *Appl. Environ. Microbiol.* **1996**, *62* (8), 2978−2987.
(16) Johnson, K. B.; Stockwell, V. O.; Burgett, D. M.; Sugar, D.; Loper, J. E. *Dispersal of Erwinia amylovora and Pseudomonas fluorescens by honey bees from hives to apple and pear blossoms*; AGRIS, 1993.
(17) Wei, N.; Whyle, R. L.; Ashman, T.-L.; Jamieson, M. A. Genotypic variation in floral volatiles influences floral microbiome more strongly than interactions with herbivores and mycorrhizae in strawberries. *Hortic. Res.* **2022**, *9*, uhab005.
(18) Schaeffer, R. N.; Vannette, R. L.; Brittain, C.; Williams, N. M.; Fukami, T. Non-target effects of fungicides on nectar-inhabiting fungi of almond flowers. *Environ. Microbiol. Rep.* **2017**, *9* (2), 79−84.
(19) Wei, N.; Russell, A. L.; Jarrett, A. R.; Ashman, T.-L. Pollinators mediate floral microbial diversity and microbial network under agrochemical disturbance. *Mol. Ecol.* **2021**, *30* (10), 2235−2247.
(20) Peay, K. G.; Belisle, M.; Fukami, T. Phylogenetic relatedness predicts priority effects in nectar yeast communities. *Proc. R. Soc. B Biol. Sci.* **2012**, *279* (1729), 749−758.
(21) Vannette, R. L.; Fukami, T. Historical contingency in species interactions: Towards niche-based predictions. *Ecol. Lett.* **2014**, *17* (1), 115−124.
(22) Hammerbacher, A.; Coutinho, T. A.; Gershenzon, J. Roles of plant volatiles in defence against microbial pathogens and microbial exploitation of volatiles. *Plant Cell Environ.* **2019**, *42* (10), 2827−2843.
(23) Schaeffer, R. N.; Rering, C. C.; Maalouf, I.; Beck, J. J.; Vannette, R. L. Microbial metabolites elicit distinct olfactory and gustatory preferences in bumblebees. *Biol. Lett.* **2019**, *15* (7), 20190132.
(24) Cellini, A.; Giacomuzzi, V.; Donati, I.; Farneti, B.; Rodriguez-Estrada, M. T.; Savioli, S.; Angeli, S.; Spinelli, F. Pathogen-induced changes in floral scent may increase honeybee-mediated dispersal of *Erwinia amylovora*. *ISME J.* **2019**, *13* (4), 847−859.
(25) Rering, C. C.; Beck, J. J.; Hall, G. W.; McCartney, M. M.; Vannette, R. L. Nectar-inhabiting microorganisms influence nectar volatile composition and attractiveness to a generalist pollinator. *New Phytol.* **2018**, *220* (3), 750−759.
(26) Weisskopf, L.; Schulz, S.; Garbeva, P. Microbial volatile organic compounds in intra-kingdom and inter-kingdom interactions. *Nat. Rev. Microbiol.* **2021**, *19* (6), 391−404.
(27) Shade, A.; McManus, P. S.; Handelsman, J. Unexpected diversity during community succession in the apple flower microbiome. *mBio* **2013**, *4* (2), e00602−12.
(28) Thomson, S. V. The role of the stigma in fire blight infections. *Phytopathol.* **1986**, *76* (5), 476−482.
(29) Bubán, T.; Orosz-Kovács, Zs. The nectary as the primary site of infection by *Erwinia amylovora* (Burr.) Winslow et al.: A mini review. *Plant Syst. Evol.* **2003**, *238* (1), 183−194.
(30) Prusky, D. Pathogen quiescence in postharvest diseases. *Annu. Rev. Phytopathol.* **1996**, *34* (1), 413−434.
(31) Schroth, M. N.; Thomson, S. V.; Moller, W. J. Streptomycin resistance in *Erwinia amylovora* [Detected in California pear orchards]. *Phytopathol.* **1979**, *69*, 565.
(32) Ma, Z.; Michailides, T. J. Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. *Crop Prot.* **2005**, *24* (10), 853−863.
(33) Fones, H. N.; Bebber, D. P.; Chaloner, T. M.; Kay, W. T.; Steinberg, G.; Gurr, S. J. Threats to global food security from emerging fungal and oomycete crop pathogens. *Nat. Food* **2020**, *1* (6), 332−342.
(34) Sundin, G. W.; Wang, N. Antibiotic resistance in plant-pathogenic bacteria. *Annu. Rev. Phytopathol.* **2018**, *56* (1), 161−180.
(35) Kopit, A. M.; Pitts-Singer, T. L. Routes of pesticide exposure in solitary, cavity-nesting bees. *Environ. Entomol.* **2018**, *47* (3), 499−510.
(36) Daisley, B. A.; Chmiel, J. A.; Pitek, A. P.; Thompson, G. J.; Reid, G. Missing microbes in bees: How systematic depletion of key symbionts erodes immunity. *Trends Microbiol.* **2020**, *28* (12), 1010−1021.
(37) Siviter, H.; Bailes, E. J.; Martin, C. D.; Oliver, T. R.; Koricheva, J.; Leadbeater, E.; Brown, M. J. F. Agrochemicals interact synergistically to increase bee mortality. *Nature* **2021**, *596* (7872), 389−392.
(38) Straw, E. A.; Thompson, L. J.; Leadbeater, E.; Brown, M. J. F. 'Inert' ingredients are understudied, potentially dangerous to bees and deserve more research attention. *Proc. R. Soc. B Biol. Sci.* **2022**, *289* (1970), 20212353.
(39) Syed Ab Rahman, S. F.; Singh, E.; Pieterse, C. M. J.; Schenk, P. M. Emerging microbial biocontrol strategies for plant pathogens. *Plant Sci.* **2018**, *267*, 102−111.
(40) Parker, K. G. *Fire Blight: Overwintering, Dissemination, and Control of the Pathogene*; New York (Cornell) Agricultural Experiment Station Memo, 1936, *193*, 1−42.
(41) Johnson, K. B.; Temple, T. N.; Kc, A.; Elkins, R. B. Refinement of nonantibiotic spray programs for fire blight control in organic pome fruit. *Plant Dis.* **2022**, *106* (2), 623−633.
(42) Crowley-Gall, A.; Trouillas, F.; Nino, E. L.; Schaeffer, R. N.; Nouri, M. T.; Crespo, M.; Vannette, R. Floral microbes suppress growth of *Monilinia laxa* with minimal effects on honey bee feeding. *Plant Dis.* **2022**, *106*, 432.
(43) Köhl, J.; Kolnaar, R.; Ravensberg, W. J. Mode of action of microbial biological control agents against plant diseases: Relevance beyond efficacy. *Front. Plant Sci.* **2019**, *10*. DOI: 10.3389/fpls.2019.00845
(44) Johnson, K. B.; Stockwell, V. O. Management of fire blight: A case study in microbial ecology. *Annu. Rev. Phytopathol.* **1998**, *36* (1), 227−248.
(45) Wilson, M.; Lindow, S. E. Interactions between the biological control agent *Pseudomonas fluorescens* A506 and *Erwinia amylovora* in pear blossoms. *Phytopathol.* **1993**, *83* (1), 117−123.
(46) Legein, M.; Smets, W.; Vandenheuvel, D.; Eilers, T.; Muyshondt, B.; Prinsen, E.; Samson, R.; Lebeer, S. Modes of action of microbial biocontrol in the phyllosphere. *Front. Plant Sci.* **2020**, 1619.
(47) Temple, T. N.; Thompson, E. C.; Uppala, S.; Granatstein, D.; Johnson, K. B. Floral Colonization dynamics and specificity of *Aureobasidium pullulans* strains used to suppress fire blight of pome fruit. *Plant Dis.* **2020**, *104* (1), 121−128.
(48) Sundin, G. W.; Werner, N. A.; Yoder, K. S.; Aldwinckle, H. S. Field evaluation of biological control of fire blight in the Eastern United States. *Plant Dis.* **2009**, *93* (4), 386−394.
(49) Sare, A. R.; Jijakli, M. H.; Massart, S. Microbial ecology to support integrative efficacy improvement of biocontrol agents for postharvest diseases management. *Postharvest Biol. Technol.* **2021**, *179*, 111572.
(50) Cui, Z.; Huntley, R. B.; Schultes, N. P.; Steven, B.; Zeng, Q. Inoculation of stigma-colonizing microbes to apple stigmas alters microbiome structure and reduces the occurrence of fire blight disease. *Phytobiomes J.* **2021**, *5* (2), 156−165.
(51) Stockwell, V. O.; Johnson, K. B.; Sugar, D.; Loper, J. E. Mechanistically compatible mixtures of bacterial antagonists improve biological control of fire blight of pear. *Phytopathol.* **2011**, *101* (1), 113−123.

(52) Nelson, E. B. The seed microbiome: Origins, interactions, and impacts. *Plant Soil* **2018**, *422* (1), 7−34.
(53) Musacchi, S.; Serra, S. Apple fruit quality: Overview on pre-harvest factors. *Sci. Hortic.* **2018**, *234*, 409−430.
(54) Sobhy, I. S.; Baets, D.; Goelen, T.; Herrera-Malaver, B.; Bosmans, L.; Van den Ende, W.; Verstrepen, K. J.; Wäckers, F.; Jacquemyn, H.; Lievens, B. Sweet scents: Nectar specialist yeasts enhance nectar attraction of a generalist aphid parasitoid without affecting survival. *Front. Plant Sci.* **2018**, *9*. DOI: 10.3389/fpls.2018.01009
(55) Goelen, T.; Sobhy, I. S.; Vanderaa, C.; Boer, J. G. de; Delvigne, F.; Francis, F.; Wäckers, F.; Rediers, H.; Verstrepen, K. J.; Wenseleers, T.; Jacquemyn, H.; Lievens, B. Volatiles of bacteria associated with parasitoid habitats elicit distinct olfactory responses in an aphid parasitoid and its hyperparasitoid. *Funct. Ecol.* **2020**, *34* (2), 507−520.
(56) Colda, A.; Bossaert, S.; Verreth, C.; Vanhoutte, B.; Honnay, O.; Keulemans, W.; Lievens, B. Inoculation of pear flowers with *Metschnikowia reukaufii* and *Acinetobacter nectaris* enhances attraction of honeybees and hoverflies, but does not increase fruit and seed set. *PLoS One* **2021**, *16* (4), e0250203.
(57) Christensen, S. M.; Munkres, I.; Vannette, R. L. Nectar bacteria stimulate pollen germination and bursting to enhance microbial fitness. *Curr. Biol.* **2021**, *31* (19), 4373−4380. e6.
(58) Ramanaidu, K.; Cutler, G. C. Different toxic and hormetic responses of *Bombus impatiens* to *Beauveria bassiana*, *Bacillus subtilis* and spirotetramat. *Pest Manag. Sci.* **2013**, *69* (8), 949−954.
(59) Pozo, M.; Vendeville, J.; Mommaerts, V.; Wackers, F. Flying Doctors for a Better Quality in Fruit Production. *Entomovectoring for Precision Biocontrol and Enhanced Pollination of Crops* **2020**, 263−277.
(60) Lenaerts, M.; Goelen, T.; Paulussen, C.; Herrera-Malaver, B.; Steensels, J.; Van den Ende, W.; Verstrepen, K. J.; Wäckers, F.; Jacquemyn, H.; Lievens, B. Nectar bacteria affect life history of a generalist aphid parasitoid by altering nectar chemistry. *Funct. Ecol.* **2017**, *31* (11), 2061−2069.
(61) Pozo, M. I.; Kemenade, G.; Oystaeyen, A.; Aledon-Catala, T.; Benavente, A.; Van den Ende, W.; Wackers, F.; Jacquemyn, H. The impact of yeast presence in nectar on bumble bee behavior and fitness. *Ecol. Monogr.* **2020**, *90* (1), e01393.
(62) Pozo, M. I.; Mariën, T.; van Kemenade, G.; Wäckers, F.; Jacquemyn, H. Effects of pollen and nectar inoculation by yeasts, bacteria or both on bumblebee colony development. *Oecologia* **2021**, *195* (3), 689−703.
(63) Sasu, M. A.; Seidl-Adams, I.; Wall, K.; Winsor, J. A.; Stephenson, A. G. Floral transmission of *Erwinia tracheiphila* by cucumber beetles in a wild *Cucurbita pepo*. *Environ. Entomol.* **2010**, *39* (1), 140−148.
(64) Donati, I.; Cellini, A.; Buriani, G.; Mauri, S.; Kay, C.; Tacconi, G.; Spinelli, F. Pathways of flower infection and pollen-mediated dispersion of Pseudomonas syringae pv. actinidiae, the causal agent of kiwifruit bacterial canker. *Hortic. Res.* **2018**, *5*, 56.
(65) Kapongo, J. P.; Shipp, L.; Kevan, P.; Sutton, J. C. Co-vectoring of *Beauveria bassiana* and *Clonostachys rosea* by bumble bees (*Bombus impatiens*) for control of insect pests and suppression of grey mould in greenhouse tomato and sweet pepper. *Biol. Control* **2008**, *46* (3), 508−514.
(66) Bristow, P. R.; McNicol, R. J.; Williamson, B. Infection of strawberry flowers by *Botrytis cinerea* and its relevance to grey mould development. *Ann. Appl. Biol.* **1986**, *109* (3), 545−554.
(67) Peres, N. A.; Timmer, L. W.; Adaskaveg, J. E.; Correll, J. C. Lifestyles of *Colletotrichum acutatum*. *Plant Dis.* **2005**, *89* (8), 784−796.
(68) Batra, L. R. *Monilinia vaccinii-corymbosi* (Sclerotiniaceae): Its biology on blueberry and comparison with related species. *Mycologia* **1983**, *75* (1), 23.
(69) Boulé, J.; Sholberg, P. L.; Lehman, S. M.; O'gorman, D. T.; Svircev, A. M. Isolation and characterization of eight bacteriophages infecting *Erwinia amylovora* and their potential as biological control agents in British Columbia, Canada. *Canad. J. Plant Pathol.* **2011**, *33* (3), 308−317.
(70) Born, Y.; Fieseler, L.; Thöny, V.; Leimer, N.; Duffy, B.; Loessner, M. J. Engineering of bacteriophages Y2:: dpoL1-C and Y2:: luxAB for efficient control and rapid detection of the fire blight pathogen, *Erwinia amylovora*. *Appl. Environ. Microbiol.* **2017**, *83* (12), e00341−17.
(71) Bristow, P. R.; Martin, R. R. Transmission and the role of honeybees in field spread of *blueberry shock ilarvirus*, a pollen-borne virus of highbush blueberry. *Phytopathol.* **1999**, *89* (2), 124−130.
(72) Amari, K.; Burgos, L.; Pallas, V.; Sanchez-Pina, M. A. *Prunus necrotic ringspot virus* early invasion and its effects on apricot pollen grain performance. *Phytopathol.* **2007**, *97* (8), 892−899.
(73) Isogai, M.; Yoshida, T.; Shimura, T.; Yoshikawa, N. Pollen tubes introduce *Raspberry bushy dwarf virus* into embryo sacs during fertilization processes. *Virology* **2015**, *484*, 341−345.