Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Insect Science

Floral nectar and honeydew microbial diversity and their role in biocontrol of insect pests and pollination

Sergio Álvarez-Pérez[1,*], Bart Lievens[2,#] and Clara de Vega[3,$]

Sugar-rich plant-related secretions, such as floral nectar and honeydew, that are commonly used as nutrient sources by insects and other animals, are also the ecological niche for diverse microbial communities. Recent research has highlighted the great potential of nectar and honeydew microbiomes in biological pest control and improved pollination, but the exploitation of these microbiomes requires a deep understanding of their community dynamics and plant–microbe–insect interactions. Additionally, the successful application of microbes in crop fields is conditioned by diverse ecological, legal, and ethical challenges that should be taken into account. In this article, we provide an overview of the nectar and honeydew microbiomes and discuss their potential applications in sustainable agricultural practices.

Addresses
[1] Department of Animal Health, Complutense University of Madrid, 28040 Madrid, Spain
[2] Laboratory for Process Microbial Ecology and Bioinspirational Management (PME&BIM), Department of Microbial and Molecular Systems, KU Leuven, B-3001 Heverlee, Belgium
[3] Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, 41012 Sevilla, Spain

Corresponding author: Álvarez-Pérez, Sergio (sergioaperez@ucm.es)
[*] http://orcid.org/0000-0002-6587-8995
[#] https://orcid.org/0000-0002-7698-6641
[$] https://orcid.org/0000-0003-4553-798X

Current Opinion in Insect Science 2024, **61**:101138

This review comes from a themed issue on **Parasites/Parasitoids/ Biological control**

Edited by **Pablo Urbaneja-Bernat, Alejandro Tena** and **Cesar Rodriguez-Saona**

Available online 4 November 2023

https://doi.org/10.1016/j.cois.2023.101138

Introduction

Present-day pest control relies vastly on chemical pesticides. However, their pernicious global effects on the natural environment and health risks to humans have resulted in an increasing trend toward a more environmentally sustainable agriculture [1]. This need of eco-friendly solutions has raised the screening of biological control agents (BCAs), including macroorganisms (usually insect parasitoids and arthropod predators) and microorganisms such as nematodes, fungi, bacteria, and viruses [2,3]. Recently, the microbiomes of flowers and plant-related secretions have been reported to be a reservoir of diverse fungi and bacteria that play an important role in plant–insect interactions [4–6]. Exploitation of these microbes may provide novel opportunities to develop new sustainable pest management strategies.

Floral nectar (further referred to as 'nectar') and honeydew, which represent the predominant plant-related sugar sources in agroecosystems, are extensively consumed by insects [7,8]. Nectar is an aqueous secretion containing sugars, amino acids, and trace amounts of many other compounds (e.g. secondary metabolites), that animal-pollinated plants offer as a reward to pollinators [9••]. Although nectar is initially sterile, it is rapidly colonized by diverse fungi and bacteria [4,6,10,11]. Honeydew, in turn, is a plant-related liquid secreted by a great variety of phloem- and xylem-feeding insects of the order Hemiptera, including aphids, leafhoppers, mealybugs, planthoppers, psyllids, scale insects, treehoppers, and whiteflies [8,12]. While honeydew mostly comprises of sugars and amino acids, this excretion product can also contain a highly diverse set of proteins that originate from the insect host and its microbiota [8,13]. Furthermore, once excreted, honeydew represents a nutrient-rich habitat prone to be colonized by other microorganisms [8,14].

In this article, we provide an overview of the diversity of the nectar and honeydew microbiomes and discuss their potential use in biological control of pests and other agricultural applications. Additionally, we indicate some ecological, legal, and ethical challenges that should be taken into account before releasing biocontrol microbes to crop fields.

Nectar and honeydew microbiomes and their insect vectors

Both nectar and honeydew are sugar-rich habitats (reaching sugar concentrations up to 70% w/v), so the microorganisms inhabiting these secretions should be able to tolerate high osmotic pressures [15]. Furthermore, nectar and honeydew microbes face other hurdles often found in these habitats, including a highly unbalanced carbon-to-nitrogen ratio, limited oxygen (e.g. due to intense microbial metabolism), and the presence of reactive oxygen species and toxins of plant origin [4,8,10]. Depending on their capacity to overcome these factors, nectar and honeydew microbes are typically classified into two groups: (i) specialists, defined as those species that have been almost exclusively found in nectar or honeydew and their animal vectors (see below) and that show a broad tolerance to the aforementioned hurdles; and (ii) generalists, including species widely present in other habitats and that are less tolerant to growth-limiting factors [4,10,16].

The nectar microbiome

Culture-dependent and culture-independent studies have revealed that nectar microbial diversity at the flower level is dominated by a few yeast and bacterial genera (see Table S1 and references therein). The most abundant nectar specialist yeasts are members of genus *Metschnikowia*, followed by *Starmerella* and *Wickerhamiella* (all Ascomycota). In particular, *Metschnikowia reukaufii* has been highlighted as the most frequent nectar specialist in diverse ecosystems around the world. Additionally, the generalist genera *Aureobasidium* and *Cryptococcus* (Ascomycota and Basidiomycota, respectively) are widely distributed in nectar, but occur at lower densities (Table S1). Regarding bacteria, there is no clear dominance of a single genus in nectar, and the most common genera (e.g. *Acinetobacter*, *Bacillus*, *Pantoea*, *Pseudomonas*, and *Rosenbergiella*) have a cosmopolitan distribution (Table S1). Nevertheless, some bacterial taxa (e.g. members of the *Acinetobacter nectaris*/*Acinetobacter boissieri* clade and the genus *Rosenbergiella*) have been repeatedly described from nectar in recent years, suggesting a habitat specificity for these microbes [17,18].

The honeydew microbiome

Research on honeydew microbes has traditionally focused on culture-based approaches and the description of new (mostly fungal) taxa. Available studies highlight the so-called 'sooty molds' as core members of the honeydew microbiome. Sooty molds are a complex and widespread group of saprophytic fungi that feed on honeydew and coat plant tissue with their mycelia [5,19]. To date, more than 200 species of sooty molds have been described, which are mostly classified into the Dothideomycete and Eurotiomycete classes of the phylum Ascomycota [5,19]. Furthermore, the implementation of next-generation sequencing approaches has revealed a previously unknown fungal diversity in honeydew [19–21]. The characterization of bacterial species from honeydew is also growing in recent years and has revealed bacteria from different genera such as *Acinetobacter*, *Pseudomonas*, *Pantoea*, and *Staphylococcus* [14,22].

Role of insects as vectors and shelters of nectar and honeydew microbes

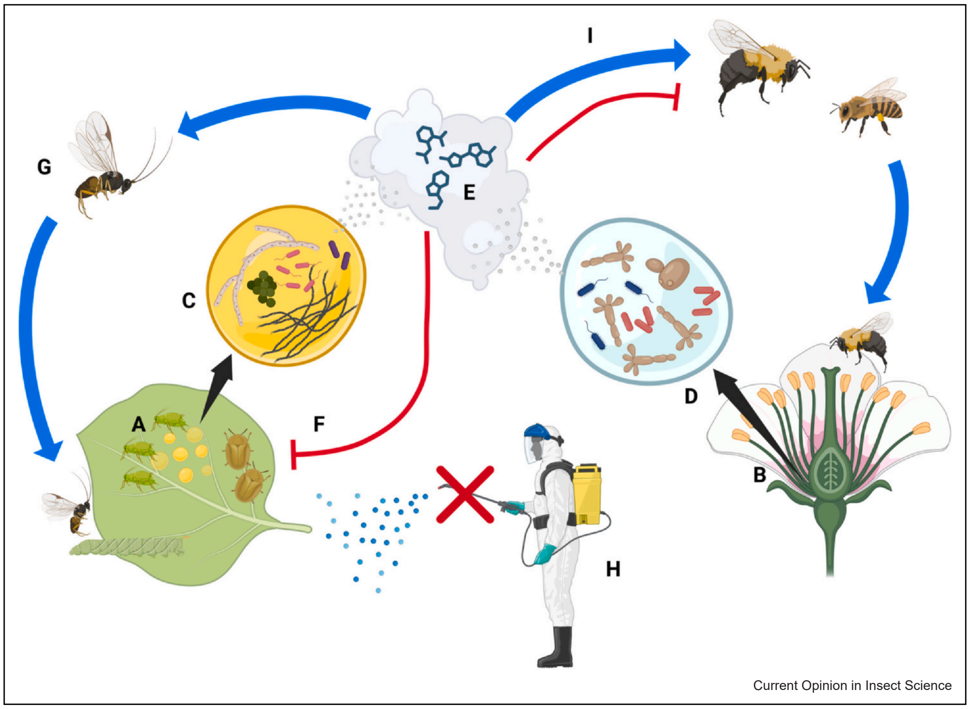
Nectar and honeydew microbes are known to be closely associated with insects, with whom they may communicate through the production of volatile organic compounds (VOCs) that result from the fermentation of sugars available in the medium (see Cusumano and Lievens [23] in this issue). Nectar microbes are vectored by pollinators and other floral visitors, and some insects may even serve as the overwintering site of nectar specialists between consecutive flowering seasons [24]. Some of these microorganisms live symbiotically within the insect and shelter in its gut, where they might also mate [25]. Although, to our knowledge, there is no proof that *M. reukauffi* or other nectar yeasts mate within the insect gut, Stefanini et al. [26] found that *Saccharomyces paradoxus* is unable to survive in social wasp intestines unless it mates and forms hybrids with *Saccharomyces cerevisiae*. Similarly, given that some of the most common nectar and honeydew bacterial inhabitants are closely related to taxa prone to horizontal gene transfer (HGT) in diverse habitats (e.g. the members of genera *Acinetobacter* and *Pseudomonas* and diverse enterobacteria, see, e.g. Refs. [27–29]), it is reasonable to think that HGT might also take place between members of the insect gut microbiota.

In return for the dispersal and shelter services offered by the insects, it has been suggested that insect-associated microbes play an important role in host physiology and nutrition, for example, by providing digestive enzymes and essential nutrients that insects cannot produce by themselves [25,30]. Microorganisms might also participate in the detoxification of toxic plant metabolites that are present in the insect's diet, suppression of pathogens that might interfere with insect development, and localization of suitable food resources [30]. The complementary benefits of these interactions are the basis of the 'dispersal-encounter' hypothesis, as proposed for yeast–insect interactions by Madden et al. [31], whereby microorganisms are dispersed by plant-visiting animals between different sugar-rich ephemeral patches, and animals obtain the benefits of an honest signal for the sugar resources and potential benefits for their health.

Applications of nectar and honeydew microbes in biological control and pollination: current state and opportunities

Nectar and honeydew are promising sources for the isolation of microbial BCAs, particularly those that act indirectly via the attraction of natural enemies of pest insects (Figure 1). Likewise, these microbes may have an additional positive effect on pollination (Figure 1). Although other beneficial insects may be affected by the presence of microbial inhabitants in nectar or honeydew, we will focus here on the indirect benefit of microbes on plant health via effects on parasitoids. Besides, given the

Figure 1



Application of the honeydew and nectar microbiomes to biological pest control and improved pollination of agricultural plants. Both honeydew, secreted by aphids and other phloem-feeding insects **(A)**, and floral nectar **(B)** are rapidly colonized by sooty molds and other microorganisms **(C)** and specialist and opportunistic nectar yeasts and bacteria **(D)**, respectively. Honeydew and nectar microbes produce diverse VOCs **(E)** that can deter pest insects **(F)** and/or attract their natural enemies **(G)** and, thus, constitute a sustainable and nontoxic alternative to traditional chemical pest control **(H)**. Furthermore, microbial VOCs can attract or deter bumble bees, honey bees, and other floral visitors **(I)**, and could be used to improve natural pollination. Figure created with BioRender.com.

outstanding agricultural and ecological importance of insect pollination, and considering that pollinators are becoming less abundant in many (agro)ecosystems [32], we will comment on the use of the nectar microbiome in pollination management.

Biological pest control

There is increasing evidence that plant- and/or insect-associated microbiomes can be exploited in indirect biological pest control [2]. For example, it has been shown that feeding microbe-free laboratory-bred populations of the egg parasitoid *Trichogramma chilonis* (Hymenoptera) with some microbial taxa commonly found in nectar, including *Acinetobacter*, *Pseudomonas*, and *Metschnikowia*, enhances the proportion of females of this wasp and its parasitism efficiency against larvae of *Helicoverpa armigera*, an important lepidopteran pest infesting several economically important crops [33]. Furthermore, it has been demonstrated that feeding on nectars fermented by microbes can have a significant impact (either positive or detrimental, depending on the microbial species involved) on the longevity and survival of the generalist aphid parasitoid *Aphidius ervi* (Hymenoptera) [34,35]. Additionally, it has been demonstrated that the microorganisms that inhabit nectar and honeydew produce diverse VOCs that deter pest insects or attract their natural enemies [2,14,23,36••]. Therefore, selection of companion plants that are attractive to beneficial insects offering sugar-rich resources such as nectar and honeydew and that are colonized by VOC-producing microorganisms should be considered as an additional strategy in biological control programs [36••]. Alternatively, nectar or honeydew could be enriched (e.g. by artificial inoculation) with microorganisms producing VOCs that attract natural enemies, as supported by the evidence gathered in several experimental setups [14,22,35,37]. However, a major drawback of these studies is that they typically analyze the volatilome of monospecific microbial cultures, without considering the effects that interspecific interactions might have on VOC production [38].

Despite their promising potential, the eventual application of nectar or honeydew microbes in biological control is still facing important challenges such as developing feasible mass-production techniques, finding suitable inoculum forms and persistent and stable formulations, designing simple application tools, assessing the survival of the microorganisms in the field, and ensuring the biosafety of the product for humans and ecosystems (see next section). In addition, it is important to consider that the potential benefits of microbial-mediated biological control may be counteracted if the used microorganisms also attract and/or enhance in some way the fitness of pest insects or hyperparasitoids (i.e. secondary parasites of immature stages of primary parasitoids) [36••,39]. Therefore, there is still much research to be done before the nectar and honeydew microbiomes can be efficiently and safely used in biological pest control.

Improved pollination

Pest regulation and successful pollination are two critical and complementary services for natural and agricultural ecosystems. Several laboratory and field studies have demonstrated that nectar yeasts (in particular, *M. reukaufii*) and bacteria can impact pollinator foraging preferences, mostly through the alteration of nectar chemistry and the production of VOCs [38,40–44]. Most research carried out so far has focused on simple systems, including monocultures of a few microbial species (or mixed cultures of a yeast and a bacterial species [38]) and a limited number of insect species (mostly honey bees and bumble bees), even when most plants are visited by diverse pollinators [45••]. Since responses of pollinators to nectar microbes could vary across insect taxa and depend on the behavioral assay employed [40,46], more studies are needed on whether patterns observed in simple systems can be applied to an improved field pollination. Furthermore, the few studies dealing with crop plants have yielded variable results (e.g. increased flower visitation after spraying blossoms with a mixture of nectar yeasts and bacteria [47] vs. no effect of microbial treatment on pollination [48]), so further research is clearly needed to fully characterize the benefits, limitations, and potential risks of using nectar microbes in improved pollination.

One potential, but so far minimally explored, use of microbes is to improve the health of key pollinators such as bumble bees and honey bees. Recent studies have shown that nectar microorganisms can have a positive impact on bumble bees' fitness and colony development [49,50]. The potential of beneficial microbes naturally occurring in plants to improve the health of commercial bee colonies, widely affected by different parasites and pathogens [51], has not been explored yet but deserves attention.

Risk assessment, perspectives, and conclusions

The application of biocontrol microorganisms in protected environments such as glasshouses is relatively straightforward due to their delivery in a more isolated and controlled environment and its potentially lower ecological impact. In contrast, the release of biocontrol agents to the open field should consider a myriad of abiotic and biotic factors that might determine their optimal growth and performance. For example, it has been demonstrated that the use of agricultural fungicides can significantly alter the composition of nectar microbial communities [52–54], and that the floral microbiome can respond to different orchard management schemes [55]. Furthermore, despite its great potential, the use of nectar and honeydew microbes for pest control and improved pollination raises a number of ecological, legal, and ethical issues that should be addressed before new products are released to the field.

The successful application of nectar and honeydew microorganisms as BCAs or to improve pollination will depend on achieving a good understanding of the biology, ecology, and population dynamics of the target insects. It must also consider all possible side effects that the use of these microorganisms can have on target and nontarget insects, which may include the alteration of their health status (e.g. through modification of their normal microbiota) and/or their foraging preferences (e.g. through modification of the chemical composition of nectar and honeydew, including the release of VOCs) [56•]. Although registered formulations of microbial BCAs undergo toxicology tests to assess their lethal effects on emblematic insects such as honey bees, very little is known yet about their effects on other potential pollinators and other beneficial insects [57••]. Similarly, it is essential to guarantee that BCAs are nonpathogenic for both crop and natural plants. Additionally, nectar and honeydew microorganisms can exert a strong influence on other community members by diverse competitive, antagonistic, and mutualistic interactions [5,10,19], which should be kept in mind when introducing new species or microbial consortia with biocontrol and/or pollination purposes.

Regarding the legal issues, it is important to note that each country/region has its own legislation on BCAs. Besides, since microorganisms are genetic resources, their use in biocontrol should meet the requirements of the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity [58]. While the legal issues can be relatively easily overcome, the long and expensive registration process of new BCAs seems to be the main hurdle for the commercialization of these products. Finally, one must keep in mind the ethical aspects of microbiome research, use, and knowledge management. So far, most advances in this field refer to human microbiome research, but similar guiding principles (e.g. open-access policies, consideration of microbiomes as global common heritage, stimulation of international and public–private collaboration, etc.) should be applied to the characterization and utilization of environmental microbiomes [59•].

In conclusion, the nectar and honeydew microbiomes have a great potential to provide sustainable solutions to biological pest control and pollination management. However, the successful application of these microbiomes in sustainable agriculture requires a deep understanding of their community dynamics and multikingdom interactions, and the proper recognition of diverse ecological, legal, and ethical aspects.

Funding
Open Access funding provided thanks to the CRUE-CSIC agreement with Elsevier. This work was supported by funding from the Spanish Ministry of Science and Innovation (to SA-P, "Ramón y Cajal" contract, RYC2018-023847-I). Work in the lab of BL is financially supported by Fonds Wetenschappelijk Onderzoek – Vlaanderen (FWO), Vlaio, and internal KU Leuven funds. The funders had no role in the preparation of the paper or in the decision to publish.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Acknowledgements
Sergio Álvarez-Pérez dedicates this article in loving memory to Ms. Pilar Pérez Sanz, who taught him the importance of courage, perseverance, and kindness in life. The authors thank Prof. Antonino Cusumano for his constructive feedback on the paper drafts.

Supporting information
Supplementary data associated with this article can be found in the online version at doi:10.1016/j.cois.2023.101138.

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Horrigan L, Lawrence RS, Walker P: **How sustainable agriculture can address the environmental and human health harms of industrial agriculture**. *Environ Health Perspect* 2002, **110**:445-456.
2. Francis F, Jacquemyn H, Delvigne F, Lievens B: **From diverse origins to specific targets: role of microorganisms in indirect pest biological control**. *Insects* 2020, **11**:533.
3. Syed Ab Rahman SF, Singh E, Pieterse CMJ, Schenk PM: **Emerging microbial biocontrol strategies for plant pathogens**. *Plant Sci* 2018, **267**:102-111.
4. Álvarez-Pérez S: **Ecology: yeasts on their natural environment**. In *Yeasts: from Nature to Bioprocesses*. Edited by Alves Júnior SL, Treichel H, Basso TO, Stambuk BU. Bentham Science Publishers; 2022:27-57.
5. Chomnunti P, Hongsanan S, Aguirre-Hudson B, Tian Q, Peršoh D, Dhami MK, Alias AS, Xu J, Liu X, Stadler M, *et al.*: **The sooty moulds**. *Fungal Divers* 2014, **66**:1-36.
6. Vannette RL: **The floral microbiome: plant, pollinator, and microbial perspectives**. *Annu Rev Ecol Evol Syst* 2020, **51**:363-386.
7. Guezen JM, Forrest JR: **Seasonality of floral resources in relation to bee activity in agroecosystems**. *Ecol Evol* 2021, **11**:3130-3147.
8. Tena A, Wäckers FL, Heimpel GE, Urbaneja A, Pekas A: **Parasitoid nutritional ecology in a community context: the importance of honeydew and implications for biological control**. *Curr Opin Insect Sci* 2016, **14**:100-104.
9. •• Nicolson SW: **Sweet solutions: nectar chemistry and quality**. *Philos Trans R Soc Lond B Biol Sci* 2022, **377**:20210163.
This is a must-read review on floral nectar's chemistry that emphasizes the role of water as a nectar nutrient, which has been traditionally neglected in the literature due to the variability in water content and diverse methodological challenges of nectar sampling.
10. Álvarez-Pérez S, Lievens B, Fukami T: **Yeast–bacterium interactions: the next frontier in nectar research**. *Trends Plant Sci* 2019, **24**:393-401.
11. de Vega C, Álvarez-Pérez S, Albaladejo RG, Steenhuisen SL, Lachance MA, Johnson SD, Herrera CM: **The role of plant–pollinator interactions in structuring nectar microbial communities**. *J Ecol* 2021, **109**:3379-3395.
12. Lanan M: **Honeydew**. In *Encyclopedia of Social Insects*. Edited by Starr C. Springer; 2020.
13. Sabri A, Vandermoten S, Leroy PD, Haubruge E, Hance T, Thonart P, De Pauw E, Francis F: **Proteomic investigation of aphid honeydew reveals an unexpected diversity of proteins**. *PLoS One* 2013, **8**:e74656.
14. Leroy PD, Sabri A, Heuskin S, Thonart P, Lognay G, Verheggen FJ, Francis F, Brostaux Y, Felton GW, Haubruge E: **Microorganisms from aphid honeydew attract and enhance the efficacy of natural enemies**. *Nat Commun* 2011, **2**:348.
15. Lievens B, Hallsworth JE, Pozo MI, Belgacem ZB, Stevenson A, Willems KA, Jacquemyn H: **Microbiology of sugar-rich environments: diversity, ecology and system constraints**. *Environ Microbiol* 2015, **17**:278-298.
16. Jacquemyn H, Pozo MI, Álvarez-Pérez S, Lievens B, Fukami T: **Yeast-nectar interactions: metacommunities and effects on pollinators**. *Curr Opin Insect Sci* 2021, **44**:35-40.
17. Álvarez-Pérez S, Baker LJ, Morris MM, Tsuji K, Sanchez VA, Fukami T, Vannette RL, Lievens B, Hendry TA: ***Acinetobacter pollinis* sp. nov., *Acinetobacter baretiae* sp. nov. and *Acinetobacter rathckeae* sp. nov., isolated from floral nectar and honey bees**. *Int J Syst Evol Microbiol* 2021, **71**.
18. Álvarez-Pérez S, de Vega C, Vanoirbeek K, Tsuji K, Jacquemyn H, Fukami T, Michiels C, Lievens B: **Phylogenomic analysis of the genus *Rosenbergiella* and description of *Rosenbergiella gaditana* sp. nov., *Rosenbergiella metrosideri* sp. nov., *Rosenbergiella epipactidis* subsp. *epipactidis* subsp. nov., *Rosenbergiella epipactidis* subsp. *californiensis* subsp. nov., *Rosenbergiella epipactidis* subsp. *japonicus* subsp. nov., *Rosenbergiella nectarea* subsp. *nectarea* subsp. nov. and *Rosenbergiella nectarea* subsp. *apis* subsp. nov., isolated from floral nectar and insects**. *Int J Syst Evol Microbiol* 2023, **73**.
19. Lv Z, Hu C, Hu Y, Wu D, Zhou H, Tian L: **Identification of dominant taxa of sooty moulds and their impact on the leaf microbiome**. *Environ Microbiol* 2023, **25**:853-866.

20. Dhami MK, Weir BS, Taylor MW, Beggs JR: **Diverse honeydew-consuming fungal communities associated with scale insects**. *PLoS One* 2013, **8**:e70316.

21. Flessa F, Harjes J, Cáceres MES, Rambold G: **Comparative analyses of sooty mould communities from Brazil and Central Europe**. *Mycol Prog* 2021, **20**:869-887.

22. Goelen T, Sobhy IS, Vanderaa C, de Boer JG, Delvigne F, Francis F, Wäckers F, Rediers H, Verstrepen KJ, Wenseleers T, *et al*.: **Volatiles of bacteria associated with parasitoid habitats elicit distinct olfactory responses in an aphid parasitoid and its hyperparasitoid**. *Funct Ecol* 2020, **34**:507-520.

23. Cusumano A, Lievens B: **Effects of nectar-inhabiting microbes on the foraging behavior and performance of parasitoids**. *Curr Opin Insect Sci* 2023, **60**:101116 (submitted to this Special Issue).

24. Pozo MI, Bartlewicz J, van Oystaeyen A, Benavente A, van Kemenade G, Wäckers F, Jacquemyn H: **Surviving in the absence of flowers: do nectar yeasts rely on overwintering bumblebee queens to complete their annual life cycle?** *FEMS Microbiol Ecol* 2018, **94**:fiy196.

25. Stefanini I: **Yeast-insect associations: it takes guts**. *Yeast* 2018, **35**:315-330.

26. Stefanini I, Dapporto L, Berná L, Polsinelli M, Turillazzi S, Cavalieri D: **Social wasps are a *Saccharomyces* mating nest**. *Proc Natl Acad Sci USA* 2016, **113**:2247-2251.

27. Job V, Gomez-Valero L, Renier A, Rusniok C, Bouillot S, Chenal-Francisque V, Gueguen E, Adrait A, Robert-Genthon M, Jeannot K, *et al*.: **Genomic erosion and horizontal gene transfer shape functional differences of the ExlA toxin in *Pseudomonas* spp**. *iScience* 2022, **25**:104596.

28. Mindlin S, Maslova O, Beletsky A, Nurmukanova V, Zong Z, Mardanov A, Petrova M: **Ubiquitous conjugative mega-plasmids of *Acinetobacter* species and their role in horizontal transfer of multi-drug resistance**. *Front Microbiol* 2021, **12**:728644.

29. Naum M, Brown EW, Mason-Gamer RJ: **Phylogenetic evidence for extensive horizontal gene transfer of type III secretion system genes among enterobacterial plant pathogens**. *Microbiology* 2009, **155**:3187-3199.

30. Blackwell M: **Made for each other: ascomycete yeasts and insects**. *Microbiol Spectr* 2017, **5** (FUNK-0081-2016).

31. Madden AA, Epps MJ, Fukami T, Irwin RE, Sheppard J, Sorger DM, Dunn RR: **The ecology of insect-yeast relationships and its relevance to human industry**. *Proc Biol Sci* 2018, **285**:20172733.

32. Bartomeus I, Dicks LV: **The need for coordinated transdisciplinary research infrastructures for pollinator conservation and crop pollination resilience**. *Environ Res Lett* 2019, **14**:045017.

33. Srinatha HS, Jalali SK, Sriram S, Chakravarthy AK: **Isolation of microbes associated with field-collected populations of the egg parasitoid, *Trichogramma chilonis* capable of enhancing biotic fitness**. *Biocontrol Sci Technol* 2015, **25**:789-802.

34. Lenaerts M, Goelen T, Paulussen C, Herrera-Malaver B, Steensels J, Van den Ende W, Verstrepen KJ, Wäckers F, Jacquemyn H, Lievens B: **Nectar bacteria affect life history of a generalist aphid parasitoid by altering nectar chemistry**. *Funct Ecol* 2017, **31**:2061-2069.

35. Sobhy IS, Baets D, Goelen T, Herrera-Malaver B, Bosmans L, Van den Ende W, Verstrepen KJ, 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**:1009.

36. •• Colazza S, Peri E, Cusumano A: **Chemical ecology of floral resources in conservation biological control**. *Annu Rev Entomol* 2023, **68**:13-29.

This article addresses the prospects for the chemical ecology of floral resources, including the role of microbial VOCs, to enhance conservation biological control and suggests several strategies to selectively favor third-trophic level parasitoids over fourth-trophic-level hyperparasitoids.

37. Sobhy IS, Goelen T, Herrera-Malaver B, Verstrepen KJ, Wäckers F, Jacquemyn H, Lievens B: **Associative learning and memory retention of nectar yeast volatiles in a generalist parasitoid**. *Anim Behav* 2019, **153**:137-146.

38. Rering CC, Vannette RL, Schaeffer RN, Beck JJ: **Microbial co-occurrence in floral nectar affects metabolites and attractiveness to a generalist pollinator**. *J Chem Ecol* 2020, **46**:659-667.

39. Jones R, Fountain MT, Günther CS, Eady PE, Goddard MR: **Separate and combined *Hanseniaspora uvarum* and *Metschnikowia pulcherrima* metabolic volatiles are attractive to *Drosophila suzukii* in the laboratory and field**. *Sci Rep* 2021, **11**:1201.

40. de Vega C, Albaladejo RG, Álvarez-Pérez S, Herrera CM: **Contrasting effects of nectar yeasts on the reproduction of Mediterranean plant species**. *Am J Bot* 2022, **109**:393-405.

41. Herrera CM, Pozo MI, Medrano M: **Yeasts in nectar of an early-blooming herb: sought by bumble bees, detrimental to plant fecundity**. *Ecology* 2013, **94**:273-279.

42. Schaeffer RN, Phillips CR, Duryea MC, Andicoechea J, Irwin RE: **Nectar yeasts in the tall Larkspur *Delphinium barbeyi* (Ranunculaceae) and effects on components of pollinator foraging behavior**. *PLoS One* 2014, **9**:e108214.

43. Vannette RL, Gauthier MP, Fukami T: **Nectar bacteria, but not yeast, weaken a plant-pollinator mutualism**. *Proc Biol Sci* 2012, **280**:20122601.

44. Yang M, Deng GC, Gong YB, Huang SQ: **Nectar yeasts enhance the interaction between *Clematis akebioides* and its bumblebee pollinator**. *Plant Biol* 2019, **21**:732-737.

45. •• Cullen NP, Fetters AM, Ashman TL: **Integrating microbes into pollination**. *Curr Opin Insect Sci* 2021, **44**:48-54.

This article reviews previous studies addressing the (potential) roles of microorganisms in pre-pollination (i.e. pollination attraction), pollination (i.e. pollen dispersal and receipt), and post-pollination (i.e. pollen germination and fertilization) processes and highlights some pending questions in this research field.

46. Crowley-Gall A, Rering CC, Rudolph AB, Vannette RL, Beck JJ: **Volatile microbial semiochemicals and insect perception at flowers**. *Curr Opin Insect Sci* 2021, **44**:23-34.

47. 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**:e0250203.

48. Schaeffer RN, Crowder DW, Illán JG, Beck JJ, Fukami T, Williams NM, Vannette RL: **Disease management during bloom affects the floral microbiome but not pollination in a mass-flowering crop**. *J Appl Ecol* 2023, **60**:64-76.

49. Pozo MI, van Kemenade G, van Oystaeyen A, Aledón-Catalá T, Benavente A, Van den Ende W, Wäckers F, Jacquemyn H: **The impact of yeast presence in nectar on bumble bee behavior and fitness**. *Ecol Monogr* 2020, **90**:e01393.

50. Pozo MI, 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**:689-703.

51. Evans E, Strange J, Sadd B, Tripodi A, Figueroa L, Adams L, Colla S, Duennes M, Lehmann D, Moylett H, *et al*.: **Parasites, parasitoids, and hive products that are potentially deleterious to wild and commercially raised bumble bees (*Bombus* spp.) in North America**. *J Poll Ecol* 2023, **32**:37-53.

52. Bartlewicz J, Pozo MI, Honnay O, Lievens B, Jacquemyn H: **Effects of agricultural fungicides on microorganisms associated with floral nectar: susceptibility assays and field experiments**. *Environ Sci Pollut Res Int* 2016, **23**:19776-19786.

53. Schaeffer RN, Vannette RL, Brittain C, Williams NM, Fukami T: **Non-target effects of fungicides on nectar-inhabiting fungi of almond flowers**. *Environ Microbiol Rep* 2017, **9**:79-84.

54. Voß AC, Hauertmann M, Laufer MC, Lach A, Junker RR, Eilers EJ: **Fungicides and strawberry pollination-effects on floral scent, pollen attributes and bumblebee behavior**. *PLoS One* 2023, **18**:e0289283.

55. Schaeffer RN, Pfeiffer VW, Basu S, Brousil M, Strohm C, DuPont ST, Vannette RL, Crowder DW: **Orchard management and landscape context mediate the pear floral microbiome**. *Appl Environ Microbiol* 2021, **87**:e0004821.

56. • Martin VN, Schaeffer RN, Fukami T: **Potential effects of nectar microbes on pollinator health**. *Philos Trans R Soc Lond B Biol Sci* 2022, **377**:20210155.
This article presents a comprehensive overview of previous research on plant–microbe–pollinator interactions and analyzes the consequences of such interactions for pollinator health.

57. •• Burgess EC, Schaeffer RN: **The floral microbiome and its management in agroecosystems: a perspective**. *J Agric Food Chem* 2022, **70**:9819-9825.
This is a nice perspective article that reviews the current state of research concerning floral microbiome assembly and management in agroecosystems and elaborates on future developments of the field to improve the sustainability of disease control and insect-mediated ecosystem services.

58. Smith D, Hinz H, Mulema J, Weyl P, Ryan MJ: **Biological control and the Nagoya Protocol on access and benefit sharing – a case of effective due diligence**. *Biocontrol Sci Technol* 2018, **28**:914-926.

59. • Lange L, Berg G, Cernava T, Champomier-Vergès MC, Charles T, Cocolin L, Cotter P, D'Hondt K, Kostic T, Maguin E, *et al.*: **Microbiome ethics, guiding principles for microbiome research, use and knowledge management**. *Environ Micro* 2022, **17**:50.
This article evaluates the current state of microbiome ethics on the basis of four cornerstone principles (*Do Good, Don't Harm, Respect, and Act Justly*) and proposes a set of guiding principles for future microbiome research.