Bidirectional interaction between phyllospheric microbiotas and plant volatile emissions

Gerard Farré-Armengol[1,2,*], Iolanda Filella[1,2], Joan Llusia[1,2] and Josep Peñuelas[1,2]

[1]CSIC, Global Ecology Unit CREAF-CSIC-UAB, Cerdanyola del Vallès, 08193 Barcelona, Catalonia, Spain.

[2]CREAF, Cerdanyola del Vallès, 08193 Barcelona, Catalonia, Spain.

*Correspondence: g.farre@creaf.uab.cat (G. Farré-Armengol)

Keywords: phyllosphere, microorganisms, VOCs, bacteria, fungi.

Abstract

Due to their antimicrobial effects and their potential role as carbon sources, plant VOC emissions play significant roles in determining the characteristics of the microbial communities that can establish on plant surfaces. Furthermore, epiphytic microorganisms, including bacteria and fungi, can affect plant VOC emissions in different ways: by producing and emitting their own VOCs, which are added to and mixed with the plant VOC blend; by affecting plant physiology and modifying the production and emission of VOCs; and by metabolizing the VOCs emitted by the plant. The study of the interactions between plant VOC emissions and phyllospheric microbiotas is thus of great interest and deserves more attention.

The phyllosphere

The phyllosphere includes all aboveground plant surfaces that provide habitats for microorganisms. The total surface area of the global phyllosphere has been estimated to represent approximately 10^9 km^2, which could be colonized by bacterial populations of approximately 10^{26} cells [1,2]. Aboveground plant surfaces harbor hundreds of species of bacteria and fungi with deleterious or beneficial effects on plants [2,3]. Among these, bacteria are by far the most abundant phyllospheric colonists and can reach densities of 10^7 cells/cm^2 of leaf surface [4]. These microbial communities are very biodiverse and can vary their composition between and within plant species, depending on several environmental factors [5–8]. The phyllosphere consists of various aboveground surfaces of plants, including the surfaces of stems (caulosphere), flowers (anthosphere), fruits (carposphere) and leaves (phylloplane), all of which can significantly differ in their microbial composition [9,10].

Bidirectional effects between plant volatiles and phyllospheric microbiota

Plant VOC (volatile organic compound) emissions play a relevant role in determining the characteristics of the microbial communities that inhabit plant surfaces, through their antimicrobial effects and their role as carbon sources for some microorganisms (Figure 1A). By contrast, plant phyllospheric microbiotas have the potential to affect plant physiology and modify plant biochemistry. Phyllospheric microorganisms reside at the interface between the plant surface and the atmosphere, where gases are exchanged, so the organisms can significantly modify the specific conditions of this microhabitat and interfere with plant VOC emissions [11] (1B). We review the current information on the bidirectional effects established between VOC emissions from aboveground plant surfaces and phyllospheric microbiotas. The interaction between plants and bacteria through VOCs is a research topic that warrants an increased research effort for providing useful information for understanding the emission of VOCs from vegetation.

Effects of plant VOC emissions on phyllospheric microbiotas

Plant VOCs can affect the phyllospheric microbiota by serving as carbon sources [12–18]. Microorganisms such as the yeast *Candida boidinii* and the bacteria *Methylobacterium extorquens* use plant VOCs such as methanol or methane as substrates for their growth, although they are facultative methylotrophs, so they do not rely only or mostly on the consumption of methanol for their subsistence [12–14]. Methylotrophic metabolism thus represents a selective advantage for bacterial colonization of the phyllosphere [12]. This advantage allows the microorganisms that use plant VOCs as substrates to grow preferentially on the surface of plants that are abundant emitters of these compounds.

Plant VOCs play a significant role in determining the characteristics of the microbial communities that can establish on each plant tissue, also through their antimicrobial effects [10]. Several studies have reported growth-inhibiting effects of VOCs on microbes [19,20]. Terpenoids, phenylpropanoids and benzenoids are major constituents of plant VOC emissions that have antimicrobial properties and strongly influence phyllospheric microbial colonization [21–23]. Main constituents of plant VOC extracts such as the common monoterpenes limonene and β-pinene have inhibiting effects on bacterial growth [21]. Aldehydes such as benzaldehyde, acetaldehyde, and cinnamaldehyde also strongly inhibit microbial growth [21,24].

Although the VOC concentrations tested in most studies revealing antimicrobial effects of VOCs are high compared to the amounts that are probably present on plant surfaces, because these works test doses from leaf extracts, strong evidence suggests that plant VOC emissions play a relevant role in structuring plant-microbe interactions on aboveground plant surfaces [10,25–27]. The antibacterial and antifungal properties of plant VOC emissions may play a significant role in selecting the microorganisms that can establish on plant surfaces by limiting their ability to colonize and grow. Furthermore, different plant parts have developed different degrees of chemical protection by emitting different amounts and profiles of VOCs that may depend on the relevance and function of each organ. It has been observed that the composition of the epiphytic bacterial communities of *Saponaria officinalis* and *Lotus corniculatus* plants differed significantly between petals and leaves, with less bacterial diversity on petals that was attributable to the antibacterial VOCs released by the floral tissues [10].

Effects of phyllospheric microbiotas on plant VOC emissions

Emission of microbial VOCs

Phyllospheric microbiotas consisting of bacteria and fungi contribute to plant volatile blends with their own emissions of VOCs from de novo biosynthesis [28–30] and biotransformation [15,31–33]. In general, bacterial VOC emission profiles have been described to be rich in alkenes, alcohols, ketones and terpenes, while in contrast, fungal VOC profiles are dominated by alcohols, benzenoids, aldehydes, and ketones (Figure 2) [30]. However, these VOC emission profiles were obtained from bacteria and fungi grown on rich media, and microorganisms growing on the phyllosphere that is much more limited in nutrients can probably show different profiles [34,35]. The composition of VOCs emitted by microorganisms is species-specific and can show different levels of complexity. The relative composition of microbial VOC blends varies with growth conditions (temperature, oxygen availability, pH), the carbon source availability and the age of the culture. Ultimately, the microbial VOC profile is a consequence of specific metabolic activities of the particular microorganism. Floral microbiotas, for example, can significantly affect the composition and the amounts of volatile terpenes emitted by flowers, which play crucial ecological roles in pollinator attraction. A comparison of floral terpene emissions and contents in untreated *Sambucus nigra* plants and plants submitted to fumigation with antibiotics showed that the removal of the microbiota significantly decreased the rates of floral terpene emission, even though the floral terpene contents did not change. This suggests that the microbiota of the anthosphere significantly contributes to floral VOC emissions [31]. Microorganisms that live on fruit surfaces also produce and emit VOCs that can significantly contribute to fruit aroma. This was demonstrated by the clearly distinguishable patterns of VOC emission produced by the bacteria and fungi from the carposphere of wine grapes [32].

Effects of microbial VOCs on plants

Phyllosphere microbes emit different types of VOCs [30] and therefore have a great potential to affect plant physiology. Some microbial VOCs enhance plant growth and stress resistance [36]. The VOCs emitted by some non pathogenic microbes also prevent the colonization of plant tissues by fungal and bacterial pathogens [36]. The endophytic bacterium *Enterobacter aerogenes* increased plant pathogen resistance and affected tritrophic interactions in maize (*Zea mays*) plants by the production and release of 2,3-butanediol, a VOC that acts as a phytohormone [37]. Microbial VOCs can mediate several interactions between bacteria and fungi that have negative or neutral effects on plants [38]. These studies indicate that phyllospheric microbiotas have significant effects on the host plant and its interactions with other organisms by emitting their own VOC profiles.

Microbes induce plant VOC emission

Microbes can also alter the plant VOC emissions by inducing plant defensive responses. Some pathogenic microbes such as the fungi *Melampsora epitea* and *Fusarium* sp., or the bacteria *Pseudomonas syringae* affect VOC production when they elicit an immune response on the plant [39–41], but other species can be tolerated by the immune system of the host plant [42]. Some studies have reported the induction of plant VOC emission by bacterial and fungal pathogens. Among these, terpenoids play a major role as defensive VOCs that are emitted in greater amounts after fungal infection [39,40,43,44]. Also, VOCs from the lipoxygenase pathway are emitted from green leaves in considerable amounts after infection and play a relevant role in inducing defensive responses in neighboring plants [39,40,44,45]. This induction has a positive effect on total VOC emissions and may also change VOC composition when the production and emission of new compounds that are not among the constitutively emitted VOCs are elicited [46].

Transformation of plant VOCs

Microbes can change the VOC compositions of plants by degrading and consuming plant VOCs as carbon sources [42]. Ubiquitous VOCs that are abundant in the atmosphere also accumulate in significant amounts on plant surfaces by uptake and deposition [47] and can thereby become accessible to phyllospheric microorganisms. Some foliar microorganisms can degrade these VOCs that are released by the plant or are adsorbed to the leaf cuticle in considerable amounts, such as methanol, methane, phenol and toluene [14,48–51]. Methanol, for example, is a prominent carbon source for epiphytic components of microbiotas, such as the methylotrophic bacterium *Methylobacterium extorquens* [12] or the methylotrophic yeast *Candida boidinii* [13].

Bacteria in the genus *Methylobacterium*, facultative methylotrophs found on the surface of strawberry leaves, can consume the methanol that is constantly emitted by the plant [14]. Diverse common soil microorganisms that are also ubiquitous on plant surfaces can degrade other VOCs such as monoterpenes [15–17] and aromatic compounds [18]. Phyllospheric microorganisms are thus able to degrade the plant-emitted VOCs that play significant roles in the plant biotic and abiotic environments [52–54].

Concluding remarks and future directions

Phyllospheric microbiology is an emerging research field at an initial stage. The inclusion of phyllospheric microbiota into ecological studies will allow making a key step forward in terrestrial ecology. The consideration of phyllospheric communities on the understanding of plant and community ecology will open the doors to a vast field of work. The composition of phyllospheric communities and their effects on plant physiology and on many ecological processes remain to be elucidated or investigated in more detail by exploiting the current genomic and metabolomic techniques [3]. Plant VOC emissions that play multiple relevant roles in plant and community ecology and also in atmospheric chemistry can be significantly affected by the activities of microorganisms living on the phyllosphere. Future research efforts should thus be devoted to continuing the study of the modes in which microorganisms can affect plant VOC emissions in various aboveground plant tissues, while also characterizing the magnitude of the changes and the resulting impacts on ecological interactions that are mediated through VOCs. This information may be of relevant interest for assessing the adequacy of different treatments applied in crop management to control fungal and bacterial plant pathogens (see also outstanding questions). Assuming that pesticide application changes or removes the natural phyllospheric microbiota from crop plants, then plant VOC emissions as well as other plant traits may be affected, and as a result, interactions with other organisms such as pollinators, herbivores or parasitoids will be affected. Pesticides have strong effects on community composition in the phyllosphere, suggesting that pesticide treatments could interfere with the natural interactions between phyllospheric microbiotas and plant defenses [55–57], or even with flower scent [46]. Addressing this question in future experiments may reveal indirect impacts of antifungal and antibacterial pesticides on herbivory and pollination, which are very relevant for crop production.

New studies should also assess the role of plant VOC emissions on determining the types and numbers of microorganisms that can establish and grow on the phyllosphere, relative to many other environmental variables. For example, the use of modified plant lines in which the expression of specific VOCs is suppressed can be used to assess this question in a more realistic way than exposing cultured monospecific microbial colonies to the VOCs. Tests with microbial cultures, however, can also complement and support the experiments by providing direct evidence of the antimicrobial effects of individual VOCs or complex VOC mixtures [19,20].

A better knowledge of the effects of phyllospheric microbiotas on VOC emissions from vegetation may also help to better understand and estimate the impacts of these microorganisms on atmospheric chemistry and even climate. A few recent studies indicate that phyllospheric microbiotas contribute greatly to the composition and amount of VOCs emitted by plants [46,58]. After better characterizing the effects of phyllospheric microbiotas on VOC emissions from a variety of plant species and under different conditions, future models of VOC emission should implement this information to better predict VOC emissions from terrestrial ecosystems and vegetation.

Acknowledgements

This research was supported by the Spanish Government grant CGL2013-48074-P, the Catalan Government grant SGR 2014-274, the European Research Council Synergy grant ERC-2013-SyG-610028 IMBALANCE-P, and by the Air Liquide Foundation (AIRLICOVS grant).

References

1. Vorholt, J.A. (2012) Microbial life in the phyllosphere. *Nat. Rev. Microbiol.* 10, 828–40
2. Lindow, S.E. and Brandl, M.T. (2003) Microbiology of the Phyllosphere. *Appl. Environ. Microbiol.* 69, 1875–1883
3. Peñuelas, J. and Terradas, J. (2014) The foliar microbiome. *Trends Plant Sci.* 19, 278–280
4. Lindow, S.E. and Leveau, J.H.. (2002) Phyllosphere microbiology. *Curr. Opin. Biotechnol.* 13, 238–243
5. Whipps, J.M. *et al.* (2008) Phyllosphere microbiology with special reference to diversity and plant genotype. *J. Appl. Microbiol.* 105, 1744–55
6. Izhaki, I. *et al.* (2013) Variability of bacterial community composition on leaves between and within plant species. *Curr. Microbiol.* 66, 227–235
7. Peñuelas, J. *et al.* (2012) Summer season and long-term drought increase the richness of bacteria and fungi in the foliar phyllosphere of Quercus ilex in a mixed Mediterranean forest. *Plant Biol.* 14, 565–575
8. Rico, L. *et al.* (2014) Community structures of N2-fixing bacteria associated with the phyllosphere of a Holm oak forest and their response to drought. *Plant Biol.* 16, 586–593
9. Ottesen, A.R. *et al.* (2013) Baseline survey of the anatomical microbial ecology of an important food plant: Solanum lycopersicum (tomato). *BMC Microbiol.* 13, 114
10. Junker, R.R. *et al.* (2011) Composition of epiphytic bacterial communities differs on petals and leaves. *Plant Biol.* 13, 918–924
11. Bringel, F. and Couée, I. (2015) Pivotal roles of phyllosphere microorganisms at the interface between plant functioning and atmospheric trace gas dynamics. *Front. Microbiol.* 6, 486
12. Sy, A. *et al.* (2005) Methylotrophic Metabolism Is Advantageous for Methylobacterium extorquens during Colonization of Medicago truncatula under Competitive Conditions. *Appl. Environ. Microbiol.* 71, 7245–7252
13. Kawaguchi, K. *et al.* (2011) Yeast methylotrophy and autophagy in a methanol-oscillating environment on growing Arabidopsis thaliana leaves. *PLoS One* 6, e25257
14. Abanda-Nkpwatt, D. *et al.* (2006) Molecular interaction between Methylobacterium extorquens and seedlings: growth promotion, methanol consumption, and localization of the methanol emission site. *J. Exp. Bot.* 57, 4025–4032
15. Marmulla, R. and Harder, J. (2014) Microbial monoterpene transformations-a review. *Front. Microbiol.* 5, 346
16. Misra, G. *et al.* (1996) Aerobic biodegradation of selected monoterpenes. *Appl. Microbiol. Biotechnol.* 45, 831–838
17. Harder, J. and Probian, C. (1995) Microbial Degradation of Monoterpenes in the Absence of Molecular Oxygen. *Appl. Environ. Microbiol.* 61, 3804–3808
18. Fuchs, G. *et al.* (2011) Microbial degradation of aromatic compounds - from one strategy to four. *Nat. Rev. Microbiol.* 9, 803–816
19. Tripathi, N.N. *et al.* (2011) Antibacterial potential of plant volatile oils: A review potential. *Proc. Natl. Acad. Sci. India Sect. B Biol.* 81, 23–68
20. Kalemba, D. and Kunicka, A. (2003) Antibacterial and Antifungal Properties of Essential Oils. *Curr. Med. Chem.* 10, 813–829
21. Gao, Y. *et al.* (2005) Volatile Organic Compounds and Their Roles in Bacteriostasis in Five Conifer Species. *J. Integr. Plant Biol.* 47, 499–507
22. Ashour, H.M. (2008) Antibacterial, antifungal, and anticancer activities of volatile oils and extracts from stems, leaves, and flowers of Eucalyptus sideroxylon and Eucalyptus torquata. *Cancer Biol. Ther.* 7, 399–403
23. Rossi, P.-G. *et al.* (2007) (E)-Methylisoeugenol and Elemicin: Antibacterial Components of

Daucus carota L. Essential Oil against Campylobacter jejuni. *J. Agric. Food Chem.* 55, 7332–7336

24 Utama, I.M.S. *et al.* (2002) In Vitro Efficacy of Plant Volatiles for Inhibiting the Growth of Fruit and Vegetable Decay Microorganisms. *J. Agric. Food Chem.* 50, 6371–6377

25 Karamanoli, K. *et al.* (2005) Bacterial colonization of the phyllosphere of nineteen plant species and antimicrobial activity of their leaf secondary metabolites against leaf associated bacteria. *Chemoecology* 15, 59–67

26 Del Giudice, L. *et al.* (2008) The microbial community of Vetiver root and its involvement into essential oil biogenesis. *Environ. Microbiol.* 10, 2824–2841

27 Huang, M. *et al.* (2012) The major volatile organic compound emitted from Arabidopsis thaliana flowers, the sesquiterpene (E) - b -caryophyllene , is a defense against a bacterial pathogen. *New Phytol.* 193, 997–1008

28 Davis, T.S. *et al.* (2013) Microbial volatile emissions as insect semiochemicals. *J. Chem. Ecol.* 39, 840–59

29 Schulz, S. and Dickschat, J.S. (2007) Bacterial volatiles: the smell of small organisms. *Nat. Prod. Rep.* 24, 814–42

30 Lemfack, M.C. *et al.* (2014) mVOC: a database of microbial volatiles. *Nucleic Acids Res.* 42, D744–8

31 Ponzoni, C. *et al.* (2008) Biotransformation of acyclic monoterpenoids by Debaryomyces sp., Kluyveromyces sp., and Pichia sp. strains of environmental origin. *Chem. Biodivers.* 5, 471–83

32 Mirata, M.A. *et al.* (2008) Fungal Biotransformation of (±)-Linalool. *J. Agric. Food Chem.* 56, 3287–3296

33 de Carvalho, C.C.C.R. and da Fonseca, M.M.R. (2006) Biotransformation of terpenes. *Biotechnol. Adv.* 24, 134–42

34 Blom, D. *et al.* (2011) Production of plant growth modulating volatiles is widespread among rhizosphere bacteria and strongly depends on culture conditions. *Environ. Microbiol.* 13, 3047–3058

35 Wheatley RE, Hackett C, B. a & K. a (1997) Effect of substrate composition on production and inhibitory activity against wood decay fungi of volatile organic compounds from Trichoderma spp. *Int. J. Biodeterior. Biodegrad.* 39, 199–205

36 Bitas, V. *et al.* (2013) Sniffing on Microbes: Diverse Roles of Microbial Volatile Organic Compounds in Plant Health. *Mol. Plant Microbe Interact.* 26, 835–843

37 D'Alessandro, M. *et al.* (2014) Volatiles produced by soil-borne endophytic bacteria increase plant pathogen resistance and affect tritrophic interactions. *Plant. Cell Environ.* 37, 813–826

38 Wheatley, R.E. (2002) The consequences of volatile organic compound mediated bacterial and fungal interactions. *Antonie Van Leeuwenhoek* 81, 357–364

39 Toome, M. *et al.* (2010) Leaf rust induced volatile organic compounds signalling in willow during the infection. *Planta* 232, 235–243

40 Wenda-Piesik, A. *et al.* (2010) Volatile organic compounds (VOCs) from cereal plants infested with crown rot: their identity and their capacity for inducing production of VOCs in uninfested plants. *Int. J. Pest Manag.* 56, 377–383

41 Huang, J. *et al.* (2003) Differential volatile emissions and salicylic acid levels from tobacco plants in response to different strains of Pseudomonas syringae. *Planta* 217, 767–775

42 Junker, R.R. and Tholl, D. (2013) Volatile organic compound mediated interactions at the plant-microbe interface. *J. Chem. Ecol.* 39, 810–825

43 Achotegui-Castells, A. *et al.* (2015) Strong Induction of Minor Terpenes in Italian Cypress, Cupressus sempervirens, in Response to Infection by the Fungus Seiridium cardinale. *J. Chem. Ecol.* 41, 224–243

44 Jansen, R.M.C. *et al.* (2009) Release of lipoxygenase products and monoterpenes by tomato plants as an indicator of Botrytis cinerea-induced stress. *Plant Biol.* 11, 859–868

45 Castelyn, H.D. *et al.* (2014) Volatiles emitted by leaf rust infected wheat induce a defence

response in exposed uninfected wheat seedlings. *J. Australas. Plant Pathol. Soc.* 44, 245–254

46 Peñuelas, J. *et al.* (2014) Removal of floral microbiota reduces floral terpene emissions. *Sci. Rep.* 4, 6727

47 Niinemets, Ü. *et al.* (2014) Bidirectional exchange of biogenic volatiles with vegetation: emission sources, reactions, breakdown and deposition. *Plant. Cell Environ.* 37, 1790–1809

48 Sandhu, A. *et al.* (2007) Bacterial degradation of airborne phenol in the phyllosphere. *Environ. Microbiol.* 9, 383–92

49 De Kempeneer, L. *et al.* (2004) Bioaugmentation of the phyllosphere for the removal of toluene from indoor air. *Appl. Microbiol. Biotechnol.* 64, 284–288

50 Barac, T. *et al.* (2004) Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants. *Nat. Biotechnol.* 22, 583–588

51 Iguchi, H. *et al.* (2012) Distribution of methanotrophs in the phyllosphere. *Biosci. Biotechnol. Biochem.* 76, 1580–1583

52 Peñuelas, J. and Llusià, J. (2003) BVOCs: plant defense against climate warming? *Trends Plant Sci.* 8, 105–109

53 Niinemets, U. *et al.* (2010) The emission factor of volatile isoprenoids: stress, acclimation, and developmental responses. *Biogeosciences* 7, 2203–2223

54 Dudareva, N. *et al.* (2006) Plant Volatiles: Recent Advances and Future Perspectives. *CRC. Crit. Rev. Plant Sci.* 25, 417–440

55 Ottesen, A.R. *et al.* (2014) The impact of systemic and copper pesticide applications on the phyllospheremicroflora of tomatoes. *J. Sci. Food Agric.* 95, 1116–1125

56 Karlsson, I. *et al.* (2014) Fungicide Effects on Fungal Community Composition in the Wheat Phyllosphere. *PLoS One* 9, e111786

57 Glenn, D.M. *et al.* (2015) Effect of pest management system on “Empire” apple leaf phyllosphere populations. *Sci. Hortic. (Amsterdam).* 183, 58–65

58 Verginer, M. *et al.* (2010) Production of Volatile Metabolites by Grape-Associated Microorganisms. *J. Agric. Food Chem.* 58, 8344–8350

GLOSSARY

Aromatic compounds: the second largest class of plant VOCs, comprising phenylpropanoid and benzenoid compounds. They are synthesized from the aromatic amino acid phenylalanine via the shikimate biosynthesis pathway.

Benzenoids: chemical group of VOCs that are characterized by containing a benzene ring. They are aromatic compounds.

Biotransformation: chemical modification (or modifications) made by an organism on a chemical compound.

Epiphytic: those organisms that live on the surface of a plant.

Monoterpenes: a group of volatile terpenes that consist of two isoprene units.

Phenylpropanoids: VOCs that are synthesized by plants from the amino acid phenylalanine through the shikimate/phenylpropanoid biosynthesis pathway. They are aromatic compounds.

Terpenoids: the largest and most diversified class of secondary metabolites with many volatile constituents. They are synthesized through the mevalonic acid (MVA) and the methylerythritol phosphate (MEP) pathways.

Figure Legends

Figure 1. (A) Effects of volatile organic compounds (VOCs) emitted by plants on phyllospheric microbiotas. Plant VOCs that have antimicrobial properties, such as some terpenoids and aldehydes, can inhibit the growth of microorganisms on the phyllosphere. Some plant VOCs, such as methanol or methane, can serve as substrates for bacteria and fungi that use them as carbon sources. **(B) Effects of phyllospheric microbiotas on the emission of plant VOCs.** Phyllospheric microorganisms can consume plant VOCs or biotransform them into new VOCs. Microorganisms can affect the physiology of the host plant, with resulting changes to their VOC emissions, and can also produce and emit their own VOCs. Microbial VOCs can have antimicrobial effects on potential plant pathogens that can colonize the phyllosphere and can also enhance plant growth and resistance to stress.

Figure 2. Distribution of microbial volatile organic compound (VOC) emissions. Richness of VOCs emitted by bacteria (yellow columns) and by fungi (red columns) for different chemical classes. Chemical classes are ordered according to the number of different compounds within a class. Data from *mVOC: a database of microbial volatiles* [30].