Bioprospecting bacterial and fungal volatiles for sustainable agriculture

Bioprospecting bacterial and fungal volatiles for sustainable agriculture

Chidananda Nagamangala Kanchiswamy[1*], Mickael Malnoy[1], Massimo E. Maffei[2]

[1] Research and Innovation Centre Genomics and Biology of Fruit Crop Department, Fondazione Edmund Mach (FEM), Istituto Agrario San Michele (IASMA), Via Mach 1, 38010 San Michele all'Adige (TN), Italy; E-Mail: mickael.malnoy@fmach.it (MM)

[2]Department of Life Sciences and Systems Biology, Innovation Centre, University of Turin, Via Quarello 15/A, 10135 Turin, Italy; E-Mail: massimo.maffei@unito.it (MEM)

[*] To whom correspondence should be addressed: E-mail: chidananda.nagamangala@fmach.it ; Tel.: +39 0461 61 51 33; Fax: +39 0461 65 09 56

Keywords

Microbial Volatile Organic Compounds, sustainable agriculture, plant growth promotion, plant protection, plant-microbe interactions.

Abstract

Current agricultural practice depends upon a wide use of pesticides, bactericides and fungicides. Increased demand for organic products indicates consumer preference for reduced chemical use. Therefore, there is a need to develop novel sustainable strategies for crop protection and

enhancement that do not rely on harmful chemicals and/or genetic modification. Microbial (bacterial and fungal) volatile organic compounds (MVOCs) are intriguingly complex and dynamic, and can modulate the physiology of plants and microorganisms by regulating metabolomics, genomics and proteomics status. Hence, MVOCs can be exploited to use as an ecofriendly, cost effective and sustainable strategy for agricultural practices. An increasing body of evidence indicates that MVOCs might become alternative to harmful pesticides, fungicides and bactericides as well as genetic modification.

Introduction

Bacteria and fungi are the major inhabitants of soil rhizosphere, the narrow zone of soil that surrounds and is influenced by plant roots and which is considered to be one of the most dynamic interfaces on Earth. In agro-ecosystems, the rhizosphere microbiota have been shown to have a profound influence on plant growth, nutrition and health [1, 2]. Numerous organisms are responsible for these processes, partaking in innumerable interactions between plants, antagonists and mutualistic symbionts, both below and above ground [3-5]. To help plants to defend against attack from multiple pathogens, sophisticated alternative interactions involving plant growth promoting rhizobacteria (PGPR) and fungi (PGPF) occur, through the activation of induced systemic resistance (ISR) [6].

Many of the current insights into the above mentioned interactions and processes have originated from direct physical contact between interacting partners. However, in the last decade considerable progress is also being made in understanding the role microbial signals and microbial volatile organic compounds (MVOCs) in below- and above-ground multitrophic interactions and their roles in modulating growth, nutrition and health of interacting partners [7-13].

Microorganisms produce a plethora of intriguingly complex and dynamic MVOCs, which are defined as compounds that have high enough vapor pressures under normal conditions to significantly vaporize and enter the atmosphere [1]. Despite increasing attention on the importance of MVOCs in both atmospheric ("above-ground") and soil ("below-ground") ecosystems [7, 14-17], their functional role remains elusive. Only recently, a few studies have

shown the wealth of MVOCs for the modulation of crop growth, development, defense, inter- and intra-specific communication [2]. Recent literature reports the documentation of MVOCs produced by just 400 microorganisms of the 10,000 described microbial species and millions of species existing on earth [10].

At the plant-microbe community level, substantial progress has been made in studying various strains of PGPR, PGPF and phytopathogen MVOCs multifaceted role in agro-ecosystems. Chemical ecologists consider MVOCs as potential semiochemicals that function as attractants and repellants to insects and other invertebrates. For agriculture scientists, MVOCs are seen as bio-control agents to control various phytopathogens and as bio-fertilizers for plant growth promotion. In the food industries, the MVOCs bio-control properties are used to prevent post-harvest plant diseases. Most recently, MVOCs have been considered as a potential source of biofuel.

Because many recent reviews have considered the multifaceted importance of MVOCs, including the regulation of VOC emissions, the role of VOC in plant rhizosphere processes (i.e. competence, pathogenesis, symbiosis) and their potential functions as quorum sensing signals both for microbial growth and regulation of root development [8-10, 18], we will not repeat this in detail. Instead, this article will focus on the role of MVOCs in plant growth, nutrients and health perspectives and possible exploitation of MVOCs significance role from lab conditions to the open field conditions. Here, we review recent progress in MVOCs research for crop welfare and suggest that a conceptual framework is needed to stimulate adoption of MVOCs at open field condition as a possible substitute for the hazardous chemical pesticides and fertilizer.

MVOCs in the field for crop welfare

Under highly competitive but symbiotic conditions, MVOCs are particularly important for antibiosis and signaling, and may serve as regulators of plant growth and development. The ecological functions of microbial volatiles are not understood in detail, but several functions such as inter and intra species communication, defense and plant growth-promotion/priming have been suggested. Research over the last 10 years has led to an increasingly clear conceptual understanding of the role MVOCs for the crop welfare. These studies demonstrated the

modulation of metabolomics, genomics and proteomics of crop plants upon MVOCs treatment. MVOCs influence on modulation of phytohormones, induction of systemic acquired resistance, defense and priming response, multiple pathogen resistance, and change in plant biomass, growth and development have been extensively studied and reviewed elsewhere [7, 9, 14, 15, 17, 19-23]. Here, we emphasize selected examples of how microbial MVOCs modulate above mentioned multifaceted interactions.

Exposure of Arabidopsis plants to MOVOCs from rhizosphere strains of *Bacillus subtilis* and *B. amyloliquefaciens* resulted in significant growth promotion. Further investigation on the volatile profile revealed that 2,3-butanediol is the major volatile compound contributing to this phenotypic effect [13, 24]. Similarly, exposure of tobacco plants to *Pseudomonas chlororaphis* MVOCs promoted growth via GacS kinase-dependent production of 2,3-butanediol [25]. These GacS kinases also regulate the synthesis of signal molecules such as acyl-homoserine lactones (AHL), suggesting that 2,3-butanediol and other MVOCs may belongs to a novel class of chemical signals that bacteria utilize to communicate with neighboring organisms [25]. *B. subtilis* emitted 2,3-butanediol contributes to salt tolerance and ISR in Arabidopsis, whereas the same compound produced by *P. chlororaphis* resulted in Arabidopsis drought tolerance and enhanced disease resistance against *Erwinia caratovora* but not against *P. syringae* pv. *tabaci* [12, 25-27]. Many other bacterial volatiles from species which are present in the plant rhizosphere, such as *Burkholderia cepaci* and *Staphylococcus,* show growth promoting features although their chemical structures are yet to be determined [21]. There are certain bacterial genera including *Burkholderia, Chromobacterium, Pseudomonas, Serratia* and *Stenotrophomans,* whose volatile profiles have shown to have adverse effects on plant growth and development [19, 22]. Transcriptional and molecular analysis of Arabidopsis exposed to growth inhibiting volatile profiles of *Serratia plymuthica* and *Stenotrophomnas maltophilia* suggest an important role of the WRKY18 transcription factor in volatile-mediated plant growth inhibition [28]. Growth modulation, ISR and drought tolerance observed in plants after microbial volatile exposure depend on genomic, metabolomic and proteomic changes, which are largely attributed to alterations on phytohormone levels. The influence of 2,3-butanediol from *B. subtilis* on plant growth and ISR is due to modulation of ethylene and auxin homeostasis. Similarly, drought tolerance induced by 2,3-butanediol from *P. chlororaphis* depends on jasmonic and salicylic acid, although the involvement other phytohormones and their cross talk could not be

ruled out [12, 13, 27, 29]. Transcriptomic, proteomic and metabolomic analyses of Arabidopsis exposed to *B.subtilis* suggests the involvement of different signaling pathways for enhanced growth, involving activity of cell wall modification, stress responses, hormone regulation, antioxidant enzymes activity and photosynthesis [29-31].

Similar studies were conducted to understand the role fungal volatile profile on plant growth, nutrients and health. *Trichoderma viride* volatiles induce significant changes in Arabidopsis, including increased lateral roots, taller, bigger and early flowering phenotypic changes [32]. 1-octen-3-ol is commonly produced by many fungi and contributes to enhance plant resistance to the necrotropic fungal pathogen *Botrytis cinerea* by inducing defense signaling cascades [33, 34]. *Alternaria alternata, Penicillium charlesii* and *P. aurantiogriseum* volatile profile promote growth and starch accumulation in several plant species [35]. Interestingly, volatiles from a non-pathogenic strain of *Fusarium oxysporum*, MSA35, associated with a group of ectosymbiotic bacteria promotes lettuce growth [36, 37]. Further studies on this strain revealed that sequiterpenes such as β-caryophyllene produced by the ectosymbiotic bacterial species are the major volatile compounds responsible for the enhanced growth [37]. Ectomycorrhizal truffles such as *Tuber borchii, T. indicum* and *T.melanopsorum* produce volatiles that mediated inhibition of leaf growth and root development in Arabidopsis [38].

Collectively, these studies demonstrate that MVOCs have profound effects on plant metabolism, growth and health. However, many of the current insights into the role of MVOCs in modulating plant growth and defense are obtained from either laboratory or greenhouse experiments. Quite recently, a study has been conducted at the field level to induce crop defense against multiple pathogens and to attract natural enemies of aphids. This study provided useful insights of possible implementation of MVOCs as crop protection and biocontrol agents in open field conditions [39]. We now have the means to begin a new era of MVOCs that might potentially replace costly and unsustainable chemical pesticides and fertilizers and limit the use of genetically modified crops. Table 1 lists some bioactive MVOCs and their effects on plants.

Deployment of MVOCs in the open field

The search for novel molecules with biotechnological applications is termed “bioprospecting”. For most of the 20th century, fungal and bacterial bioprospecting has focused on the search for

traditional secondary metabolites with drug value (e.g. penicillin, lovastatin) or for enzymes with new applications (e.g. biomass degrading enzymes from thermophiles). A concerted search for new biotechnological products among MVOCs will require a paradigm shift in the scientific community's thinking [15]. MVOCs represent a new frontier in bioprospecting. However, although considerable progress has been made in our understanding of MVOCs for crop welfare at lab conditions, we are still far from implementing them under field conditions. Relatively recent studies conducted on volatile application at open field condition suggest that MVOCs can be applied to trigger defense against both pathogens and herbivores [39]. This is just the beginning but we still need to optimize proper conditions for the effective implementation of MVOCs at the field level.

There are many limitations of MVOCs for field applications: a) identification of bioactive MVOCs; b) optimization of concentration of specific volatiles or blend of volatile compounds; and c) application at the field level. The latter, by considering MVOCs physical and chemical properties, is the most difficult and challenging task. For instance, 2,3-butandiol field treatment on tobacco led to significant reduction in disease symptoms, whereas no significant results were observed when cucumber plants were treated to fight the biotrophic pathogen *Pseudomonas syringae* [39-41]. However, an artificial VOC mixture prepared on the basis of the composition of the VOCs (mainly alcohols and esters) mimicked the inhibitory effects of the natural MVOCs released by *Saccharomyces cerevisiae* on citrus black spot, caused by the fungus *Guignardia citricarpa* at postharvest. Thus, MVOCs produced by the yeast or the artificial mixtures might be a promising control method for citrus black spot or others postharvest diseases [42, 43]. So far, MVOCs are successfully applied at field level as a foliar spray and soil dumping [39-41] but there are no comparative studies using different methods of field application to provide a better understanding of effective and optimized methods.

Conclusions and future perspectives

Studies of MVOCs application at the field level are still in their infancy. More experiments and field trials are needed to prove their worth and provide sustained industry pipelines leading to a commercial production that meets farmers' needs. Consumers are well aware of the hazardous effect caused to the environment and human health by pesticides and chemical fertilizers. Alternative to this, genetically modified crop plants and recently proposed genetically edited

crops [44] could provide a solution, but most countries have lengthy, cumbersome and expensive regulatory frameworks, which slow down the use of genetically modified crop plants. Now it is time to adopt emerging MVOCs, a new sustainable approach that can be available in a cheaper, efficient, effective and ecofriendly manner. MVOCs are equivalent to biopesticides or biofertlizers. The market breadth and demand for these naturally derived compounds increased considerably in the recent years around the world but their use is still only 4% of the global pesticide market [45, 46]. Researchers realized the importance of MVOCs for the crop welfare under lab conditions and recently extended their studies to field level with certain success. We are now beginning to understand the multi-facet interaction of MVOCs with microorganisms and crop plants and further studies should be done by field level testing different crop species and obtaining reproducible results which could satisfy farmers' and consumers' needs. However, several questions remain unsolved (see Box 1).

In our opinion, MVOCs possess a high potential impact for crop welfare and sustainable agriculture but we are just beginning to understand their role and still far from agricultural applications. In the coming years we assume MVOCs will outperform chemical pesticides and fertilizers and will become novel candidate for sustainable agriculture.

References

1. Chaparro JM, Badri DV, Vivanco JM: **Rhizosphere microbiome assemblage is affected by plant development**. *The ISME journal* 2014, **8**(4):790-803.
2. Schreiter S, Ding GC, Heuer H, Neumann G, Sandmann M, Grosch R, Kropf S, Smalla K: **Effect of the soil type on the microbiome in the rhizosphere of field-grown lettuce**. *Frontiers in microbiology* 2014, **5**:144.
3. Bennett AE, Bever JD: **Mycorrhizal species differentially alter plant growth and response to herbivory**. *Ecology* 2007, **88**(1):210-218.
4. Hol WH, de Boer W, Termorshuizen AJ, Meyer KM, Schneider JH, van Dam NM, van Veen JA, van der Putten WH: **Reduction of rare soil microbes modifies plant-herbivore interactions**. *Ecology letters* 2010, **13**(3):292-301.
5. Behie SW, Zelisko PM, Bidochka MJ: **Endophytic insect-parasitic fungi translocate nitrogen directly from insects to plants**. *Science* 2012, **336**(6088):1576-1577.
6. Nadeem SM, Ahmad M, Zahir ZA, Javaid A, Ashraf M: **The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments**. *Biotechnology advances* 2014, **32**(2):429-448.

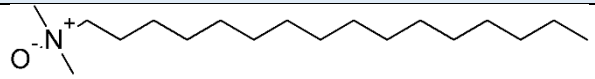
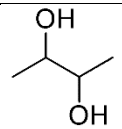
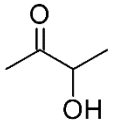
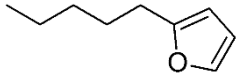
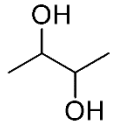
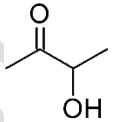
7. Piechulla B, Degenhardt J: **The emerging importance of microbial volatile organic compounds**. *Plant, cell & environment* 2013.
8. Ortiz-Castro R, Diaz-Perez C, Martinez-Trujillo M, del Rio RE, Campos-Garcia J, Lopez-Bucio J: **Transkingdom signaling based on bacterial cyclodipeptides with auxin activity in plants**. *Proceedings of the National Academy of Sciences of the United States of America* 2011, **108**(17):7253-7258.
9. Ortiz-Castro R, Contreras-Cornejo HA, Macias-Rodriguez L, Lopez-Bucio J: **The role of microbial signals in plant growth and development**. *Plant signaling & behavior* 2009, **4**(8):701-712.
10. Ortiz-Castro R, Martinez-Trujillo M, Lopez-Bucio J: **N-acyl-L-homoserine lactones: a class of bacterial quorum-sensing signals alter post-embryonic root development in Arabidopsis thaliana**. *Plant, cell & environment* 2008, **31**(10):1497-1509.
11. Ortiz-Castro R, Valencia-Cantero E, Lopez-Bucio J: **Plant growth promotion by Bacillus megaterium involves cytokinin signaling**. *Plant signaling & behavior* 2008, **3**(4):263-265.
12. Ryu CM, Farag MA, Hu CH, Reddy MS, Kloepper JW, Pare PW: **Bacterial volatiles induce systemic resistance in Arabidopsis**. *Plant physiology* 2004, **134**(3):1017-1026.
13. Ryu CM, Farag MA, Hu CH, Reddy MS, Wei HX, Pare PW, Kloepper JW: **Bacterial volatiles promote growth in Arabidopsis**. *Proceedings of the National Academy of Sciences of the United States of America* 2003, **100**(8):4927-4932.
14. Effmert U, Kalderas J, Warnke R, Piechulla B: **Volatile mediated interactions between bacteria and fungi in the soil**. *Journal of chemical ecology* 2012, **38**(6):665-703.
15. Shannon U. Morath RH, Joan W. Bennett: **Fungal volatile organic compounds: A review with emphasis on their biotechnological potential**. *Fungal Biology Reviews* 2012, **26**(2-3):73-83.
16. Junker RR, Tholl D: **Volatile organic compound mediated interactions at the plant-microbe interface**. *Journal of chemical ecology* 2013, **39**(7):810-825.
17. Bitas V, Kim HS, Bennett JW, Kang S: **Sniffing on microbes: diverse roles of microbial volatile organic compounds in plant health**. *Molecular plant-microbe interactions : MPMI* 2013, **26**(8):835-843.
18. Chernin L, Toklikishvili N, Ovadis M, Kim S, Ben-Ari J, Khmel I, Vainstein A: **Quorum-sensing quenching by rhizobacterial volatiles**. *Environmental microbiology reports* 2011, **3**(6):698-704.
19. Bailly A, Weisskopf L: **The modulating effect of bacterial volatiles on plant growth: current knowledge and future challenges**. *Plant signaling & behavior* 2012, **7**(1):79-85.
20. Bennett JW, Hung R, Lee S, Padhi S: **18 Fungal and Bacterial Volatile Organic Compounds: An Overview and Their Role as Ecological Signaling Agents**. In: *Fungal Associations.* Edited by Hock B, vol. 9: Springer Berlin Heidelberg; 2012: 373-393.
21. Vespermann A, Kai M, Piechulla B: **Rhizobacterial volatiles affect the growth of fungi and Arabidopsis thaliana**. *Applied and environmental microbiology* 2007, **73**(17):5639-5641.
22. Kai M, Haustein M, Molina F, Petri A, Scholz B, Piechulla B: **Bacterial volatiles and their action potential**. *Applied microbiology and biotechnology* 2009, **81**(6):1001-1012.
23. Penuelas J, Asensio D, Tholl D, Wenke K, Rosenkranz M, Piechulla B, Schnitzler JP: **Biogenic volatile emissions from the soil**. *Plant, cell & environment* 2014.
24. Farag MA, Ryu CM, Sumner LW, Pare PW: **GC-MS SPME profiling of rhizobacterial volatiles reveals prospective inducers of growth promotion and induced systemic resistance in plants**. *Phytochemistry* 2006, **67**(20):2262-2268.
25. Han SH, Lee SJ, Moon JH, Park KH, Yang KY, Cho BH, Kim KY, Kim YW, Lee MC, Anderson AJ *et al*: **GacS-dependent production of 2R, 3R-butanediol by Pseudomonas chlororaphis O6 is a major determinant for eliciting systemic resistance against Erwinia carotovora but not against Pseudomonas syringae pv. tabaci in tobacco**. *Molecular plant-microbe interactions : MPMI* 2006, **19**(8):924-930.

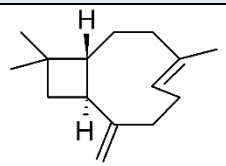
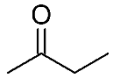
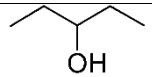
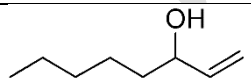
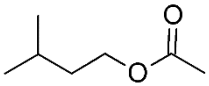
26. Zhang H, Kim MS, Sun Y, Dowd SE, Shi H, Pare PW: **Soil bacteria confer plant salt tolerance by tissue-specific regulation of the sodium transporter HKT1**. *Molecular plant-microbe interactions : MPMI* 2008, **21**(6):737-744.
27. Cho SM, Kang BR, Han SH, Anderson AJ, Park JY, Lee YH, Cho BH, Yang KY, Ryu CM, Kim YC: **2R,3R-butanediol, a bacterial volatile produced by Pseudomonas chlororaphis O6, is involved in induction of systemic tolerance to drought in Arabidopsis thaliana**. *Molecular plant-microbe interactions : MPMI* 2008, **21**(8):1067-1075.
28. Wenke K, Wanke D, Kilian J, Berendzen K, Harter K, Piechulla B: **Volatiles of two growth-inhibiting rhizobacteria commonly engage AtWRKY18 function**. *The Plant journal : for cell and molecular biology* 2012, **70**(3):445-459.
29. Zhang H, Kim MS, Krishnamachari V, Payton P, Sun Y, Grimson M, Farag MA, Ryu CM, Allen R, Melo IS *et al*: **Rhizobacterial volatile emissions regulate auxin homeostasis and cell expansion in Arabidopsis**. *Planta* 2007, **226**(4):839-851.
30. Kwon YS, Ryu CM, Lee S, Park HB, Han KS, Lee JH, Lee K, Chung WS, Jeong MJ, Kim HK *et al*: **Proteome analysis of Arabidopsis seedlings exposed to bacterial volatiles**. *Planta* 2010, **232**(6):1355-1370.
31. Zhang H, Xie X, Kim MS, Kornyeyev DA, Holaday S, Pare PW: **Soil bacteria augment Arabidopsis photosynthesis by decreasing glucose sensing and abscisic acid levels in planta**. *The Plant journal : for cell and molecular biology* 2008, **56**(2):264-273.
32. Hung R, Lee S, Bennett JW: **Arabidopsis thaliana as a model system for testing the effect of Trichoderma volatile organic compounds**. *Fungal Ecology* 2013, **6**(1):19-26.
33. Kishimoto K, Matsui K, Ozawa R, Takabayashi J: **Volatile 1-octen-3-ol induces a defensive response in Arabidopsis thaliana**. *J Gen Plant Pathol* 2007, **73**(1):35-37.
34. Contreras-Cornejo H, Macías-Rodríguez L, Herrera-Estrella A, López-Bucio J: **The 4-phosphopantetheinyl transferase of Trichoderma virens plays a role in plant protection against Botrytis cinerea through volatile organic compound emission**. *Plant Soil* 2014, **379**(1-2):261-274.
35. Ezquer I, Li J, Ovecka M, Baroja-Fernandez E, Munoz FJ, Montero M, Diaz de Cerio J, Hidalgo M, Sesma MT, Bahaji A *et al*: **Microbial volatile emissions promote accumulation of exceptionally high levels of starch in leaves in mono- and dicotyledonous plants**. *Plant & cell physiology* 2010, **51**(10):1674-1693.
36. Minerdi D, Bossi S, Gullino ML, Garibaldi A: **Volatile organic compounds: a potential direct long-distance mechanism for antagonistic action of Fusarium oxysporum strain MSA 35**. *Environmental microbiology* 2009, **11**(4):844-854.
37. Minerdi D, Bossi S, Maffei ME, Gullino ML, Garibaldi A: **Fusarium oxysporum and its bacterial consortium promote lettuce growth and expansin A5 gene expression through microbial volatile organic compound (MVOC) emission**. *FEMS microbiology ecology* 2011, **76**(2):342-351.
38. Splivallo R, Novero M, Bertea CM, Bossi S, Bonfante P: **Truffle volatiles inhibit growth and induce an oxidative burst in Arabidopsis thaliana**. *The New phytologist* 2007, **175**(3):417-424.
39. Song GC, Ryu CM: **Two Volatile Organic Compounds Trigger Plant Self-Defense against a Bacterial Pathogen and a Sucking Insect in Cucumber under Open Field Conditions**. *International journal of molecular sciences* 2013, **14**(5):9803-9819.
40. Cortes-Barco AM, Goodwin PH, Hsiang T: **Comparison of induced resistance activated by benzothiadiazole, (2R,3R)-butanediol and an isoparaffin mixture against anthracnose of Nicotiana benthamiana**. *Plant Pathology* 2010, **59**(4):643-653.
41. Cortes-Barco AM, Hsiang T, Goodwin PH: **Induced systemic resistance against three foliar diseases of Agrostis stolonifera by (2R,3R)-butanediol or an isoparaffin mixture**. *Annals of Applied Biology* 2010, **157**(2):179-189.

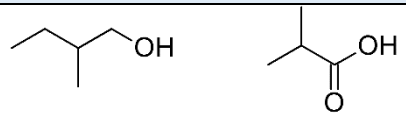
42. Fialho MB, Ferreira LFR, Monteiro RTR, Pascholati SF: **Antimicrobial volatile organic compounds affect morphogenesis-related enzymes in Guignardia citricarpa, causal agent of citrus black spot**. *Biocontrol Science and Technology* 2011, **21**(7):797-807.
43. Fialho M, Toffano L, Pedroso M, Augusto F, Pascholati S: **Volatile organic compounds produced by Saccharomyces cerevisiae inhibit the in vitro development of Guignardia citricarpa, the causal agent of citrus black spot**. *World Journal of Microbiology and Biotechnology* 2010, **26**(5):925-932.
44. Nagamangala Kanchiswamy C, Sargent DJ, Velasco R, Maffei ME, Malnoy M: **Looking forward to genetically edited fruit crops**. *Trends in biotechnology* 2014.
45. Glare T, Caradus J, Gelernter W, Jackson T, Keyhani N, Kohl J, Marrone P, Morin L, Stewart A: **Have biopesticides come of age?** *Trends in biotechnology* 2012, **30**(5):250-258.
46. Wilson K, Benton TG, Graham RI, Grzywacz D: **Pest control: biopesticides' potential**. *Science* 2013, **342**(6160):799.
47. Velázquez-Becerra C, Macías-Rodríguez L, López-Bucio J, Altamirano-Hernández J, Flores-Cortez I, Valencia-Cantero E: **A volatile organic compound analysis from Arthrobacter agilis identifies dimethylhexadecylamine, an amino-containing lipid modulating bacterial growth and Medicago sativa morphogenesis in vitro**. *Plant Soil* 2011, **339**(1-2):329-340.
48. Zou C, Li Z, Yu D: **Bacillus megaterium strain XTBG34 promotes plant growth by producing 2-pentylfuran**. *Journal of microbiology* 2010, **48**(4):460-466.
49. Blom D, Fabbri C, Eberl L, Weisskopf L: **Volatile-mediated killing of Arabidopsis thaliana by bacteria is mainly due to hydrogen cyanide**. *Applied and environmental microbiology* 2011, **77**(3):1000-1008.
50. Åström B, Gerhardson B: **Wheat cultivar reactions to deleterious rhizosphere bacteria under gnotobiotic conditions**. *Plant Soil* 1989, **117**(2):157-165.
51. Blom D, Fabbri C, Connor EC, Schiestl FP, Klauser DR, Boller T, Eberl L, Weisskopf L: **Production of plant growth modulating volatiles is widespread among rhizosphere bacteria and strongly depends on culture conditions**. *Environmental microbiology* 2011, **13**(11):3047-3058.
52. Gutiérrez-Luna F, López-Bucio J, Altamirano-Hernández J, Valencia-Cantero E, de la Cruz H, Macías-Rodríguez L: **Plant growth-promoting rhizobacteria modulate root-system architecture in Arabidopsis thaliana through volatile organic compound emission**. *Symbiosis* 2010, **51**(1):75-83.
53. Wheatley RE: **The consequences of volatile organic compound mediated bacterial and fungal interactions**. *Antonie van Leeuwenhoek* 2002, **81**(1-4):357-364.
54. Scholler CE, Gurtler H, Pedersen R, Molin S, Wilkins K: **Volatile metabolites from actinomycetes**. *Journal of agricultural and food chemistry* 2002, **50**(9):2615-2621.
55. Funes H, Zerba E, Gonzalez Audino P: **Comparison of three types of traps baited with sexual pheromones for ambrosia beetle Megaplatypus mutatus (Coleoptera: Platypodinae) in poplar plantations**. *Journal of economic entomology* 2009, **102**(4):1546-1550.
56. Manrique G, Vitta AC, Ferreira RA, Zani CL, Unelius CR, Lazzari CR, Diotaiuti L, Lorenzo MG: **Chemical communication in Chagas disease vectors. Source, identity, and potential function of volatiles released by the metasternal and Brindley's glands of Triatoma infestans adults**. *Journal of chemical ecology* 2006, **32**(9):2035-2052.
57. Vitta AC, Lorenzo MG: **Copulation and mate guarding behavior in Triatoma brasiliensis (Hemiptera: Reduviidae)**. *Journal of medical entomology* 2009, **46**(4):789-795.
58. Bukovinszky T, Gols R, Posthumus MA, Vet LE, Van Lenteren JC: **Variation in plant volatiles and attraction of the parasitoid Diadegma semiclausum (Hellen)**. *Journal of chemical ecology* 2005, **31**(3):461-480.

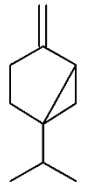
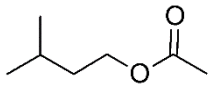
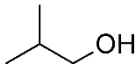
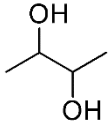
59. Strobel GA, Dirkse E, Sears J, Markworth C: **Volatile antimicrobials from Muscodor albus, a novel endophytic fungus**. *Microbiology* 2001, **147**(Pt 11):2943-2950.
60. Mitchell AM, Strobel GA, Moore E, Robison R, Sears J: **Volatile antimicrobials from Muscodor crispans, a novel endophytic fungus**. *Microbiology* 2010, **156**(Pt 1):270-277.
61. Singh SK, Strobel GA, Knighton B, Geary B, Sears J, Ezra D: **An endophytic Phomopsis sp. possessing bioactivity and fuel potential with its volatile organic compounds**. *Microbial ecology* 2011, **61**(4):729-739.
62. Strobel G, Singh SK, Riyaz-Ul-Hassan S, Mitchell AM, Geary B, Sears J: **An endophytic/pathogenic Phoma sp. from creosote bush producing biologically active volatile compounds having fuel potential**. *FEMS microbiology letters* 2011, **320**(2):87-94.
63. Macias-Rubalcava ML, Hernandez-Bautista BE, Oropeza F, Duarte G, Gonzalez MC, Glenn AE, Hanlin RT, Anaya AL: **Allelochemical effects of volatile compounds and organic extracts from Muscodor yucatanensis, a tropical endophytic fungus from Bursera simaruba**. *Journal of chemical ecology* 2010, **36**(10):1122-1131.
64. Mercier J, Jiménez JI: **Control of fungal decay of apples and peaches by the biofumigant fungus Muscodor albus**. *Postharvest Biology and Technology* 2004, **31**(1):1-8.
65. Thakeow P, Angeli S, Weißbecker B, Schütz S: **Antennal and Behavioral Responses of Cis boleti to Fungal Odor of Trametes gibbosa**. *Chemical Senses* 2008, **33**(4):379-387.
66. Drilling K, Dettner K: **Electrophysiological responses of four fungivorous coleoptera to volatiles of Trametes versicolor: implications for host selection**. *Chemoecology* 2009, **19**(2):109-115.

Table 1. List of bioactive MVOCs and their effects on plants

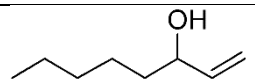
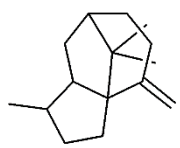
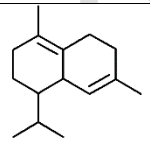
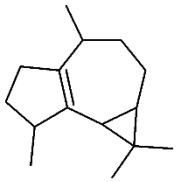
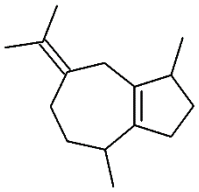
Bacterial or Fungal species and strain	Identified volatile compounds	Effects on interacting organisms	Ref.
Arthobacter agilis UMCV2	 N,N-dimethyl-hexadecanamine	Growth promotion	[47]
Bacillus amyloliquefaciens IN937a	 2,3-Butanediol  Acetoin	Growth promotion and induced systemic resistance (ISR)	[12, 13]
Bacillus megaterium XTBG34	 2-penthylfuran	Growth promotion	[48]
Bacillus subtilis GBO3	 2,3-Butanediol  Acetoin	Growth promotion and ISR	[12, 13]

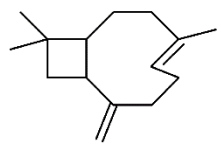
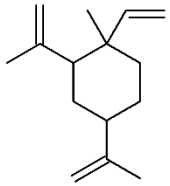
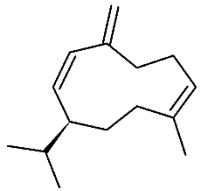
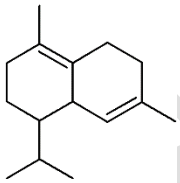
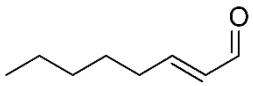
Bacterial or Fungal species and strain	Identified volatile compounds	Effects on interacting organisms	Ref.
Fusarium oxysporum MSA 35	 β-caryophyllene	Induced shoot length, root length and fresh weight of lettuce seedlings	[37]
Many species of bacteria, fungi and plants	 2-butanone	ISR, emission of green leaf volatiles to attract natural enemies of Aphid	[24, 39, 53, 54]
Many species of bacteria, fungi and plants	 3-pentanol	ISR, pheromone, in response to herbivore produced by plant to attract natural enemies	[39, 55-58]
Mold fungi	 1-octen-3-ol	Induced defense and protection against *Botrytis cinerea*	[33]
Muscodor albus	 Isoamyl acetate	Collectively they acted synergistically to kill a broad range of plant- and human-pathogenic fungi and bacteria	[59]

Bacterial or Fungal species and strain	Identified volatile compounds	Effects on interacting organisms	Ref.
Muscodor albus	 2-methyl butanol isobutyric acid	Volatile mixture were effectively used to control postharvest plant diseases	[64]
Muscodor crispans	Mixture of volatile compounds	Effective against a wide range of plant pathogens, including the fungi *Pythium ultimum*, *Phytophthora cinnamomi*, *Sclerotinia sclerotiorum* and *Mycosphaerella fijiensis* (the black sigatoka pathogen of bananas), and the serious bacterial pathogen of citrus, *Xanthomonas axonopodis* pv. citri. In addition, the VOCs of *M. crispans* killed several human pathogens, including *Yersinia pestis*, *Mycobacterium tuberculosis* and *Staphylococcus aureus*.	[60]
Muscodor yucatanensis	Mixture of volatile organic compounds	Mixture of volatile organic compounds produced by *M. yucatanensis* have allelochemical effects against other endophytic fungi, phytopathogenic fungi and plants.	[63]
Phoma sp	Unique mixture of volatile organic compounds, including a series of sesquiterpenoids, some alcohols and several reduced naphthalene derivatives.	The volatiles of *Phoma sp.* possess antifungal and fuel properties Some of the test organisms with the greatest sensitivity to the *Phoma sp.* Volatiles were *Verticillium*, *Ceratocystis*, *Cercospora* and *Sclerotinia*.	[62]

Bacterial or Fungal species and strain	Identified volatile compounds	Effects on interacting organisms	Ref.
Phomopsis sp	 sabinene  isoamyl alcohol  2-propanone  2-methyl propanol	Volatile mixture of Phomopsis sp. possess antifungal properties and an artificial mixture of the VOCs mimicked the antibiotic effects of this organism with the greatest bioactivity against a wide range of plant pathogenic test fungi including: *Pythium*, *Phytophthora*, *Sclerotinia*, *Rhizoctonia*, *Fusarium*, *Botrytis*, *Verticillium*, and *Colletotrichum*.	[61]
Pseudomanas aeruginosa PAO1, PAO14, Tb, TBCF10839 and PUPa3	HCN	Growth inhibition	[49]
Pseudomanas chlororaphis O6	 2,3-Butanediol	Growth promotion, ISR and drought stress tolerant	[25, 27]
Pseudomanas fluorescens A112	Not determined	Growth inhibition (shoot and root)	[50]
Pseudomanas	Not determined	Growth inhibition	[21]

Bacterial or Fungal species and strain	Identified volatile compounds	Effects on interacting organisms	Ref.
trivialis 3Re2-7			
Rhizosphere strains (isolated from rhizosphere of lemon plants) L263, L266, L272a, L254, L265a and L265b	Volatile mixture	Growth promoting and modulation of root architecture	[52]
Rhizosphere strains (more than 42 strains predominantly from *Burkholderia* genus)	Not determined	Growth inhibition or promotion (dose dependent)	[51]
Serratia marcescens MG-1	Not determined	Growth inhibition	[21]
Serratia plymuthica 3Re4-18, HRO-C48, IC14	Not determined	Growth inhibition	[21, 49]

Bacterial or Fungal species and strain	Identified volatile compounds	Effects on interacting organisms	Ref.
Stenotrophomanas maltophilia R3089	Not determined	Growth inhibition	[21]
Stenotrophomanas rhizospehila P69	Not determined	Growth inhibition	[21]
Trametes gibbosa	 1-octen-3-ol	Serves as attractant for fungus eating beetles	[65]
Trametes versicolor	 γ-patchoulene  δ-cadinene  Isoledene  β-guaiene	Serves as attractant for fungus eating beetles	[66]

Bacterial or Fungal species and strain	Identified volatile compounds	Effects on interacting organisms	Ref.
Trichoderma virens	 β-caryophyllene  β-elemene  Germacrene D  δ-cadinene	Growth promotion and induction of defense responses of *Arabidopsis thaliana* against *Botrytis cinerea*	[34]
Tuber melanosporum, Tuber indicum and *Tuber borchii* (truffles)	 2-octenal	Growth inhibition	[38]

Outstanding Questions Box 1

VOCs play important signaling roles for bacteria and fungi but also for other organisms in their natural environments. Many ecological interactions are mediated by VOCs, including those between fungi and plant, bacteria and plants, plant-plants, arthropods-plants, ect. The diverse functions of MVOCs can be exploited in biotechnological applications for biofuel, biocontrol, and mycofumigation. MVOCs represent a new frontier in bioprospecting, and the study of these gas-phase compounds promises the discovery of new products for human exploitation (medical, agricultural and industrial arenas) and will generate new hypotheses in fundamental biology. However, the mechanisms through which MVOC respond to their surrounding must be better understood in order to be more predictive about which role and effect on their surrounding. Some key questions remain to be answered:

- What is the advantage of the plant to perceive (M)VOCs?
- Which plant proteins participate in the perception of MVOCs?
- What is the identity of MVOCs responsible for induction of plant growth/defense?
- Are plants able to perceive MVOCs from their bacterial and fungal pathogens, and are they able to induce defense mechanism?
- Are plants able make the difference between MVOCs produce by host or non host pathogens?
- Can MVOCs be used as biopesticides?

Glossary

Above-ground: a position measured with respect to the underlying ground surface.

Agrochemicals: a generic term for the various chemical products used in agriculture. In most cases, agrichemical refers to the broad range of pesticides, including insecticides, herbicides, and fungicides. It may also include synthetic fertilizers, hormones and other chemical growth agents, and concentrated stores of raw animal manure.

Below-ground: a position measured with respect to the upper ground surface.

Biofertlizer: a substance containing living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant.

Biofilm: any group of microorganisms in which cells stick to each other on a surface.

Biopesticides: include several types of pest management intervention: through predatory, parasitic, or chemical relationships. The term has been associated historically with biological control and the manipulation of living organisms.

Bioprospecting: the search for new natural and sustainable molecules in the hope of finding novel biotechnological applications

Crop welfare: is the provision of a minimal level of well-being and social support for all crops.

Info-chemical: information-conveying chemicals including kairomones, allelochemicals or pheromones that play a crucial role in food web interactions.

Microorganism: a very diverse kingdom that includes all the bacteria and archaea and almost all the protozoa. They also include some members of the fungi, algae, and animals such as rotifers.

Multitrophic interactions: incorporation of species from different trophic or nutritional levels interacting in the same system.

MVOCs: microbial volatile organic compounds that have high enough vapor pressures under normal conditions to significantly vaporize and enter the atmosphere.

Mycofumigation: the use of gas-producing fungi to kill other microorganisms via production of MVOCs.

Plant growth inhibition: reduction of plant growth determined by environmental factors, such as temperature, available water, available light, carbon dioxide and available nutrients in the soil or by the actions of pathogenic and saprophytic organisms and herbivores.

Priming: exposure to conditions by which the processing of a target stimulus is aided or altered by the presentation of a previously presented stimulus.

Rhizobacteria: root-colonizing bacteria that form symbiotic relationships with many plants. Though parasitic varieties of rhizobacteria exist, the term usually refers to bacteria that form a relationship beneficial for both parties (mutualism).

Rhizosphere: a narrow region of soil that is directly influenced by root secretions and associated soil microorganisms. It contains many bacteria that feed on sloughed-off plant cells, termed rhizodeposition, and the proteins and sugars released by roots.

Sustainable agriculture: an integrated system of plant and animal production practices having a site-specific application that will last over the long term.