Trends in Plant Science

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Letter

Circumvent CO_2 Effects in Volatile-Based Microbe–Plant Interactions

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More than a decade ago, Ryu *et al.* [1] observed that volatile metabolites emitted from bacteria promote plant growth. Since then, many researchers and research groups have studied these volatile-based microbe–plant interactions. From the beginning, arabidopsis (*Arabidopsis thaliana*) was almost exclusively used as the test organism, and mostly positive growth and development-stimulating effects were registered when bacteria were grown in the vicinity of the plant. Simple dual-culture systems as well as sophisticated experimental set-ups were used to not only ensure the physical separation of plants and bacteria or fungi, but also prevent metabolite flow through media or soil, thus only allowing volatile metabolites to function as bioactive compounds (summarized in [2]).

Bacteria and fungi produce a wealth of volatile organic compounds (VOCs), some of which are ubiquitously emitted and some of which are uniquely released by microbes (summarized in [3]). Unraveling the biological and ecological functions of these microbial (m)VOCs is a major future task. They may act alone and/or in combination with other VOCs of the species/strain-specific blend, and it will be exciting to study their functional potentials when directly or indirectly applied to plants. The phenotypic plant responses observed thus far range from increase of biomass and cell expansion, elevation of photosynthesis via ABA signaling, increase of starch accumulation, tolerance to biotic stress (e.g., drought, salt, choline, and osmotic stress), and enhanced sulfur nutrition, to induction of systemic resistance and iron acquisition [4–15]. These promising results were quickly turned into the hope that microbial volatiles could stimulate plant growth and help plants defend against plant diseases, resulting in a sustainable and improved agriculture [16].

While there is growing evidence that volatiles released from microorganisms have positive impacts on plant health and growth, many studies lack fundamental control experiments that must be carried out before we can proclaim the promising future resulting from bacterial and fungal volatiles.

In addition to VOCs, bacteria also release many inorganic compounds, such as CO_2, HCN, NH_3, and H_2S. While NH_3, and H_2S are only emitted under certain growth conditions, such as on sulfur- or protein-rich medium, respectively, respiratory CO_2 is ubiquitously emitted when the tricarboxylic cycle (TCC) is active in aerobically growing bacteria and fungi. In enclosed dual-culture systems or other experimental setups that are sealed to be gas-tight, or that inhibit unhindered gas exchange, microbial respiratory CO_2 can accumulate in the headspace [17]. Plants, as autotrophic organisms, love CO_2, and when the CO_2 concentration is higher than ambient atmospheric levels, plants respond with increased growth and biomass, starch accumulation, stress and pathogen resistance, etc. [18]. Therefore, CO_2 fertilization produces congruent plant phenotypes similar to plant fumigation

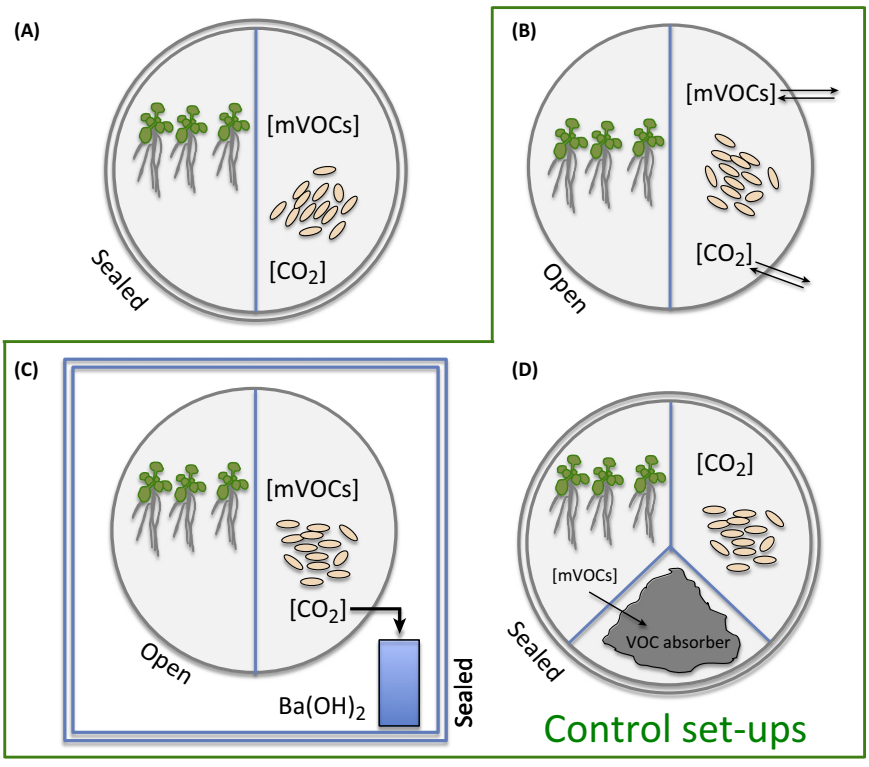

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Figure 1. Experimental Set-Ups of Commonly Used Headspace Co-Cultivation Systems. (A) Gas-tight container system (ideally glass dishes) sealed with plastic parafilm. Plant and microbial media are physically separated, avoiding diffusion of metabolites through the agar. (B) enclosed system without sealing that enables diffusion of mVOCs and CO_2 out of the container; (C) nonsealed co-cultivation container nested in a gas-tight container with $Ba(OH)_2$ to trap CO_2, leading to lower CO_2 concentrations while microbial volatile organic compounds (mVOCs) become enriched relative to CO_2; (D) gas-tight tripartite co-cultivation system with one compartment containing VOC-absorbent material (e.g., Tenax, activated charcoal) to trap mVOCs. Larger and lower letter sizes of $[CO_2]$ and [mVOCs] indicate changes in concentrations in the set-ups.

with microbial volatiles (see above). Thus, in microbe–plant co-cultivation, systems it is essential to separate the VOC-based plant growth promotion from the co-occurring effects of CO_2 fertilization [17,19].

In many publications, the information about whether a closed or sealed system was used and whether the CO_2 concentrations were ambient or higher in the test enclosure is missing. Metabolic activities and, therefore, the release of CO_2, of different bacteria and fungi vary considerably, and depend on growth conditions and the growth phase and, thus, do not serve as sufficient controls. In systems with plants in soil containers, CO_2 may also be effective when the plants are fumigated with microbial volatiles through the soil, since CO_2 can easily penetrate through the aereal spaces of the soil and reach the leaves from belowground. While this occurs continuously in nature, in most of the bioassays testing mVOCs on plants growing on media, the enclosed Petri dishes or other containers most likely prevent free gas exchange with the surrounding atmosphere, resulting in increased CO_2 levels.

Although we are convinced that the emission of VOCs from bacteria and fungi living in the phyllosphere, rhizosphere, caulosphere, carposphere, anthosphere, or endosphere impact microbe–plant, as well as microbe–microbe interactions in multiple ways, future experimental designs must include appropriate CO_2 (and HCN, NH_3, and H_2S) controls (Figure 1) to enable the dissection of the biological effects of microbial VOCs or VOC mixtures in biologically relevant concentrations, from artefacts originating from the growth stimulating effects of this inorganic volatile. It should be noted that, in nature, only very rarely closed spaces exist; therefore, it should be our goal to ensure that gas exchange is unhindered to prevent accumulation of volatiles when ecologically relevant functions of microbial VOCs are being studied.

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References

1. Ryu, C-M. *et al.* (2003) Bacterial volatiles promote growth in *Arabidopsis*. *Proc Natl Acad Sci U.S.A.* 100, 4927–4932
2. Kai, M. *et al.* (2016) Bacterial-plant-interactions: approaches to unravel the biological function of bacterial volatiles in the rhizosphere. *Front. Microbiol.* http://dx.doi.org/10.3389/fmicb.2016.00108
3. Lemfack, M.C. *et al.* (2014) mVOC: a database of microbial volatiles. *Nucleic Acid Res.* 42, D744–D748
4. Zhang, H. *et al.* (2007) Rhizobacterial volatile emissions regulate auxin homeostasis and cell expansion in *Arabidopsis*. *Planta* 226, 839–851
5. Zhang, H. *et al.* (2008) Soil bacteria augment *Arabidopsis* photosynthesis by decreasing glucose sensing and abscisic acid levels *in planta*. *Plant J.* 56, 264–273
6. Zhang, H. *et al.* (2009) A soil bacterium regulates plant acquisition of iron via deficiency-inducible mechanisms. *Plant J.* 58, 568–577
7. Zhang, H. *et al.* (2009) Soil bacteria confer plant salt tolerance by tissue-specific regulation of the sodium transporter HKT1. *Mol. Plant-Microbe Interact.* 21, 737–744
8. Zhang, H. *et al.* (2010) Choline and osmotic-stress tolerance induced in *Arabidopsis* by the soil microbe *Bacillus subtilis* (GB03). *Mol. Plant-Microbe Interact.* 23, 1097–1104
9. Cho, S.M. *et al.* (2008) 2R,3R-butanediol, a bacterial volatile produced by *Pseudomonas chlororaphis* O6, is involved in induction of systemic tolerance to drought in *Arabidopsis thaliana*. *Mol. Plant-Microbe Interact.* 21, 1067–1075
10. Ezquer, I. *et al.* (2010) Microbial volatile emissions promote accumulation of exceptionally high levels of starch in leaves in mono- and dicotyledonous plants. *Plant Cell Physiol.* 51, 1674–1693
11. Velazquez-Becerra, C. *et al.* (2010) 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* 339, 329–340
12. 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
13. Groenhagen, U. *et al.* (2013) Production of bioactive volatiles by different *Burkholderia ambifaria* strains. *J. Chem. Ecol.* 39, 892–906
14. Meldau, D. *et al.* (2013) Dimethyl disulfide produced by the naturally associated bacterium *Bacillus* sp B55 promotes *Nicotiana attenuata* growth by enhancing sulfur nutrition. *Plant Cell* 25, 2731–2747
15. Zamioudis, C. *et al.* (2015) Rhizobacterial volatiles and photosynthesis-related signals coordinate *MYB72* expression in Arabidopsis roots during onset of induced systemic resistance and iron-deficiency responses. *Plant J.* 84, 309–322
16. Kanchiswamy, C.N. *et al.* (2015) Bioprospecting bacterial and fungal volatiles for sustainable agriculture. *Trends Plant Sci.* 20, 206–211
17. Kai, M. and Piechulla, B. (2009) Plant growth promotion due to rhizobacterial volatiles–an effect of CO_2? *FEBS Letters* 583, 3473–3477
18. Jin, J. *et al.* (2015) The impact of elevated carbon dioxide on the phosphorus nutrition of plants: a review. *Ann. Bot.* 116, 987–999
19. Ditengou, F.A. *et al.* (2015) Volatile signalling by sesquiterpenes from ectomycorrhizal fungi reprograms root architecture. *Nat. Commun.* 6, 6279