to genetic modification technology. This is an important finding as it implies successful navigation of the backfire effect without mentioning GMOs, after which instruction on the safety and benefits of GMOs would almost certainly be more effective. Of course, additional research is required to determine how best to bridge hunger with GMOs or other sensitive topics in agriculture. Our experience suggests that concern about hunger may open minds to GMOs, but concurrent instruction on hunger and genetic modification could possibly backfire if the perceived source of information is distrusted or if GMOs are presented as the only potential solution to hunger.

This sort of approach to addressing emotions and intuitions before instruction on sensitive subject matter, however, may be important when teaching about socially controversial topics in general, as research indicates that lack of acceptance of a concept can, in fact, prevent students from developing an understanding of the concept [9]. Moreover, prior research has suggested that helping students reach a position of deferred judgment on such topics is paramount in overcoming cognitive barriers rooted in prior rejection [10–12]. It seems that when countering positions based on emotion and intuition, it is important to appeal to those intuitions before building a rational argument based on evidence.

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Letter

Circumvent CO_2 Effects in Volatile-Based Microbe–Plant Interactions

Birgit Piechulla[1,*] and Jörg-Peter Schnitzler[2]

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

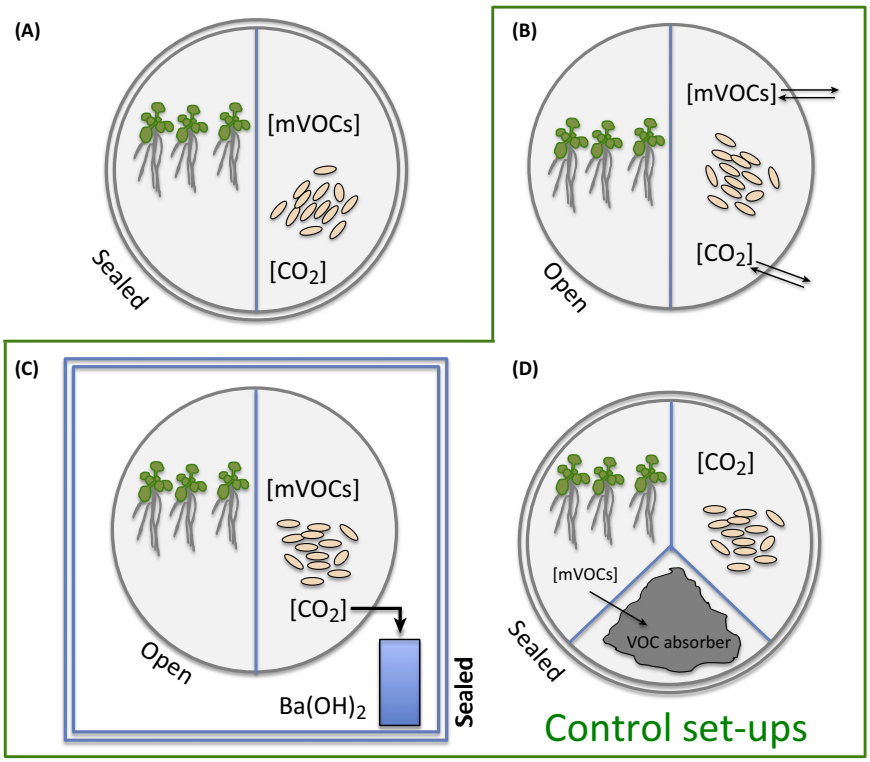

Figure 1. Experimental Set-Ups of Commonly Used Headspace Co-Cultivation Systems. (A) Gas-tight container system (ideally glass dishes) sealed with plastic parafilm. Plant and microbial media are physically separated, avoiding diffusion of metabolites through the agar. (B) enclosed system without sealing 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.

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 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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Spotlight

Phytochromes: More Than Meets the Eye

Stefan A. Rensing,[1,2,3,*] David J. Sheerin,[1] and Andreas Hiltbrunner[1,3,*]

Phytochromes play a key role in the regulation of plant growth and development. Phytochrome-related proteins also occur in some bacteria, fungi, and algae. We highlight recent findings on the evolution of phytochromes and discuss novel hypotheses on the function of phytochromes in diatoms, a group of mainly pelagic algae.

The Pigment of the Imagination

'The pigment of the imagination', as phytochromes were once referred to when their molecular nature remained elusive, has fascinated plant scientists for a century [1]. Until 1959, when purified for the first time [2], phytochromes were imagined as red/far-red **photoreversible pigments** (see Glossary) that regulate flowering, seed germination, and other plant physiological responses [3]. The ability to reversibly convert between an inactive **red light**- and a physiologically active **far-red light**-absorbing form (called Pr and Pfr) is the hallmark feature of phytochromes. Owing to this unique property, phytochromes are utilized in optogenetics, where they allow control of signaling pathways and cellular processes in non-plant cells by light.

Canonical plant phytochromes are present in **seed plants** and **non-seed plants** such as ferns, mosses, and **charophyte algae**, but not in **green algae** [4]. They consist of an N-terminal **photosensory core module** (PCM) and a C-terminal regulatory module which contains two PAS domains and a histidine kinase-related domain [3]. Historically, phytochromes were thought to be restricted to the plant kingdom, which is the reason for their Greek-derived name that translates to 'plant pigment'. The identification of bacterial and fungal proteins containing the highly conserved PCM known from plant phytochromes thus came as a surprise [5,6]. Whole-genome sequencing projects and high-throughput transcriptomic approaches further expanded the list of organisms containing phytochromes consisting of the PCM fused to different combinations of other domains [4,7,8] (Figure 1).

Phytochrome Evolution

A recent report investigated a total of 300 genomes and transcriptomes of **land plants** and charophytes, which together

Glossary

Archaeplastida (Plantae): a major group of eukaryotes comprising the red algae, the glaucophyte algae, the prasinophytes, the green algae, the streptophyte (charophyte) algae, and the land plants. They acquired their plastid by primary endosymbiosis of a cyanobacterium-like cell.
Canopy shade: sunlight contains roughly equal levels of red and far-red light, while in canopy shade the red:far-red light ratio is lower than 1 owing to photosynthetic pigments absorbing red but not far-red light. Phytochrome B is active in high red:far-red light but inactive under light conditions with a low red:far-red light ratio.
Charophyte algae: the polyphyletic charophyte algae include lineages that are sister to land plants and contain canonical plant phytochromes (e.g., *Mougeotia* spp.).
Chromophore: phytochromes depend on a chromophore for absorption of light. Canonical plant phytochromes almost exclusively contain phytochromobilin, a linear tetrapyrrole, as the chromophore; this binds to a conserved cysteine residue in the GAF domain. Non-plant phytochromes contain other linear tetrapyrrole as the chromophore (phycocyanobilin, biliverdin) and they bind the chromophore through a cysteine residue at the extreme N-terminus.
Far-red light (FR): light in the 720–740 nm wavelength range.
Green algae (chlorophyte algae): division Chlorophyta including *Chlamydomonas* spp., *Volvox* spp., and *Chlorella* spp.; canonical plant phytochromes have not been identified in green algae.
Land plants: synonym for embryophytes; these live primarily in terrestrial habitats and include seed plants, ferns, lycophytes, and bryophytes.
Neofunctionalization: the process in which a gene originating from a gene duplication event acquires a novel function not associated with the founder gene.
Non-seed plants: plants that do not produce seeds, but spores.
Photoreversible pigments: pigments that are able to reversibly convert between two states by absorption of light; the two states (Pr and Pfr in case of red/far-red reversible phytochromes) have different absorption spectra and therefore the light quality (wavelength) determines the equilibrium between the two states.
Photosensory core module (PCM): the PCM includes the phytochrome domains required for chromophore binding, light absorption via the chromophore, and photo-reversibility; it is composed of a PAS (Per/Arnt/Sim), a GAF (cGMP phosphodiesterase/adenylate cyclase/FhlA), and a PHY (phytochrome) domain in the order PAS–GAF–PHY.
PhyA and phyB: phyA responses are efficiently induced by far-red light, while activity in red light is lower. By contrast, phyB is most sensitive to red light and inactive in far-red light. The