Trends in Plant Science

CellPress REVIEWS

Spotlight

Plant Defense by VOC-Induced Microbial Priming

Hongwei Liu[1,]* and
Laura E. Brettell[1]

The plant holobiont extends the plant's capacity for nutrient acquisition and stress protection. Recent studies show that under biotic stress plants can promote the acquisition of certain beneficial bacteria to their rhizosphere. Active emission of volatile organic compounds (VOCs) is a newly identified mechanism utilized by plants for this process.

The 'Crying-for-Help' Strategy

One of the most exciting findings of recent decades has been that plant-associated microbes are not merely passengers, but instead can provide the plant with an extended functional repertoire to better facilitate multiple biological processes. The host plant and all of its microbiota thereby form an ecological unit, the holobiont. Numerous studies have highlighted increased nutrient acquisition and enhanced immunity as key benefits provided by these microbes. Plants seem to have evolved a 'crying-for-help' strategy whereby they recruit beneficial environmental microbes to their holobiome (the combined genomes of the host and all of its microbiota) to overcome stresses and facilitate adaptation to changing environments [1]. Root exudates putatively mediate such a recruitment process, with the exudation of VOCs being a newly identified mechanism [2,3]. There is now increasing evidence that it is possible to exploit the mechanisms behind the crying-for-help strategy to increase crop resistance to pests and microbial pathogens [1].

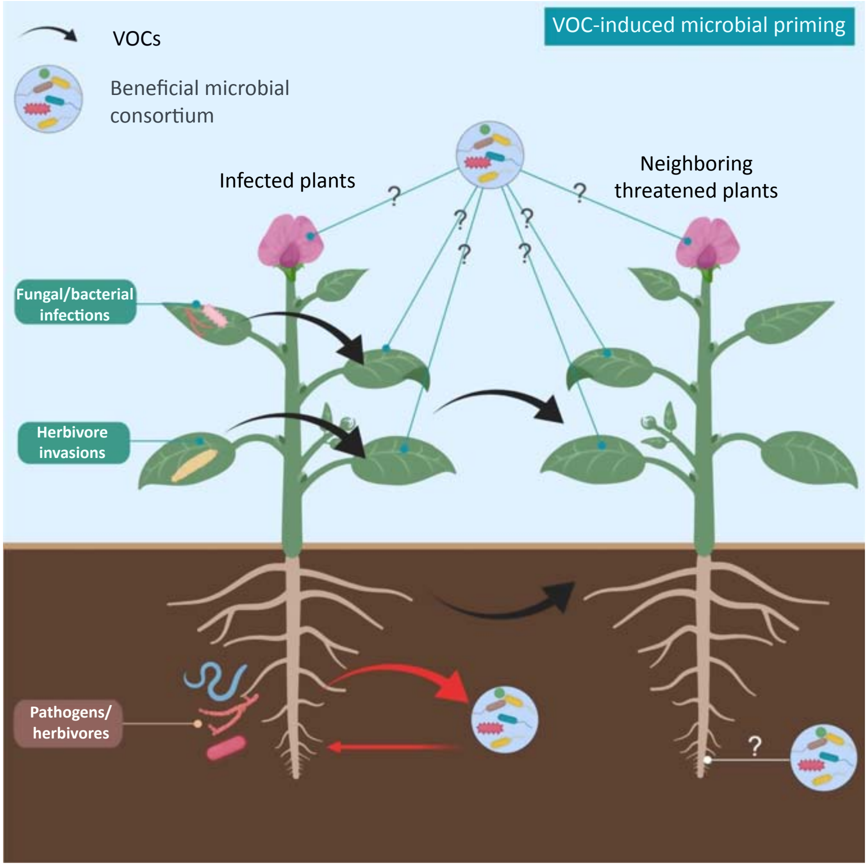
Recruiting Beneficial Bacteria into the Rhizosphere to Cope with Biotic Stresses

The rhizosphere, the narrow soil zone that surrounds and is influenced by plant roots, arguably has the most complex ecosystem on Earth. Plants invest significant resources in shaping their rhizosphere, which results in higher microbiota density and intense microbial activity compared with soil microbiota without plant root systems. Intriguingly, the living plant roots exude an array of simple and complex molecules (exudates) to recruit specific soil bacterial phylotypes (e.g., Gammaproteobacteria) and suppress others (e.g., Acidobacteria, Gemmatimonadetes) [4]. One role of the recruited rhizosphere microbiome is to protect plants against diseases and pests. This was elegantly shown in a recent study by Berendsen *et al*. [5], who found that plants recruit a bacterial consortium to fight disease. On infection of the leaf by *Hyaloperonospora arabidopsis*, the causative agent of downy mildew disease, arabidopsis (*Arabidopsis thaliana*) accumulated *Stenotrophomonas* spp., *Xanthomonas* spp., and *Microbacterium* spp. in its rhizosphere. The authors isolated these bacteria and found that, when administered in combination, they significantly reduced disease incidence in arabidopsis. Furthermore, the next generation grown in the soil were able to take advantage of the protection afforded by the altered rhizosphere microbiome. This experiment proved the theory that plants can 'cry for help' and was further confirmed with a study using *Pseudomonas syringae* as the inducing pathogen [6]. Despite recent speculation pinpointing root exudates as a driving force governing root microbiome assembly [2], how arabidopsis attracted these beneficial microbes were not investigated in these two studies.

Stenotrophomonas spp., especially *S. maltophilia*, can adhere to and form biofilms on root surfaces, exhibiting a strong capacity to colonize in the rhizosphere [7]. These species have been found to be present at an increased abundance in the rhizosphere of herbivore-challenged plants, indicating that they are efficient rhizocomponents responding to plants crying for help [8]. Many of these species are of interest for biotechnological application as plant growth stimulators [7]. However, *S. maltophilia* is a global opportunistic human pathogen, an aspect that should be taken into consideration before its use in agriculture [4]. Furthermore, there are pending questions regarding whether the crying-for-help theory applies to the rhizosphere only or also to the phyllosphere (leaf) and anthosphere (flower); that is, whether these plant tissues actively recruit beneficial microbes for the purpose of combating stresses (Figure 1). Additionally, a knowledge gap exists regarding the extent to which this theory is applicable across different types of biotic stress, such as infections by bacteria, fungi, and viruses.

Plants Attract Beneficial Microbes by Emission of VOCs

Enhancing the secretion of particular root metabolites is a known mechanism that plants employ to attract microbes. One supporting example is that, on foliar attack by *P. syringae*, tomato plants increased their secretion of L-malic acid, an intermediate of the tricarboxylic acid (TCA) cycle, into the rhizosphere [9]. Consequently, a beneficial bacterium, *Bacillus subtilis* FB17, which triggers induced systemic resistance (ISR), was attracted [9]. Recently, evidence was provided that root VOCs also play a role in attracting beneficial bacteria in soils [3]. Schulz-Bohm *et al.* discovered that the roots of *Carex arenaria* infected by *Fusarium culmorum* produced a set of VOCs, the profile of which differed from that of the healthy plants [3]. These *F. culmorum*-induced VOCs of *C. arenaria* were found to attract certain beneficial bacteria from a distance of >12 cm. Furthermore, these VOCs possessed highly variable translocation capacities, suggesting that plants



Trends in Plant Science

Figure 1. Volatile Organic Compound (VOC)-Mediated Attraction of Beneficial Bacterial Consortia. Belowground, plants can actively release blends of VOCs to attract remote biocontrol bacteria in soils, as suggested by Schulz-Bohm *et al.* [3]. This is depicted by red arrows in the illustration. Plausible but as yet unconfirmed is that, within the same plant, undamaged plant tissues can detect VOCs of disease- and/or herbivore-damaged tissues as a signal to accumulate particular biocontrol bacteria for defense before experiencing damage. Similarly, plants under biotic stress release blends of VOCs, which may signal the presence of the herbivores/pathogens to other plants in the community. The informed healthy plants therefore accumulate beneficial bacteria in the rhizosphere and the aerial portion of plants. It is postulated that such plant VOC-mediated allelopathic effects could microbially prime the defense of neighboring plants. Note: '?' represents as-yet-unconfirmed processes. This figure was created using Biorender (https://biorender.com/).

may be able to manipulate VOC composition to attract bacteria through variable soil environments. The study by Schultz-Bohm *et al.* [3] investigated six key bacterial isolates; however, in reality the rhizosphere microbial composition is much more complex, so further research is needed to understand the extent to which soil bacterial phylotypes may respond to VOCs of infected plants. While more details about this mechanism are needed, this study discovered a novel mechanism by which plants recruit beneficial microbes to the rhizosphere.

Based on the above, we propose a conceptual framework explaining how plants can attract a bacterial consortium (e.g., that observed by Berendsen *et al.* [5]) (Figure 1). (i) Disease-infected plants actively release nonvolatile root exudates (e.g., malic acid, coumarins [10]) into the rhizosphere to selectively signal and recruit certain members of the soil microbiome. Beneficial microbes recruited via this mechanism suppress pathogens directly or serve as signaling molecules to prime plant defenses. (ii) Plants actively emit blends of volatile compounds to attract beneficial microbes. Despite the fact that VOCs exist as only a minor component of root metabolites (1%), they can diffuse across long distances in soils and thus attract remote microbes. These beneficial microbes may form biofilms on leaf and root surfaces, which protect plants from pathogen/herbivore invasions and minimize damage. Following this framework, it may be hypothesized that within the same plant undamaged tissues can detect VOCs of herbivore/pathogen-challenged tissues as a signal to accumulate particular biocontrol bacteria for defenses prior to experiencing damage (Figure 1). Similarly, within a plant community, plants under biotic stress release blends of volatile compounds, which may signal the presence of herbivores/pathogens to neighboring plants. Thus, the informed healthy neighboring plants can accumulate beneficial bacteria in the rhizosphere and/or aerial portion of plants (Figure 1). The intensity and ratios of constituents of stress-induced VOCs may be plant species specific and vary on biotic stresses. Lastly, such mechanisms may play key roles in both below- and above-ground recruitment of bacteria.

Overall, positive allelopathic effects on the microbiome of neighboring plants can serve as another layer of plant defense at a community scale. However, using VOCs for plant defense is associated with potential tradeoffs with other biological processes. It is well known that plants use a diverse spectrum of VOCs to communicate with each other, to attract pollinators, or deter pests [11]. Thus, investing increased resources into defense may compromise the attraction of pollinators [12] or otherwise negatively affect the plants' reproduction process.

Plants seem to establish beneficial microbial consortia in their rhizosphere to cope with biotic stresses. This microbial consortium can also be a supreme legacy for the next generation with which future plants can cope with biotic stresses. VOCs may play a pivotal role in this process, which has only recently begun to be recognized. Collectively, biotic stress-induced recruitment of beneficial microbes may add another layer for the priming of plant defense, and the exploitation of the underlying mechanisms may foster future microbial strategies to control crop pests and diseases.

[1]Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW 2753, Australia

*Correspondence: h.liu2@westernsydney.edu.au (H. Liu).

https://doi.org/10.1016/j.tplants.2019.01.008

References

1. Bakker, P.A.H.M. *et al.* (2018) The soil-borne legacy. *Cell* 172, 1178–1180
2. Sasse, J. *et al.* (2018) Feed your friends: do plant exudates shape the root microbiome? *Trends Plant Sci.* 23, 25–41
3. Schulz-Bohm, K. *et al.* (2018) Calling from distance: attraction of soil bacteria by plant root volatiles. *ISME J.* 12, 1252–1262
4. Edwards, J. *et al.* (2015) Structure, variation, and assembly of the root-associated microbiomes of rice. *Proc. Natl. Acad. Sci. U. S. A.* 112, E911–E920
5. Berendsen, R.L. *et al.* (2018) Disease-induced assemblage of a plant-beneficial bacterial consortium. *ISME J.* 12, 1496–1507
6. Yuan, J. *et al.* (2018) Root exudates drive the soil-borne legacy of aboveground pathogen infection. *Microbiome* 6, 156
7. Hayward, A.C. *et al.* (2010) *Stenotrophomonas* and *Lysobacter*: ubiquitous plant-associated gamma-proteobacteria of developing significance in applied microbiology. *J. Appl. Microbiol.* 108, 756–770
8. Kong, H.G. *et al.* (2016) Aboveground whitefly infestation-mediated reshaping of the root microbiota. *Front. Microbiol.* 7, 1314
9. Rudrappa, T. *et al.* (2008) Root-secreted malic acid recruits beneficial soil bacteria. *Plant Physiol.* 148, 1547–1556
10. Stringlis, I.A. *et al.* (2018) MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. *Proc. Natl. Acad. Sci. U. S. A.* 115, E5213–E5222
11. Dudareva, N. *et al.* (2013) Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytol.* 198, 16–32
12. Lucas-Barbosa, D. *et al.* (2016) Visual and odour cues: plant responses to pollination and herbivory affect the behaviour of flower visitors. *Funct. Ecol* 30, 431–441

Spotlight

Tuning the Orchestra: miRNAs in Plant Immunity

Wenyi Wang [1,*] and
Gad Galili[1]

miRNAs act as negative modulators of target genes and play key roles in post-transcriptional gene regulation through sequence-specific mRNA cleavage and translational inhibition. Two recent reports highlight the orchestrated role of miRNA2111 and miRNA172b in plant innate immunity [1,2] (*Science* 2018;362:233–236; *Plant Cell* 2018;30:2779–2794).

miRNAs are a group of endogenous small noncoding RNA molecules, typically 20–24 nucleotides, that negatively regulate their target genes through RNA silencing. In the past two decades, extensive studies revealed that miRNAs play key roles in different aspects of plant growth and other biological processes such as cell proliferation, differentiation, and autophagy [3]. Accumulating evidence suggests that, apart from their role in plant development, miRNAs also orchestrate plant innate immunity, leading to antiviral immunity or viral pathogenesis.

Gene Silencing by miRNAs in Plant Immunity: For Better or Worse

In a recently published paper, Tsikou *et al*. [1] investigated the shoot-derived miR2111 target *TOO MUCH LOVE (TML)*, which is a root factor during autoregulation of nodulation, and demonstrated that in a *tml* loss mutant, knockout of cytokinin receptor *LHK1* and *miR2111* overexpression exhibited plant hyperinfection. Moreover, root-synthesized *CLE* peptides activated shoot HYPERNODULATION ABERRANT ROOT FORMATION1 (HAR1), which modified miR2111 production and/or shoot–root translocation after infection [1]. Zou *et al*. [3] revealed the role of the miR172b-TOE1/2 module in regulating plant innate immunity and the miR172b targets *TARGET OF EAT (TOE)1* and *TOE2* to downregulate their expression. Moreover, TOE1 and TOE2 directly bind to the promoter of *FLAGELLIN-SENSING2 (FLS2)* and inhibit its activity. FLS2 is a plasma membrane-localized receptor-like kinase (LRR-RLK) with the function of detecting bacterial flagellin to mount PAMP-triggered immunity (PTI). A *toe1 toe2* double mutant exhibited stronger resistance to *Pseudomonas syringae* pv. *tomato (Pst)* DC3000 infection compared with wild type plants. A similar response was detected in transgenic plants that overexpressed *miR172b* [3]. Thus, according to the studies mentioned above, miR2111 and miR172b play a key role in plant immunity (Figure 1).

Plants have evolved particular mechanisms to fight off a multitude of invading pathogenic microorganisms, including fungi, bacteria, and viruses. Host defense responses are mediated by activation and repression of a large set of genes, and miRNAs are important regulator molecules which can be induced or repressed in response to pathogen infection in order to subsequently regulate the expression of defense response genes by mRNA cleavage and translational inhibition. The studies of Tsikou *et al*. [1] and Zou *et al*. [3] showed distinct expression patterns of miRNAs: *miR2111* was downregulated, whereas *miR172b* was upregulated during infection. Plant miRNA-mediated gene silencing can occur through concomitant repression of a positive or negative immune response regulator, and/or activator of a positive or negative regulator of immune defense. In plants, miR393 was the first miRNA identified to play a