Airborne medicine: bacterial volatiles and their influence on plant health

Author for correspondence:
Laure Weisskopf
Tel: +41 26 300 88 11
Email: laure.weisskopf@unifr.ch

Paolina Garbeva[1] (iD) **and Laure Weisskopf[2]** (iD)

[1]Netherlands Institute of Ecology, Droevendaalsesteeg 10, 6708 PB, Wageningen, the Netherlands; [2]Department of Biology, University of Fribourg, Chemin du musée 10, CH-1700, Fribourg, Switzerland

Contents

Summary

Like most other eukaryotes, plants do not live alone but in close association with a diverse microflora. These plant-associated microbes contribute to plant health in many different ways, ranging from modulation of hormonal pathways to direct antibiosis of plant pathogens. Over the last 15 yr, the importance of volatile organic compounds as mediators of mutualistic interactions between plant-associated bacteria and their hosts has become evident. This review summarizes current knowledge concerning bacterial volatile-mediated plant protection against abiotic and biotic stresses. It then discusses the translational potential of such metabolites or of their emitters for sustainable crop protection, the possible ways to harness this potential, and the major challenges still preventing us from doing so. Finally, the review concludes with highlighting the most pressing scientific gaps that need to be filled in order to enable a better understanding of: the molecular mechanisms underlying the biosynthesis of bacterial volatiles; the complex regulation of bacterial volatile emission in natural communities; the perception of bacterial volatiles by plants; and the modes of actions of bacterial volatiles on their host.

I. Introduction

1. Plants' responses to stresses

During their entire life span, plants face many different types of 'stresses'; that is, external conditions preventing them from reaching optimal growth. Some of these stresses are of abiotic nature, such as poor nutrient availability or heavy metal toxicity, drought or flooding, and exposure to cold or high temperature. By contrast, stresses of biotic nature involve the interaction of plants with other organisms using the plant as food source, such as herbivores or disease-causing agents, which can be of viral, bacterial, fungal, or oomycete origin. To withstand these different stresses, plants have developed sophisticated mechanisms, which mainly reside in the production of a diverse set of chemical defences encompassing highly bioactive secondary metabolites. Some of these compounds have direct toxicity to the attacker, whereas others recruit its natural enemies or warn neighbouring plants to mount their own defences (Hare, 2011; Clavijo McCormick *et al.*, 2012; Erb, 2018). The vast richness of the plant secondary metabolome,

which still is a prolific source of bioactive molecules of relevance for plant and human health, might lead one to think that plants do not depend on other organisms for their own defence. However, we have recently learned that plants, like other organisms, are densely colonized by diverse microbes (Vorholt, 2012; Müller *et al.*, 2016; Banerjee *et al.*, 2018), which might also contribute to their host's defence (Innerebner *et al.*, 2011; Ritpitakphong *et al.*, 2016).

2. Microbial metabolites of relevance to plant health

Microbes in general, and bacteria in particular, are well known for their biochemical versatility; using a wide array of inorganic and organic compounds to fuel their metabolism, they are also prolific producers of secondary metabolites of diverse biological activities. Many of these metabolites have antimicrobial properties and are used as antibiotic and antifungal drugs (Brader *et al.*, 2014; Ait Barka *et al.*, 2016; Caulier *et al.*, 2019). This wealth of antimicrobial compounds encoded in genomes of both bacteria and fungi is not surprising considering that these mostly heterotrophic microbial life forms have competed for the same resources for millions of years before the appearance of the first terrestrial plants. Traditionally, the study of microbial metabolism and the search for bioactive molecules have focused on soluble compounds. However, mounting evidence suggests that microbes, and especially bacteria, emit diverse volatile compounds with significant biological activities on a wide range of target organisms, including plants and their pathogens (Groenhagen *et al.*, 2013; Piechulla *et al.*, 2017). Although plant-associated fungi and protists also emit volatile organic compounds (Hung *et al.*, 2015; Chen *et al.*, 2016; Li *et al.*, 2016), the present review focuses on bacterial volatiles and highlights the diverse means by which these compounds contribute to maintaining and protecting plant health.

3. Structure and function of the bacterial smell

Volatile compounds are usually defined as small (< 300 Da) molecules of low boiling point and high vapour pressure, and containing a lipophilic moiety (Schulz & Dickschat, 2007). More generally, these physical and chemical features influence the volatility of molecules, which should be seen as a continuous rather than dichotomous property, some molecules showing very high, and other very low volatility. However, all volatile molecules share the ability to move via the gaseous phase, a feature that can be of great relevance in environments such as the heterogeneous soil matrix that constitutes an important source of bacterial inoculum for the plant microbiome (Zarraonaindia *et al.*, 2015; Grady *et al.*, 2019). There, volatile signals can accumulate and spread via the air-filled soil pores, enabling exchanges between roots and microbes at a longer distance than the sole emission of diffusible compounds would allow. Beyond volatility, the chemical structures of bacterial volatiles are very diverse, ranging from small aliphatic (e.g. dimethyl disulphide, which is produced by many bacteria) or aromatic molecules (e.g. indole, the major smell of *Escherichia coli*) to large ketones, alkanes, or alkenes (e.g. the 1-undecene typical of certain *Pseudomonas*). Terpenes (e.g. geosmin, the typical smell of soil, produced by Actinobacteria), which have been abundantly

studied as plant secondary metabolites (Chen *et al.*, 2011; Riedlmeier *et al.*, 2017), are a further important class of bacterial volatiles. For detailed information on the chemical diversity of bacterial volatiles, we refer the reader to recent and excellent reviews on the topic (Schulz & Dickschat, 2007; Citron *et al.*, 2012; Effmert *et al.*, 2012; Peñuelas *et al.*, 2014).

The 'smell' or 'volatilome' of a given bacterial strain is usually constituted of a wide diversity of volatile compounds, whose composition and relative abundance change with growth phase or growth conditions (Kai *et al.*, 2010; Blom *et al.*, 2011a). Recent work indicates that the emission of particular volatiles can be induced by the presence of interacting partners (Schmidt *et al.*, 2017), suggesting complex regulation of the synthesis and/or emission of these metabolites rather than unspecific release of 'metabolic waste products'. In general, closely related strains emit more similar volatilomes than phylogenetically more distant strains do (Ryu *et al.*, 2003; Kai *et al.*, 2007; Groenhagen *et al.*, 2013). Most volatiles are commonly produced by different and unrelated bacteria (Schulz & Dickschat, 2007; Garbeva *et al.*, 2014b), but others are more specific, and the use of volatiles as markers of bacterial presence is a particularly interesting outcome of this field of research; for example, for clinical diagnostic purposes or for the detection of pathogens (Tait *et al.*, 2014).

4. Bacterial volatiles as modulators of plant health

In terms of biological functions, bacterial volatiles have been reported to affect many different types of target organisms, be it bacteria themselves, other microbes (such as fungi or protists), plants, or animals. Regarding the effect of bacterial volatiles on plants, only a few exceptions of phytotoxic volatiles have been reported, whereas most bacterial volatiles have a shown positive impact on plant health. This positive impact originated either (1) from a promoting effect on the plant, leading to better growth or to induced defences against abiotic or biotic stresses, or (2) from an inhibitory effect on the pests and pathogens responsible for the health threat. These two main modes of action are presented in the two following sections. Thereafter, these findings, which largely originate from laboratory studies in controlled conditions, are discussed in terms of their relevance for crop protection under glasshouse and field conditions. The review closes with a concluding section on the main opportunities and challenges this relatively new field of research is likely to generate in the coming years.

II. Direct effects of bacterial volatiles on plant health

1. Offensive smells: some bacterial volatiles lead to plant death

Following the first report of plant growth promotion by bacterial volatiles (Ryu *et al.*, 2003), researchers aimed at understanding whether this volatile-mediated plant growth modulation was a general phenomenon. Using simple split plate experimental setups, they therefore started to expose the model plant *Arabidopsis thaliana* to volatiles emitted by rhizosphere bacteria belonging to different taxonomic groups. Although strong growth promotion –

which turned out to be partly due to CO_2 emission (Kai & Piechulla, 2009) – was confirmed in many cases, these early studies also reported drastic growth inhibition, phytotoxic effects, and even plant killing within a few days (Vespermann *et al.*, 2007; Blom *et al.*, 2011a). The volatiles responsible were identified mainly as inorganic compounds; for example, hydrogen cyanide (HCN; Blom *et al.*, 2011b) or ammonia (Weise *et al.*, 2013). Later work demonstrated that some organic volatiles were phytotoxic as well, especially when applied in high (micromolar to millimolar) concentrations. One such example is dimethyl disulphide (Kai *et al.*, 2010), a compound otherwise shown to increase plant growth in sulphur (S)-limited conditions (Meldau *et al.*, 2013) and tested as a soil fumigant against soil-borne diseases and pests (Pecchia *et al.*, 2017; Gómez-Tenorio *et al.*, 2018). The volatile 2-phenylethanol commonly found in bacterial blends (Schulz & Dickschat, 2007) also inhibited the growth of *A. thaliana* (Wenke *et al.*, 2012) and that of *Medicago sativa* (Ulloa-Benítez *et al.*, 2016) when applied at a concentration of *c.* 0.2 mM. However, we deem it unlikely that these high concentrations are in any relation to the actual quantities emitted in nature. Indeed, when testing the same or similar compounds in a more realistic nanomolar range, dimethyl disulphide was shown to significantly promote plant growth, whereas 2-phenylethanol did not significantly affect the growth of *A. thaliana* (Groenhagen *et al.*, 2013). These examples highlight the importance of performing dose–response assays when testing biological effects of pure volatiles. Ideally, concentrations should be adjusted to those occurring in nature, but as long as these are unknown, including a wide range of concentrations starting in the nanomolar range or even lower would enhance the relevance of the effects observed under laboratory conditions.

Taken together, the aforementioned studies suggest strong phytotoxicity for the inorganic bacterial volatiles ammonia and HCN, leading to the potential use of such producers of biocidal volatiles in biological control of weeds, as tested in earlier studies (Flores-Vargas & O'Hara, 2006; Zeller *et al.*, 2007). However, it appears that only few organic volatiles emitted by bacteria might have phytotoxic activity (Tyagi *et al.*, 2018), and most of the data accumulated so far advocates for beneficial effects of bacterial volatile organic compounds, which have been shown to contribute to alleviation of both abiotic and biotic stresses, as discussed in the next section.

2. Friendly scents: helping plants to cope with stress

Impact of bacterial volatiles on abiotic stress tolerance Abiotic stresses are of multiple kinds and encompass, among others, nutrient deficiency, drought, and salinity stress. Early work focusing on the model strain *Bacillus amyloliquefaciens* GB03 has demonstrated that the growth promotion triggered by this strain's volatiles were partly due to higher iron (Fe) uptake, which was mediated by rhizosphere acidification and upregulation of the plant Fe acquisition machinery (Zhang *et al.*, 2009). These findings were later corroborated by similar observations of increased acidification and Fe acquisition in *Medicago* exposed to volatiles emitted by its symbiont *Sinorhizobium meliloti* (del Carmen Orozco-Mosqueda *et al.*, 2013). Likewise, later work focusing on another *Bacillus*

strains revealed that bacterial volatiles themselves could serve as nutrient sources, as elegantly demonstrated by plants taking up bacterial dimethyl disulphide synthetized from labelled sulphate in a split Petri dish setup (Meldau *et al.*, 2013). Salinity stress, an ever-increasing world-wide problem, is caused by both water shortage and toxic level accumulation of ions such as sodium (Na) or chloride (Isayenkov & Maathuis, 2019). Using the same *Bacillus* GB03 model strain, Zhang and colleagues demonstrated the salinity stress-alleviating potential of bacterial volatiles, which led to tissue-specific expression modulation of an Na exporter in *A. thaliana*. This ultimately resulted in significantly higher tolerance and 50% reduction in Na content of Arabidopsis exposed to the volatiles of *Bacillus* GB03 compared with nonexposed plants (Zhang *et al.*, 2008). The volatile compound responsible for such increased salt tolerance was not identified in this study. By contrast, 2,3-butanediol emitted by *Pseudomonas chlororaphis* O6 (Gammaproteobacteria) was shown to confer drought stress tolerance to Arabidopsis (Cho *et al.*, 2008). This drought stress tolerance, which was not provided by bacterial mutants impaired in butanediol fermentation, depended on the salicylic acid (SA) pathway, as well as on nitric oxide and hydrogen peroxide synthesis (Cho *et al.*, 2008, 2013). Beyond *A. thaliana*, Vaishnav *et al.* (2015) investigated the effect of the volatiles from another *Pseudomonas* strain – *Pseudomonas simiae* AU – on the salinity stress tolerance of soybean (*Glycine max*) plants. They also observed lower plant Na levels, coupled with increased proline contents, although the volatile compounds responsible were not identified. Lastly, a fourth example of drought stress tolerance by bacterial volatiles involved the broad host endophyte and plant-growth-promoting rhizobacterium (PGPR) *Paraburkholderia phytofirmans* PsJN (Betaproteobacteria; Ledger *et al.*, 2016). This strain was previously known to confer tolerance to a range of abiotic stresses, including salt, drought, high and low temperatures, and heavy metal contamination (Esmaeel *et al.*, 2018). When investigating putative determinants of such conferred tolerance using a targeted mutagenesis approach, the authors found that none of the 'usual suspects' (including 1-aminocyclopropane-1-carboxylate deaminase activity) accounted significantly for the observed effects. By contrast, even a short and early exposure of Arabidopsis to the volatile blends emitted by *Paraburkholderia* PsJN mimicked the long-term growth-promoting and stress-alleviating effects observed with the strain's inoculation on the roots. Further analysis revealed that the mixture of three volatiles produced by this strain (2-undecanone, 7-hexanol, and 3-methylbutanol) conferred similar growth promotion and stress tolerance as the strain itself, thereby identifying these volatile compounds as the major determinants in this strain's ability to confer abiotic stress tolerance to its host plant (Ledger *et al.*, 2016).

In summary, these results highlight the ability of phylogenetically diverse bacteria to emit different volatile compounds, which trigger similar increased tolerance to abiotic stress (for further examples of such volatile-induced abiotic stress tolerance, please see Liu & Zhang 2015). Compared with plants, bacteria withstand much higher deviations from their optimal environmental conditions and are therefore likely to be better equipped than their host to tolerate such abiotic stresses. Since most plant-associated bacteria

are dependent on their host for photosynthesized carbon (C)-containing nutrients, the ability to increase their host's tolerance to adverse conditions might have been a selective advantage in the course of evolution, for the establishment of long-lasting and resilient plant–bacteria interactions. However, on the plant side, the mechanisms through which bacterial volatiles trigger the observed physiological changes (e.g. proline accumulation or lower Na uptake) ultimately leading to abiotic stress tolerance remain largely mysterious. In particular, since plants can also sense their environment and respond to stressful conditions on their own, why would this response be facilitated by exposure to a bacterial volatile? Since no molecular mechanism has been described yet, we can only speculate at this point that plants might perceive bacterial volatiles as a warning of potential danger (e.g. infection by pathogenic bacteria) and mount a general tolerance response to many types of stresses that would also encompass abiotic stresses such as drought and salinity. The aforementioned lack of specificity (bacteria of diverse phylogenetic origin emitting different volatiles trigger similar effects in plants) seems to corroborate this 'general danger response' hypothesis. One could also imagine that exposure to biotic cues such as bacterial volatiles potentiate the plant sensitivity to other, biotic or abiotic stress factors, ultimately leading to a faster and stronger response, as observed for the so-called 'priming' of plant resistance against pathogens by beneficial *Pseudomonas* (Pieterse *et al.*, 2014).

Impact of bacterial volatiles on biotic stress tolerance When facing biotic stresses such as pest or pathogen attacks, plants defend themselves via two main resistance pathways, the SA-mediated systemic acquired resistance (SAR), and the jasmonic acid/ethylene-mediated induced systemic resistance (ISR) (Pieterse *et al.*, 2011; Tsuda & Somssich, 2015). Whereas SAR is mainly induced by pathogenic microbes, plants have been shown to express ISR when their roots are colonized by beneficial *Pseudomonas* secreting specific metabolites (e.g. siderophores) or harbouring molecular determinants such as flagellin or the O antigen of the outer membrane lipopolysaccharides (Meziane *et al.*, 2005). In addition to these nonvolatile molecules, 2*R*,3*R*-butanediol was shown to be involved in induction of systemic resistance against *Erwinia carotovora* by both *Bacillus* GB03 and *P. chlororaphis* O6 (Ryu *et al.*, 2004; Han *et al.*, 2006). This induction of defences was enantiomer specific, with 2*S*,3*S*-butanediol being ineffective in inducing resistance (Han *et al.*, 2006). This specificity was recently confirmed in a field study where 2*R*,3*R*-butanediol was shown to induce resistance in pepper plants exposed to viral infection, whereas the *S*-form was inefficient in both inducing defence-related genes and protecting the plants under field conditions (Kong *et al.*, 2018).

Beyond butanediol, two other bacterial volatile compounds have been shown to elicit plant defences against pathogens. The first is the long-chained (C13) alkane tridecane identified in the blends of a *Paenibacillus polymyxa* strain (Lee *et al.*, 2012). When applied as pure compounds, tridecane induced upregulation of defence-related genes and led to significantly reduced disease symptoms caused by *Pseudomonas syringae* on *A. thaliana*. However, this necessitated very high concentrations (millimolar range), which

stood in no relation to the compound's relative abundance in the complex blend of the bacterium, suggesting that other volatiles in addition to tridecane might have contributed to resistance induction by *P. polymyxa* (Lee *et al.*, 2012). The second compound is indole, which regulates a wide breadth of processes in bacteria (Lee & Lee, 2010) and is an important signal in bacteria–plant interactions (Bailly *et al.*, 2014). Although bacteria-emitted indole has not yet been shown to induce resistance, plant-emitted indole acts as a rapid priming signal from herbivore-attacked maize (*Zea mays*) plants to warn neighbouring plants and enable them to prepare their own defences (Erb *et al.*, 2015). This suggests that plants might also recognize bacteria-emitted indole as a warning signal.

In addition to the aforementioned studies, many other reports exist of health-promoting effects of complex bacterial blends. However, in most cases, they did not monitor the expression of defence genes nor use specific mutant lines impaired in the different induced resistance pathways, which makes it difficult to disentangle plant-mediated protection through ISR or SAR from direct inhibition of the pathogens, which is a very widely observed biological activity of bacterial volatiles, as detailed in the next section. One attempt to evaluate the relative proportion of direct pathogen inhibition vs induction of plant defences used an approach where plants were first exposed to the bacterial volatiles, which were then removed before infection, in order to avoid direct exposure of the pathogen to the bacterial volatiles. The authors concluded that most of the observed protection was due to induced defences, not to pathogen inhibition (Sharifi & Ryu, 2016). This relative importance of plant resistance induction vs direct pathogen inhibition is likely strain and compound dependent, although some volatile compounds were shown to display both types of effect: reduction of pathogen virulence and upregulation of plant genes involved in the SA-mediated defence pathway (Tahir *et al.*, 2017).

III. Indirect effects of bacterial volatiles on plant health: affecting the plant's enemies

1. Earthy scents: the role of bacterial volatiles in soil suppressiveness and fungistasis

Since the last decade, an increasing number of studies have revealed evidence for the production of pathogen-suppressing and fungistatic microbial volatiles in soils (Garbeva *et al.*, 2011; Effmert *et al.*, 2012; Hol *et al.*, 2015; Piechulla *et al.*, 2017). Fungistasis is defined by a restricted ability of the fungal propagules to germinate and grow. The involvement of volatiles in soil fungistasis was first revealed in 2004, when Chuankun *et al.* (2004) showed germination inhibition of fungal spores exposed to volatiles emitted from natural soils. Furthermore, this study revealed significant correlation between direct and volatile-mediated fungistasis, suggesting a prominent role of volatile compounds in the overall fungistasis. Accordingly, the authors identified volatiles commonly produced by different fungistatic soils, such as trimethylamine, dimethyl disulphide, benzaldehyde, or *N*,*N*-dimethyloctylamine, which inhibited fungal growth when applied as pure compounds (Chuankun *et al.*, 2004; Piechulla *et al.*, 2017).

Many volatiles produced by soil or rhizosphere bacteria have negative effects on the growth of a broad spectrum of fungal and oomycete pathogens (Weisskopf, 2013; Piechulla *et al.*, 2017). This suggests that these compounds might contribute to the 'disease suppressiveness' of soils (low disease incidence occurring despite the presence of a virulent pathogen), although this property has been traditionally linked to nonvolatile bacterial metabolites such as 2,4-diacetylphloroglucinol (Weller *et al.*, 2002). Indeed, a recent study revealed that changes in soil bacterial community composition caused by anaerobic disinfestation coincided with a loss of emission of *Pythium* suppressing volatiles and with an increase of hyacinth root infection by the same pathogen (van Agtmaal *et al.*, 2015).

The emission of volatiles is influenced by various abiotic factors, including nutrient availability, temperature, pH, or soil moisture (Insam & Seewald, 2010), but the soil properties and management practices that specifically influence volatile-mediated pathogen suppression are still largely unknown. To investigate this question, van Agtmaal *et al.* (2018) analysed the effect of volatiles emitted from a broad range of agricultural soils on the growth of different plant pathogens. They observed that volatile-mediated suppression of *Rhizoctonia solani* correlated positively with organic matter content, microbial biomass, and proportion of litter saprotrophs in the microbial community, whereas it correlated negatively with pH, microbial diversity (Shannon index), and the proportion of Acidobacteria in the community. By contrast to this negative correlation with Acidobacteria, Carrion and co-workers observed that members of the Burkholderiaceae family were more abundant and more active in suppressive soils than in conducive soils. They further identified *Paraburkholderia graminis* and its emission of S volatiles as important actors of this soil suppressiveness (Carrión *et al.*, 2018). These findings corroborate the results of many *in vitro* experiments that identified S-containing volatile compounds such as dimethyl disulphide and dimethyl trisulphide as powerful antifungal and anti-oomycete agents (Schöller *et al.*, 2002; Kai *et al.*, 2009; De Vrieze *et al.*, 2015).

In addition to the Pseudomonaceae and Burkholderiaceae, which have been clearly associated with disease suppressiveness, Actinobacteria are likely to be involved as well. This phylum is highly abundant in soils and known to harbour prolific volatile producers (Schöller *et al.*, 2002; Groenhagen *et al.*, 2014; Cordovez *et al.*, 2015; Ait Barka *et al.*, 2016). Recently, Cordovez and co-workers revealed that antifungal volatiles were produced by *Streptomyces* isolated from the rhizosphere of sugar beet grown in *Rhizoctonia*-suppressive soil and that these volatiles could significantly inhibit hyphal growth of *R. solani* (Cordovez *et al.*, 2015). Early studies had already demonstrated the antifungal potential of *Streptomyces* volatiles (Hora & Baker, 1972; Herrington *et al.*, 1987), and a few compounds have been identified that mediate such antifungal action, such as methyl vinyl ketone (butenone) inhibiting spore germination of *Cladosporium cladosporioides* (Herrington *et al.*, 1987) or dimethyl disulphide inhibiting mycelial growth of *Fusarium moniliforme* (Wang *et al.*, 2013). Similarly, anisole, produced by *Streptomyces albulus*, was recently shown to inhibit the growth of *Sclerotinia sclerotiorum* and *Fusarium oxysporum* (Wu *et al.*, 2015).

Although most antifungal activity tests are performed with single strains in laboratory studies, bacterial emitters of volatiles in soils are part of complex microbial communities. Community composition, diversity, and interactions can significantly influence the production of pathogen-supressing volatiles. Indeed, a synthetic community approach revealed that both microbial interactions and shifts in community composition had strong effects on volatile emission in soil microcosms (Schulz-Bohm *et al.*, 2015). Interestingly, the presence of a slow-growing, low-abundance *Paenibacillus* strain significantly affected the growth and volatile emission by other abundant members of the community. In particular, one specific volatile compound (i.e. 2,5-bis(1-methylethy)-pyrazine) that significantly inhibited the plant pathogens *R. solani* and *Fusarium culmorum* (Tyc *et al.*, 2017) was only found in the presence of this *Paenibacillus* strain (Schulz-Bohm *et al.*, 2015). This finding is in line with the observation that the loss of soil bacterial diversity can lead to a decline in the production of antifungal volatiles. By performing dilution-to-extinction experiments for seven different soils, Hol and co-workers observed that communities with high bacterial species richness produced volatiles that strongly reduced the hyphal growth of the pathogen *F. oxysporum*. For most soils, the loss of bacterial species resulted in loss of antifungal volatile production, and several known antifungal compounds, such as 2-methylfuran, 2-furaldehyde, and benzothiazole were only produced in the more diverse bacterial communities (Hol *et al.*, 2015). The importance of the microbial interactions aspect should be kept in mind when analysing emission of disease-inhibiting volatiles in controlled conditions (which have so far been restricted mostly to single strains), and the experimental setups should in future be adjusted to allow such naturally occurring interspecific interactions to take place.

In addition to regulation of bacterial volatile emission by interspecific interactions, single strains also regulate their volatile emission, and a few reports exist that identified the molecular actors of such regulation. The first regulatory mechanism identified in the context of bacterial volatile emission was 'quorum sensing', a population-density-based regulation known to control the production of a wide range of processes in bacteria, including virulence factors and production of secondary metabolites (Lazdunski *et al.*, 2004). Quorum sensing was shown to affect global volatile emission in *Serratia plymuthica* (Müller *et al.*, 2009) and to regulate the emission of the small volatile 2-acetophenone implicated in *Pseudomonas aeruginosa* lung persistence (Kesarwani *et al.*, 2011). A second major regulator of secondary metabolite production in *Pseudomonas* is the two-component system GacA/GacS (Haas & Défago, 2005). Recently, Ossowicki and co-workers revealed that volatiles emitted by the tomato rhizosphere isolate *Pseudomonas donghuensis* P482 inhibited the growth of several plant pathogens, including *R. solani*, *F. culmorum*, *Verticillium dahlia*, and *Pythium ultimum*. Interestingly, a GacA-deficient mutant of *P. donghuensis* P482 entirely lost this inhibition ability (Ossowicki *et al.*, 2017). In addition to HCN, whose production is known to be regulated by the GacA/GacS regulatory system, metabolomic analysis revealed clear differences in volatile profiles between the wild-type and the GacA mutant, with five compounds (dimethyl disulphide, *S*-methyl thioacetate, methyl thiocyanate, dimethyl trisulphide, and

1-undecane) detected only in the headspace of the wild-type. Hence, the emission of volatile compounds with strong antifungal and anti-oomycete activity could be dependent on the GacS/GacA two-component regulatory system for some plant-associated bacteria, as are other determinants of antifungal activity.

In summary, many studies have reported powerful antifungal and anti-oomycete activities of bacterial volatiles, and recent work has unveiled the importance of global metabolic regulators as well as of interspecific interactions as determinants of such pathogen-inhibiting volatiles. However, the exact modes of action of these molecules on the target organisms remain largely elusive. Few studies have investigated this question, and disruption of cell membrane integrity and perturbation of redox balance are two commonly observed consequences of bacterial volatile exposure (Giorgio *et al.*, 2015). Recently, the terpene caryolan-1-ol, commonly produced by *Streptomyces* spp., was reported to affect the endomembrane system of *Botrytis cinerea* by disrupting sphingolipid synthesis and vesicle trafficking (Cho *et al.*, 2017). More research efforts are needed to gain basic understanding on the molecular mechanisms underlying the observed effects of bacterial volatiles on the various plant pathogens.

2. Fratricide smells: bacterial volatiles against bacterial plant pathogens

If bacterial volatile emission has evolved as a way to repel competitors, one might also expect some of these compounds to have antibacterial activities. Indeed, some bacterial volatiles have been shown to affect other bacteria, although the literature about volatile-mediated bacteria–bacteria interactions is still scarce. One of the first examples of broad-range antibacterial volatiles are the sesquiterpenes albaflavenone and pentalenolactone produced by *Streptomyces coelicolor* and *Streptomyces avermitilis*, respectively (Tetzlaff *et al.*, 2006; Zhao *et al.*, 2008). Two plant-associated bacteria, *Pseudomonas fluorescens* and *S. plymuthica*, were later reported to emit volatiles that strongly suppressed the growth of *Agrobacterium tumefaciens* and *Agrobacterium vitis* (Dandurishvili *et al.*, 2011). The authors further identified dimethyl disulphide as one of the possible responsible compounds for the bacteriostatic effect. Of the few studies available that reported efficient control of bacterial phytopathogens by bacterial volatiles, two recent ones investigated the ubiquitous, broad-host pathogen *Ralstonia solanacearum*. Raza *et al.* (2016) examined the effect of volatiles emitted by a *P. fluorescens* strain on the growth and virulence traits of this pathogen using tomato as a model. The volatiles showed dose-dependent bacteriostatic effects on *R. solanacearum* in both agar medium and infested soil. Furthermore, these volatiles could repress the expression of virulence traits involved in host colonization, such as motility, root colonization, and biofilm formation and thereby efficiently control tomato wilt (Raza *et al.*, 2016).

The same *R. solanacearum* was also affected by volatiles emitted by several *Bacillus* strains, which led to inhibition of wilt on tobacco (Tahir *et al.*, 2017). This inhibition was attributed to few volatiles including benzaldehyde, 1,2-benzisothiazol-3(2*H*)-one and 1,3-butadiene. In addition to triggering expression of defence genes in

the plant, these volatiles also directly affected the bacterial pathogen by inhibiting its motility and chemotaxis. In addition, microscopy studies revealed severe morphological and ultrastructural changes in volatile-exposed *Ralstonia* cells. These phenotypic changes were linked to altered expression levels of PhcA (a global virulence regulator), and of genes related to the type III and type IV secretion systems, to extracellular polysaccharides and to chemotaxis-related genes, which are all virulence factors (Tahir *et al.*, 2017). Finally, the soil isolate *Bacillus subtilis* FA26 emitted volatiles that inhibited *Clavibacter michigansis* ssp. *sepedonicus*, which causes extensive damage in potato and tomato production. This protective effect was assigned to benzaldehyde, nonanal, benzothiazole, and acetophenone (Rajer *et al.*, 2017).

Except for the few aforementioned studies, little is known on the antibacterial potential of bacterial volatiles. By contrast, one early observation was that volatiles triggered increased resistance to antibiotics in target bacteria (Lee *et al.*, 2010; Bernier *et al.*, 2011; Groenhagen *et al.*, 2013). Since many bacterial volatiles are shared across diverse taxa, bacteria might have developed tolerance to their own metabolic products and therefore be less affected by these compounds than are more distantly related organisms such as fungi or oomycetes.

IV. Harnessing the power of bacterial volatiles to improve plant health

1. Challenges in the application of pure volatiles

Two main application techniques can be envisaged for bacterial volatile compounds: a drenching/spraying application (as used for nonvolatile plant protection products) or an application by air diffusion (e.g. as used for mating-disruption pheromones in grapevine and other perennial crops). One challenge in applying bacterial volatiles through soil drenching or leaf spraying is their lipophilic nature, and hence their poor water solubility. Indeed, the few studies that have dealt with larger scale experiments to verify the suitability of volatile application for crop protection have focused on at least partially water-soluble compounds such as dimethyl disulphide (recently commercialized for soil fumigation against nematodes and soil-borne pathogens), 2-pentanol, or 2-butanone. The latter two compounds efficiently increased the resistance of cucumber plants against both *P. syringae* and the aphid *Myzus persicae* in open field experiments (Song & Ryu, 2013). Likewise, 2,3-butanediol treatment in field-grown cucumbers led to induction of plant resistance against viruses, ultimately resulting in reduced incidence of naturally occurring viruses (Kong *et al.*, 2018). Importantly, these two studies bring the proof of concept that the power of action of bacterial volatiles is not restricted to the Petri dish environment, although volatile compounds might require more sophisticated formulation than their nonvolatile counterparts to accommodate their lipophilic nature. For instance, slow release of volatiles could rely on encapsulation into a specific matrix, as used for aroma in the food industry (Mascheroni *et al.*, 2013).

Although the latter field experiments involved drench application of volatiles dissolved in water, a recent study investigated the

crop protection efficiency of continuously released volatiles, using either a filter paper soaked with 2,3-butanediol or a live culture of its producer *B. subtilis* GB03. This continuous exposure to an atmosphere enriched in protective volatiles led in both cases to reduced leaf spot symptoms in cucumber (Song *et al.*, 2019). Although upscaling this miniature system to industrial glasshouse conditions might prove challenging, this study shows that atmosphere enrichment in specific volatiles can result in significant plant protection, and modifications of glasshouse atmosphere might enable harnessing of the protecting effect of poorly soluble volatiles. Moreover, bacterial volatiles might also be introduced into the ventilation systems of crop storage facilities and thereby contribute to post-harvest disease control. In any case, formulation efforts will be needed to adjust the carrier to the respective physical and chemical properties of each bioactive volatile, to ensure its continuous release, to prevent its premature evaporation, and to protect it from degradation.

A general feature of the effects of diverse volatile-emitting bacteria is that the strong biological activity observed upon exposure to the complex volatile blend of any given strain is rarely mimicked when applying single, pure volatile molecules. This might be due to the synergetic activity of different components of the volatile mixture, but also to the dynamic changes in the composition of the volatilomes occurring during the different growth phases of the bacterial population. Indeed, even with formulations that would enable continuous or even dynamic release of volatiles, we would still be far away from mimicking the biological variations occurring in the relative concentration of the different volatiles and in the total amount of volatiles emitted over the different growth phases (Kai *et al.*, 2010). Whether differences in relative abundance of specific compounds rather than absolute concentration of single molecules are responsible for the observed biological activity of volatiles remains to be investigated. Similarly, perception of increasing and/or decreasing concentrations of the same molecule might be necessary to trigger effects in plants exposed to volatiles. These aspects cannot be studied using the simplified experimental setup used in most studies; namely, divided Petri dishes in which the volatiles are released almost instantaneously into the atmosphere of the dish.

Finally, one major requirement of any plant-protection product – volatile or nonvolatile – is its ability to affect specifically its target without harming other, so-called 'nontarget' organisms. Hence, volatiles interfering with developmental stages or processes that are specific to the target pathogen should be preferred to those acting on widespread pathways, such as the respiratory chain (e.g. HCN). This highlights the need for more fundamental studies investigating the mode of action of efficient volatiles. Studies comparing the response of different target organisms to the same volatile blend revealed substantial differences in sensitivity (Vespermann *et al.*, 2007; Groenhagen *et al.*, 2013; Hunziker *et al.*, 2015). This suggests that, in principle, volatiles could specifically affect plant pathogens without exerting broad inhibitory effect on nontarget organisms. However, several studies provided evidence that a single class or even a single volatile could have differing or even opposing effects on different organisms (van Dam *et al.*, 2016). One such example is the intensively studied 2,3-butanediol. In addition to

triggering growth promotion and resistance in plants, this compound also acts as a virulence factor for many phytopathogenic bacteria belonging to the Enterobacteriaceae family, such as *Dickeya*, *Pectobacterium*, or *Erwinia* (reviewed in Audrain *et al.*, 2015). One first step in the analysis of the selectivity of putative disease-inhibiting volatiles is to test their innocuousness to plants themselves. Whereas the inorganic volatiles ammonia and HCN are phytotoxic, many other volatiles, such as acetophenone or different S-containing volatiles, which significantly reduced the growth of pathogenic fungi, did not negatively affect plants (Groenhagen *et al.*, 2013). However, whether these volatiles would also be harmless to more closely related organisms, such as mycorrhizal or saprophytic fungi, is largely unknown and warrants further research.

2. Challenges in the application of volatile-emitting strains

Many of the aforementioned limitations to the use of single volatiles could be overcome by applying the strains emitting the volatiles rather than the pure compounds. The synergetic effects of the volatile mixture, the natural variations in the composition, and the relative abundance of the different components of the blends, as well as the dynamic changes in overall concentrations of bacterial volatiles, would be preserved with an approach based on strain rather than pure volatile application. However, other challenges arise with such inoculation strategies. The first prerequisite for a strain inoculated either in the rhizosphere or the phyllosphere is to establish in sufficient population densities in these highly competitive environments. Once a strain is established, the main difficulty lies in ensuring that the plant health-protecting volatiles will indeed be emitted *in situ*.

Very few studies have addressed the capacity of bacteria to emit disease-inhibiting volatiles in experimental setups that come closer to natural conditions. Most of these studies have focused on the soil and rhizosphere. The ability of bacteria to emit antifungal volatiles when inoculated into soil or sand supplemented with artificial root exudates in very low concentrations brought the proof of concept that bacterial volatile emission is not restricted to the very artificial, nutrient-rich media commonly used in laboratories (Garbeva *et al.*, 2014a; Schulz-Bohm *et al.*, 2015). Likewise, significant growth promotion was observed in Arabidopsis exposed to volatiles emitted by bacteria that grew on a very nutrient-poor medium mimicking bulk soil conditions (Angle *et al.*, 1991; Blom *et al.*, 2011a). These few reports indicate that the soil could offer favourable chemical and physical conditions for the emission of bioactive volatiles by introduced bacteria. Moreover, such volatile emission might be stronger in the rhizosphere, where abundant organic C supply is expected to boost bacterial metabolism. Beyond this general metabolic boost, plant roots are likely to play an essential role in shaping the rhizosphere microbial volatile landscape. By altering their root exudate composition, they might modulate the relative abundance of specific precursors of volatile biosynthetic pathways, thereby leading to altered volatile emission by root-associated bacteria. For example, increased tryptophan exudation could lead to higher indole emission, or increased methionine exudation could lead to higher emission of S-
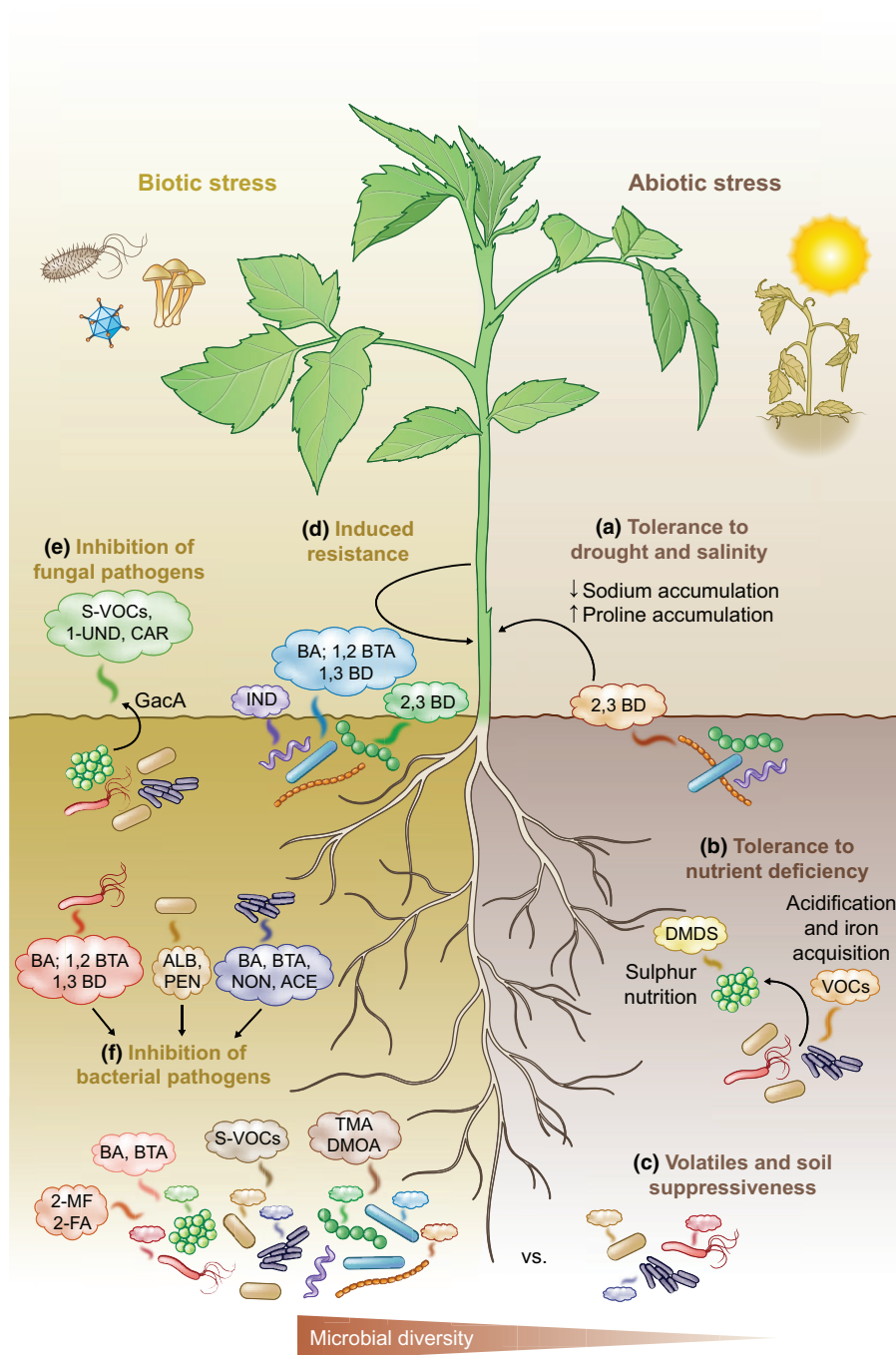
Fig. 1 Beneficial effects of bacterial volatiles on plant health. Bacterial volatiles conferring tolerance to abiotic stress are depicted on the right side of the plant, whereas those conferring tolerance to biotic stress are depicted on the left side of the plant. (a) Tolerance to drought and salinity mediated by 2,3-butanediol (2,3 BD) has been linked to both decrease of internal sodium levels and increase of proline accumulation. (b) Alleviation of nutrient deficiency mediated by complex volatile blends was shown to originate from acidification leading to increased uptake of iron. Furthermore, dimethyl disulphide (DMDS) can serve as source of sulphur. (c) Soil suppressiveness is also mediated by bacterial volatiles. The complexity and diversity of soil bacterial communities is an important determinant of the emission of disease-suppressive volatiles such as 2-methylfuran (2-MF), 2-furaldehyde (2-FA), benzaldehyde (BA), benzothiazole (BTA), trimethylamine (TMA), *N,N*-dimethyloctylamine (DMOA), and sulphur-containing volatiles (S-VOCs). (d) Induced systemic resistance can be triggered by bacterial volatiles such as 2,3 BD, indole (IND), BA, 1,2-benzisothiazol-3(2*H*)-one (1,2 BTA) and 1,3-butanediene (1,3 BD). In addition to inducing resistance in the plants, (e) bacterial volatiles such as S-VOCs, 1-undecene (1-UND), or the terpene caryolan-1-ol (CAR) exhibit direct inhibitory effects on fungal and oomycete pathogens, whereas (f) other volatiles, such as nonanal (NON), acetophenone (ACE), or the sesquiterpenes albaflavenone (ALB) and pentalenolactone (PEN), show direct inhibitory effects on bacterial pathogens.

containing volatiles such as dimethyl disulphide. As already mentioned, the ability of phyllosphere-colonizing bacteria to emit disease-protecting volatiles *in situ* remains to be demonstrated. One study brought indirect proof of such ability: Dandurishvili *et al.* (2011) reported increased dimethyl disulphide emission by tomato plants inoculated with an *S. plymuthica* strain whereas dimethyl disulphide was not detected in noninoculated plants. However, dimethyl disulphide can also be produced by plants, and such experimental setups do not allow the distinguishing of volatiles emitted by the plants from those emitted by the bacteria (Dandurishvili *et al.*, 2011).

Most of our knowledge on the emission of volatiles by plant-associated bacteria originates from laboratory studies on single strains. However, when considering that inoculated strains will integrate into complex rhizosphere and phyllosphere communities, the fact that interspecific interactions can strongly influence volatile emission should be kept in mind (Tyc *et al.*, 2015). A recent study even demonstrated that volatile precursors released by a Gram-positive and a Gram-negative bacterium reacted chemically in the shared headspace of both strains to yield a new volatile (Kai *et al.*, 2018). This fascinating discovery highlights an added layer of complexity originating from purely chemical reactions leading to the occurrence of new compounds, in addition to the biological complexity of microbial interactions. This suggests that the changes in the volatile blend triggered by addition of volatile-emitting strains to the diverse soil, rhizosphere, or phyllosphere communities will be very difficult to predict. Moreover, since numerous volatiles are shared by many species even across significant phylogenetic distance, some redundancy in the ability to emit such volatiles is expected. Therefore, one promising approach could be to try to steer volatile emission by targeted addition of specific organic substrates acting as precursors of the desired volatiles. Along these lines, two studies demonstrated that incorporation to the soil of *Allium* and *Brassica* residues led to increased emission of S-containing volatiles, which resulted in increased disease-suppressing activities against soil-borne fungal pathogens (Wang *et al.*, 2009; Arnault *et al.*, 2013).

V. Conclusions and perspectives

As summarized in this review, bacterial volatiles can help plants cope with abiotic and biotic stresses, through induced systemic resistance or inhibition of different developmental stages of multiple fungal, oomycete, and bacterial pathogens (Fig. 1). So far, most studies have focused on model plants, such as *A. thaliana*, and a couple of microbial volatiles, such as 2,3-butanediol or dimethyl disulphide, have received most attention, despite the high chemical diversity of bacterial volatile blends. A few pioneer studies have demonstrated plant health-protecting effects of bacterial volatiles in glasshouse and field conditions, and they raise hope that bacterial volatiles might be a suitable source of new plant protection products of lesser toxicity for the environment and human health. However, many fundamental questions underlying both the bacterial and the plant sides of the volatile-mediated interaction remain unanswered. We conclude this review by highlighting what we consider the most pressing fundamental questions that need to be investigated in order to advance our basic knowledge of volatile-mediated interactions between plants and their associated bacteria (Box 1).

On the bacterial side, we still know very little about the genetic basis of volatile emission. Except for a few exceptions, we do not yet know how bacterial volatiles are synthesized and whether they pass the membrane(s) via specific transporters or through diffusion. Whether bacterial volatiles are simply waste products of primary metabolism or whether they are specifically induced by environmental cues has been a longstanding matter of debate, but recent evidence of the induction of bacterial sodorifen emission upon exposure to fungal volatiles brought the proof of concept that volatile emission can be induced (Schmidt *et al.*, 2017). Further research efforts are needed to unravel the molecular determinants of bacterial volatile emission. One important question in this context is to what extent plants can influence volatile emission by their associated bacterial microbiota, to promote emission of health-protecting volatiles and reduce emission of deleterious volatiles.

On the plant side, we do not yet know how plants perceive volatiles. Are bacterial volatiles sensed in a similar way to plant volatiles (Koeduka *et al.*, 2018), or do plants perceive volatiles as microbial elicitors? Few studies have investigated whether bacterial volatiles act as microbe-associated molecular patterns and trigger similar responses to canonical elicitors such as flagellin. Such a response has not yet been observed, although the term microbial volatile-associated molecular pattern has been proposed, and some overlap between the plant response to bacterial volatiles and to abiotic stress has been observed (Wenke *et al.*, 2012). By contrast, exposure to some volatiles seems to reduce the responsiveness of plants to such typical elicitors (Blom *et al.*, 2011a). Beyond perception and first reaction to bacterial volatiles, what are the modes of action on both the plants and their enemies ultimately leading to plant health protection? In addition to the modulation of Fe and Na uptake, what are the molecular mechanisms underlying abiotic stress tolerance conferred by bacterial volatiles? What are the

Box 1 Outstanding questions.

How do plants perceive bacterial volatiles?
Can plants distinguish between volatiles emitted by pathogenic and beneficial microorganisms?
What are the modes of action underlying the health-protecting effects of bacterial volatiles?
What are the regulatory pathways and genes involved in bacterial volatile biosynthesis and emission?
Under which ecologically relevant conditions do bacteria produce plant health-protecting volatiles?
Which are the key bacterial volatiles that are crucial for plant health, and by which bacteria are they emitted?
What is the spatial scale of volatile-mediated plant–microbe interactions?
What is the best way of applying volatiles or volatile-emitting strains?
How can we steer/stimulate plant microbiota to emit beneficial volatiles?

pathways leading to volatile-mediated induced resistance against pathogens? Which are the molecular targets in the pathogens themselves that lead to the observed inhibition of mycelial growth, sporulation of fungal and oomycete pathogens, or reduced expression of virulence factors in bacterial pathogens?

In conclusion, the ability of volatile compounds emitted by bacteria to protect plant health against abiotic and biotic stresses is undisputed. However, we need extensive research efforts to advance our basic understanding of the mechanisms underlying these protective effects and to move away from the artificial laboratory to experimental conditions that come closer to the natural plant environment. In addition to the scientific excitement of understanding the volatile-mediated interaction between plants and their associated microbiota, such added knowledge is also needed for better prediction of field efficacy and for smart integration of new protection mechanisms into increasingly sustainable agricultural practices.

ORCID

Paolina Garbeva https://orcid.org/0000-0002-2359-5719
Laure Weisskopf https://orcid.org/0000-0001-8789-3830

References

van Agtmaal M, van Os GJ, Hol WHG, Hundscheid MPJJ, Runia WT, Hordijk CA, de Boer W, Gera Hol WH, Hundscheid MPJJ, Runia WT *et al.* 2015. Legacy effects of anaerobic soil disinfestation on soil bacterial community composition and production of pathogen-suppressing volatiles. *Frontiers in Microbiology* 6: e701.

van Agtmaal M, Straathof AL, Termorshuizen A, Lievens B, Hoffland E, de Boer W. 2018. Volatile-mediated suppression of plant pathogens is related to soil properties and microbial community composition. *Soil Biology and Biochemistry* 117: 164–174.

Ait Barka E, Vatsa P, Sanchez L, Vaillant-Gaveau N, Jacquard C, Klenk H-P, Clément C, Ouhdouch Y, Van Wezel P. 2016. Taxonomy, physiology, and natural products of Actinobacteria. *Microbiology and Molecular Biology Reviews* 80: 1–43.

Angle JS, McGrath SP, Chaney RL. 1991. New culture medium containing ionic concentrations of nutrients similar to concentrations found in the soil solution. *Applied and Environmental Microbiology* 57: 3674–3676.

Arnault I, Fleurance C, Vey F, Du Fretay G, Auger J. 2013. Use of Alliaceae residues to control soil-borne pathogens. *Industrial Crops and Products* 49: 265–272.

Audrain B, Farag MA, Ryu CM, Ghigo JM. 2015. Role of bacterial volatile compounds in bacterial biology. *FEMS Microbiology Reviews* 39: 222–233.

Bailly A, Groenhagen U, Schulz S, Geisler M, Eberl L, Weisskopf L. 2014. The inter-kingdom volatile signal indole promotes root development by interfering with auxin signalling. *The Plant Journal* 80: 758–771.

Banerjee S, Schlaeppi K, van der Heijden MGA. 2018. Keystone taxa as drivers of microbiome structure and functioning. *Nature Reviews Microbiology* 16: 567–576.

Bernier SP, Létoffé S, Delepierre M, Ghigo JM. 2011. Biogenic ammonia modifies antibiotic resistance at a distance in physically separated bacteria. *Molecular Microbiology* 81: 705–716.

Blom D, Fabbri C, Connor EC, Schiestl FP, Klauser DR, Boller T, Eberl L, Weisskopf L. 2011a. Production of plant growth modulating volatiles is widespread among rhizosphere bacteria and strongly depends on culture conditions. *Environmental Microbiology* 13: 3047–3058.

Blom D, Fabbri C, Eberl L, Weisskopf L. 2011b. Volatile-mediated killing of *Arabidopsis thaliana* by bacteria is mainly due to hydrogen cyanide. *Applied and Environmental Microbiology* 77: 1000–1008.

Brader G, Compant S, Mitter B, Trognitz F, Sessitsch A. 2014. Metabolic potential of endophytic bacteria. *Current Opinion in Biotechnology* 27: 30–37.

del Carmen Orozco-Mosqueda M, Macías-Rodríguez LI, Santoyo G, Farías-Rodríguez R, Valencia-Cantero E. 2013. *Medicago truncatula* increases its iron-uptake mechanisms in response to volatile organic compounds produced by *Sinorhizobium meliloti*. *Folia Microbiologica* 58: 579–585.

Carrión VJ, Cordovez V, Tyc O, Etalo DW, de Bruijn I, de Jager VCL, Medema MH, Eberl L, Raaijmakers JM. 2018. Involvement of Burkholderiaceae and sulfurous volatiles in disease-suppressive soils. *ISME Journal* 12: 2307–2321.

Caulier S, Nannan C, Gillis A, Licciardi F, Bragard C, Mahillon J. 2019. Overview of the antimicrobial compounds produced by members of the *Bacillus subtilis* group. *Frontiers in Microbiology* 10: e302.

Chen F, Tholl D, Bohlmann J, Pichersky E. 2011. The family of terpene synthases in plants: a mid-size family of genes for specialized metabolism that is highly diversified throughout the kingdom. *The Plant Journal* 66: 212–229.

Chen X, Köllner TG, Jia Q, Norris A, Santhanam B, Rabe P, Dickschat JS, Shaulsky G, Gershenzon J, Chen F. 2016. Terpene synthase genes in eukaryotes beyond plants and fungi: occurrence in social amoebae. *Proceedings of the National Academy of Sciences, USA* 113: 12132–12137.

Cho G, Kim J, Park CG, Nislow C, Weller DM, Kwak YS. 2017. Caryolan-1-ol, an antifungal volatile produced by *Streptomyces* spp., inhibits the endomembrane system of fungi. *Open Biology* 7: e170075.

Cho SM, Kang BR, Han SH, Anderson AJ, Park J-Y, Lee Y-H, Cho BH, Yang K-Y, Ryu C-M, Kim YC. 2008. 2*R*,3*R*-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* 21: 1067–1075.

Cho SM, Kim YH, Anderson AJ, Kim YC. 2013. Nitric oxide and hydrogen peroxide production are involved in systemic drought tolerance induced by 2*R*,3*R*-butanediol in *Arabidopsis thaliana*. *Plant Pathology Journal* 29: 427–434.

Chuankun X, Minghe M, Leming Z, Keqin Z. 2004. Soil volatile fungistasis and volatile fungistatic compounds. *Soil Biology and Biochemistry* 36: 1997–2004.

Citron CA, Rabe P, Dickschat JS. 2012. The scent of bacteria: headspace analysis for the discovery of natural products. *Journal of Natural Products* 75: 1765–1776.

Clavijo McCormick A, Unsicker SB, Gershenzon J. 2012. The specificity of herbivore-induced plant volatiles in attracting herbivore enemies. *Trends in Plant Science* 17: 303–310.

Cordovez V, Carrion VJ, Etalo DW, Mumm R, Zhu H, van Wezel GP, Raaijmakers JM. 2015. Diversity and functions of volatile organic compounds produced by *Streptomyces* from a disease-suppressive soil. *Frontiers in Microbiology* 6: e1081.

van Dam NM, Weinhold A, Garbeva P. 2016. Calling in the dark: the role of volatiles for communication in the rhizosphere. In: Blande JD, Glinwood R, eds. *Deciphering chemical language of plant communication*. Cham, Switzerland: Springer International Publishing, 175–210.

Dandurishvili N, Toklikishvili N, Ovadis M, Eliashvili P, Giorgobiani N, Keshelava R, Tediashvili M, Vainstein A, Khmel I, Szegedi E *et al.* 2011. Broad-range antagonistic rhizobacteria *Pseudomonas fluorescens* and *Serratia plymuthica* suppress *Agrobacterium* crown gall tumours on tomato plants. *Journal of Applied Microbiology* 110: 341–352.

Effmert U, Kalderas J, Warnke R, Piechulla B. 2012. Volatile mediated interactions between bacteria and fungi in the soil. *Journal of Chemical Ecology* 38: 665–703.

Erb M. 2018. Volatiles as inducers and suppressors of plant defense and immunity – origins, specificity, perception and signaling. *Current Opinion in Plant Biology* 44: 117–121.

Erb M, Veyrat N, Robert CA, Xu H, Frey M, Ton J, Turlings TC. 2015. Indole is an essential herbivore-induced volatile priming signal in maize. *Nature Communications* 6: e6273.

Esmaeel Q, Miotto L, Rondeau M, Leclère V, Clément C, Jacquard C, Sanchez L, Barka EA. 2018. *Paraburkholderia phytofirmans* PsJN-plants interaction: from perception to the induced mechanisms. *Frontiers in Microbiology* 9: e2093.

Flores-Vargas RD, O'Hara GW. 2006. Isolation and characterization of rhizosphere bacteria with potential for biological control of weeds in vineyards. *Journal of Applied Microbiology* 100: 946–954.

Garbeva P, Hol WHGG, Termorshuizen AJ, Kowalchuk GA, De Boer W. 2011. Fungistasis and general soil biostasis – a new synthesis. *Soil Biology and Biochemistry* 43: 469–477.

Garbeva P, Hordijk C, Gerards S, de Boer W. 2014a. Volatiles produced by the mycophagous soil bacterium *Collimonas*. *FEMS Microbiology Ecology* 87: 639–649.

Garbeva P, Hordijk C, Gerards S, De Boer W. 2014b. Volatile-mediated interactions between phylogenetically different soil bacteria. *Frontiers in Microbiology* 5: e289.

Giorgio A, De Stradis A, Lo Cantore P, Iacobellis NS. 2015. Biocide effects of volatile organic compounds produced by potential biocontrol rhizobacteria on *Sclerotinia sclerotiorum*. *Frontiers in Microbiology* 6: e1056.

Gómez-Tenorio MA, Tello JC, Zanón MJ, de Cara M. 2018. Soil disinfestation with dimethyl disulfide (DMDS) to control *Meloidogyne* and *Fusarium oxysporum* f. sp. *radicis-lycopersici* in a tomato greenhouse. *Crop Protection* 112: 133–140.

Grady KL, Sorensen JW, Stopnisek N, Guittar J, Shade A. 2019. Assembly and seasonality of core phyllosphere microbiota on perennial biofuel crops. *Nature Communications* 10: e4135.

Groenhagen U, Baumgartner R, Bailly A, Gardiner A, Eberl L, Schulz S, Weisskopf L. 2013. Production of bioactive volatiles by different *Burkholderia ambifaria* strains. *Journal of Chemical Ecology* 39: 892–906.

Groenhagen U, Maczka M, Dickschat JS, Schulz S. 2014. Streptopyridines, volatile pyridine alkaloids produced by *Streptomyces* sp. FORM5. *Beilstein Journal of Organic Chemistry* 10: 1421–1432.

Haas D, Défago G. 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Reviews Microbiology* 3: 307–319.

Han SH, Lee SJ, Moon JH, Park KH, Yang KY, Cho BH, Kim KY, Kim YW, Lee MC, Anderson AJ *et al.* 2006. GacS-dependent production of 2*R*,3*R*-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* 19: 924–930.

Hare JD. 2011. Ecological role of volatiles produced by plants in response to damage by herbivorous insects. *Annual Review of Entomology* 56: 161–180.

Herrington PR, Craig JT, Sheridan JE. 1987. Methyl vinyl ketone: a volatile fungistatic inhibitor from *Streptomyces griseoruber*. *Soil Biology and Biochemistry* 19: 509–512.

Hol WHG, Garbeva P, Hordijk C, Hundscheid MPJ, Klein Gunnewiek PJA, Van Agtmaal M, Kuramae EE, De Boer W. 2015. Non-random species loss in bacterial communities reduces antifungal volatile production. *Ecology* 96: 2042–2048.

Hora TS, Baker R. 1972. Soil fungistasis: microflora producing a volatile inhibitor. *Transactions of the British Mycological Society* 59: 491–500.

Hung R, Lee S, Bennett JW. 2015. Fungal volatile organic compounds and their role in ecosystems. *Applied Microbiology and Biotechnology* 99: 3395–3405.

Hunziker L, Bönisch D, Groenhagen U, Bailly A, Schulz S, Weisskopf L. 2015. *Pseudomonas* strains naturally associated with potato plants produce volatiles with high potential for inhibition of *Phytophthora infestans*. *Applied and Environmental Microbiology* 81: 821–830.

Innerebner G, Knief C, Vorholt JA. 2011. Protection of *Arabidopsis thaliana* against leaf-pathogenic *Pseudomonas syringae* by *Sphingomonas* strains in a controlled model system. *Applied and Environmental Microbiology* 77: 3202–3210.

Insam H, Seewald MSA. 2010. Volatile organic compounds (VOCs) in soils. *Biology and Fertility of Soils* 46: 199–213.

Isayenkov SV, Maathuis FJM. 2019. Plant salinity stress: many unanswered questions remain. *Frontiers in Plant Science* 10: e80.

Kai M, Crespo E, Cristescu SM, Harren FJM, Francke W, Piechulla B. 2010. *Serratia odorifera*: analysis of volatile emission and biological impact of volatile compounds on *Arabidopsis thaliana*. *Applied Microbiology and Biotechnology* 88: 965–976.

Kai M, Effmert U, Berg G, Piechulla B. 2007. Volatiles of bacterial antagonists inhibit mycelial growth of the plant pathogen *Rhizoctonia solani*. *Archives of Microbiology* 187: 351–360.

Kai M, Effmert U, Lemfack MC, Piechulla B. 2018. Interspecific formation of the antimicrobial volatile schleiferon. *Scientific Reports* 8: e16852.

Kai M, Haustein M, Molina F, Petri A, Scholz B, Piechulla B. 2009. Bacterial volatiles and their action potential. *Applied Microbiology and Biotechnology* 81: 1001–1012.

Kai M, Piechulla B. 2009. Plant growth promotion due to rhizobacterial volatiles – an effect of CO_2? *FEBS Letters* 583: 3473–3477.

Kesarwani M, Hazan R, He J, Que Y, Apidianakis Y, Lesic B, Xiao G, Dekimpe V, Milot S, Deziel E *et al.* 2011. A quorum sensing regulated small volatile molecule reduces acute virulence and promotes chronic infection phenotypes. *PLoS Pathogens* 7: e1002192.

Koeduka T, Matsui K, Hosokawa S, Watanabe H, Nagashima A, Hasezawa S, Ishigami K, Touhara K, Higaki T. 2018. Transcriptional regulators involved in responses to volatile organic compounds in plants. *Journal of Biological Chemistry* 294: 2256–2266.

Kong HG, Shin TS, Kim TH, Ryu C-M. 2018. Stereoisomers of the bacterial volatile compound 2,3-butanediol differently elicit systemic defense responses of pepper against multiple viruses in the field. *Frontiers in Plant Science* 9: e90.

Lazdunski AM, Ventre I, Sturgis JN. 2004. Regulatory circuits and communication in Gram-negative bacteria. *Nature Reviews Microbiology* 2: 581–592.

Ledger T, Rojas S, Timmermann T, Pinedo I, Poupin MJ, Garrido T, Richter P, Tamayo J, Donoso R. 2016. Volatile-mediated effects predominate in *Paraburkholderia phytofirmans* growth promotion and salt stress tolerance of *Arabidopsis thaliana*. *Frontiers in Microbiology* 7: e1838.

Lee B, Farag MA, Park HB, Kloepper JW, Lee SH, Ryu CM. 2012. Induced resistance by a long-chain bacterial volatile: elicitation of plant systemic defense by a C13 volatile produced by *Paenibacillus polymyxa*. *PLoS ONE* 7: e48744.

Lee HH, Molla MN, Cantor CR, Collins JJ. 2010. Bacterial charity work leads to population-wide resistance. *Nature* 467: 82–85.

Lee JH, Lee J. 2010. Indole as an intercellular signal in microbial communities. *FEMS Microbiology Reviews* 34: 426–444.

Li N, Alfiky A, Vaughan MM, Kang S. 2016. Stop and smell the fungi: fungal volatile metabolites are overlooked signals involved in fungal interaction with plants. *Fungal Biology Reviews* 30: 134–144.

Liu X-M, Zhang H. 2015. The effects of bacterial volatile emissions on plant abiotic stress tolerance. *Frontiers in Plant Science* 6: e774.

Mascheroni E, Fuenmayor CA, Cosio MS, Di Silvestro G, Piergiovanni L, Mannino S, Schiraldi A. 2013. Encapsulation of volatiles in nanofibrous polysaccharide membranes for humidity-triggered release. *Carbohydrate Polymers* 98: 17–25.

Meldau DG, Meldau S, Hoang LH, Underberg S, Wünsche H, Baldwin IT, Wunsche H, Baldwin IT. 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.

Meziane H, Van Der Sluis I, Van Loon LC, Höfte M, Bakker PAHM. 2005. Determinants of *Pseudomonas putida* WCS358 involved in inducing systemic resistance in plants. *Molecular Plant Pathology* 6: 177–185.

Müller DB, Vogel C, Bai Y, Vorholt JA. 2016. The plant microbiota: systems-level insights and perspectives. *Annual Review of Genetics* 50: 211–234.

Müller H, Westendorf C, Leitner E, Chernin L, Riedel K, Schmidt S, Eberl L, Berg G. 2009. Quorum-sensing effects in the antagonistic rhizosphere bacterium *Serratia plymuthica* HRO-C48. *FEMS Microbiology Ecology* 67: 468–478.

Ossowicki A, Jafra S, Garbeva P. 2017. The antimicrobial volatile power of the rhizospheric isolate *Pseudomonas donghuensis* P482. *PLoS ONE* 12: e0174362.

Pecchia S, Franceschini A, Santori A, Vannacci G, Myrta A. 2017. Efficacy of dimethyl disulfide (DMDS) for the control of chrysanthemum *Verticillium* wilt in Italy. *Crop Protection* 93: 28–32.

Peñuelas J, Asensio D, Tholl D, Wenke K, Rosenkranz M, Piechulla B, Schnitzler JP, Penuelas J, Asensio D, Tholl D *et al.* 2014. Biogenic volatile emissions from the soil. *Plant, Cell & Environment* 37: 1866–1891.

Piechulla B, Lemfack M-C, Kai M. 2017. Effects of discrete bioactive microbial volatiles on plants and fungi. *Plant, Cell & Environment* 40: 2042–2067.

Pieterse CMJ, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SCM. 2011. Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology* 28: 489–521.

Pieterse CMJ, Zamioudis C, Berendsen RL, Weller DM, Van Wees SCMM, Bakker PAHMHM, Pieterse MJ, Zamioudis C, Berendsen RL, Weller DM *et al.* 2014. Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology* 52: 347–375.

Rajer FU, Wu H, Xie Y, Xie S, Raza W, Tahir HAS, Gao X. 2017. Volatile organic compounds produced by a soil-isolate, *Bacillus subtilis* FA26 induce adverse ultra-structural changes to the cells of *Clavibacter michiganensis* ssp. *sepedonicus*, the causal agent of bacterial ring rot of potato. *Microbiology* 163: 523–530.

Raza W, Ling N, Yang L, Huang Q, Shen Q. 2016. Response of tomato wilt pathogen *Ralstonia solanacearum* to the volatile organic compounds produced by a biocontrol strain *Bacillus amyloliquefaciens* SQR-9. *Scientific Reports* 6: e24856.

Riedlmeier M, Ghirardo A, Wenig M, Knappe C, Koch K, Georgii E, Dey S, Parker JE, Schnitzler J-P, Vlot C. 2017. Monoterpenes support systemic acquired resistance within and between plants. *Plant Cell* 29: 1440–1459.

Ritpitakphong U, Falquet L, Vimoltust A, Berger A, Métraux J-PP, L'Haridon F. 2016. The microbiome of the leaf surface of Arabidopsis protects against a fungal pathogen. *New Phytologist* 210: 1033–1043.

Ryu C-M, Farag MA, Hu C-H, Reddy MS, Kloepper JW, Paré PW. 2004. Bacterial volatiles induce systemic resistance in Arabidopsis. *Plant Physiology* 134: 1017–1026.

Ryu CM, Farag MA, Hu CH, Reddy MS, Wei HX, Paré PW, Kloepper JW. 2003. Bacterial volatiles promote growth in *Arabidopsis*. *Proceedings of the National Academy of Sciences, USA* 100: 4927–4932.

Schmidt R, de Jager V, Zühlke D, Wolff C, Bernhardt J, Cankar K, Beekwilder J, van Ijcken W, Sleutels F, de Boer W *et al.* 2017. Fungal volatile compounds induce production of the secondary metabolite sodorifen in *Serratia plymuthica* PRI-2C. *Scientific Reports* 7: e862.

Schöller CEG, Gürtler H, Pedersen R, Molin S, Wilkins K. 2002. Volatile metabolites from Actinomycetes. *Journal of Agricultural and Food Chemistry* 50: 2615–2621.

Schulz-Bohm K, Zweers H, de Boer W, Garbeva P. 2015. A fragrant neighborhood: volatile mediated bacterial interactions in soil. *Frontiers in Microbiology* 6: e1212.

Schulz S, Dickschat JS. 2007. Bacterial volatiles: the smell of small organisms. *Natural Product Reports* 24: 814–842.

Sharifi R, Ryu CM. 2016. Are bacterial volatile compounds poisonous odors to a fungal pathogen *Botrytis cinerea*, alarm signals to *Arabidopsis* seedlings for eliciting induced resistance, or both? *Frontiers in Microbiology* 7: e196.

Song GC, Riu M, Ryu C-M. 2019. Beyond the two compartments Petri-dish: optimising growth promotion and induced resistance in cucumber exposed to gaseous bacterial volatiles in a miniature greenhouse system. *Plant Methods* 15: e9.

Song GC, Ryu CM. 2013. 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* 14: 9803–9819.

Tahir HAS, Gu Q, Wu H, Niu Y, Huo R, Gao X. 2017. *Bacillus* volatiles adversely affect the physiology and ultra-structure of *Ralstonia solanacearum* and induce systemic resistance in tobacco against bacterial wilt. *Scientific Reports* 7: e40481.

Tait E, Perry JD, Stanforth SP, Dean JR. 2014. Use of volatile compounds as a diagnostic tool for the detection of pathogenic bacteria. *TrAC – Trends in Analytical Chemistry* 53: 117–125.

Tetzlaff CN, You Z, Cane DE, Takamatsu S, Omura S, Ikeda H. 2006. A gene cluster for biosynthesis of the sesquiterpenoid antibiotic pentalenolactone in *Streptomyces avermitilis*. *Biochemistry* 45: 6179–6186.

Tsuda K, Somssich IE. 2015. Transcriptional networks in plant immunity. *New Phytologist* 206: 932–947.

Tyagi S, Mulla SI, Lee KJ, Chae JC, Shukla P. 2018. VOCs-mediated hormonal signaling and crosstalk with plant growth promoting microbes. *Critical Reviews in Biotechnology* 38: 1277–1296.

Tyc O, de Jager VCL, van den Berg M, Gerards S, Janssens TKS, Zaagman N, Kai M, Svatos A, Zweers H, Hordijk C *et al.* 2017. Exploring bacterial interspecific interactions for discovery of novel antimicrobial compounds. *Microbial Biotechnology* 10: 910–925.

Tyc O, Zweers H, de Boer W, Garbeva P. 2015. Volatiles in inter-specific bacterial interactions. *Frontiers in Microbiology* 6: e1412.

Ulloa-Benítez Medina-Romero YM, Sánchez-Fernández RE, Lappe-Oliveras P, Roque-Flores G, Duarte Lisci G, Herrera Suárez T, Macías-Rubalcava ML. 2016. Phytotoxic and antimicrobial activity of volatile and semi-volatile organic compounds from the endophyte *Hypoxylon anthochroum* strain Blaci isolated from *Bursera lancifolia* (Burseraceae). *Journal of Applied Microbiology* 121: 380–400.

Vaishnav A, Kumari S, Jain S, Varma A, Choudhary DK. 2015. Putative bacterial volatile-mediated growth in soybean (*Glycine max* L. Merrill) and expression of induced proteins under salt stress. *Journal of Applied Microbiology* 119: 539–551.

Vespermann A, Kai M, Piechulla B. 2007. Rhizobacterial volatiles affect the growth of fungi and *Arabidopsis thaliana*. *Applied and Environmental Microbiology* 73: 5639–5641.

Vorholt JA. 2012. Microbial life in the phyllosphere. *Nature Reviews Microbiology* 10: 828–840.

De Vrieze M, Pandey P, Bucheli TD, Varadarajan AR, Ahrens CH, Weisskopf L, Bailly A. 2015. Volatile organic compounds from native potato-associated *Pseudomonas* as potential anti-oomycete agents. *Frontiers in Microbiology* 6: e1295.

Wang C, Wang Z, Qiao X, Li Z, Li F, Chen M, Wang Y, Huang Y, Cui H. 2013. Antifungal activity of volatile organic compounds from *Streptomyces alboflavus* TD-1. *FEMS Microbiology Letters* 341: 45–51.

Wang D, Rosen C, Kinkel L, Cao A, Tharayil N, Gerik J. 2009. Production of methyl sulfide and dimethyl disulfide from soil-incorporated plant materials and implications for controlling soilborne pathogens. *Plant and Soil* 324: 185–197.

Weise T, Kai M, Piechulla B. 2013. Bacterial ammonia causes significant plant growth inhibition. *PLoS ONE* 8: e63538.

Weisskopf L. 2013. The potential of bacterial volatiles for crop protection against phytophathogenic fungi. In: Méndez-Vilas A, ed. *Microbial pathogens and strategies for combating them: science, technology and education*. Bardajoz, Spain: Formatex Research Center, 1352–1363.

Weller DM, Raaijmakers JM, Gardener BBM, Thomashow LS. 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology* 40: 309–348.

Wenke K, Wanke D, Kilian J, Berendzen K, Harter K, Piechulla B. 2012. Volatiles of two growth-inhibiting rhizobacteria commonly engage AtWRKY18 function. *The Plant Journal* 70: 445–459.

Wu Y, Yuan J, E Y, Raza W, Shen Q, Huang Q. 2015. Effects of volatile organic compounds from *Streptomyces albulus* NJZJSA2 on growth of two fungal pathogens. *Journal of Basic Microbiology* 55: 1104–1117.

Zarraonaindia I, Owens SM, Weisenhorn P, West K, Hampton-Marcell J, Lax S, Bokulich NA, Mills DA, Martin G, Taghavi S *et al.* 2015. The soil microbiome influences grapevine-associated microbiota. *mBio* 6: e02527-14.

Zeller SL, Brandl H, Schmid B. 2007. Host-plant selectivity of rhizobacteria in a crop/weed model system. *PLoS ONE* 2: e846.

Zhang H, Kim M-S, Sun Y, Dowd SE, Shi H, Paré PW. 2008. Soil bacteria confer plant salt tolerance by tissue-specific regulation of the sodium transporter HKT1. *Molecular Plant–Microbe Interactions* 21: 737–744.

Zhang H, Sun Y, Xie X, Kim MS, Dowd SE, Paré PW. 2009. A soil bacterium regulates plant acquisition of iron via deficiency-inducible mechanisms. *The Plant Journal* 58: 568–577.

Zhao B, Lin X, Lei L, Lamb DC, Kelly SL, Waterman MR, Cane DE. 2008. Biosynthesis of the sesquiterpene antibiotic albaflavenone in *Streptomyces coelicolor* A3(2). *Journal of Biological Chemistry* 283: 8183–8189.