

1 Harnessing microbial volatiles to replace 2 pesticides and fertilizers

3 Gareth Thomas^{1,2,3}, David Withall¹, Michael Birkett¹

4 1. Biointeractions and Crop Protection Department, Rothamsted Research, Harpenden,
5 Hertfordshire, AL5 2JQ, UK

6 2. School of Biosciences, College of Life and Environmental Sciences, University of
7 Exeter, Exeter, EX4 4QD, UK

8 3. Current address: Microbiomes, Microbes and Informatics Group, Organisms and
9 Environment Division, School of Biosciences, Cardiff University, Cardiff, Wales, CF10
10 3AX, UK

11 Corresponding author: Gareth Thomas; electronic mail: ThomasG61@cardiff.ac.uk

12 Summary

13 **Global agricultural systems are under increasing pressure to deliver sufficient, healthy**
14 **food for a growing population. Seasonal inputs, including synthetic pesticides and**
15 **fertilizers, are applied to reduce losses by pathogens, and enhance crop biomass,**
16 **although their production and application can also incur several economic and**
17 **environmental penalties. New solutions are therefore urgently required to enhance crop**
18 **yield whilst reducing dependence on these seasonal inputs. Volatile Organic**
19 **Compounds (VOCs) produced by soil microorganisms may provide alternative**
20 **solutions, due to their ability to inhibit fungal pathogens, induce plant resistance**
21 **against pathogens, and enhance plant growth promotion. This review will highlight**
22 **recent advances in our understanding of these biological activities of microbial VOCs**
23 **(mVOCs), providing perspectives on research required to develop them into viable**
24 **alternatives to current unsustainable seasonal inputs. This can identify potential new**

25 **avenues for mVOC research and stimulate discussion across the academic community**
26 **and agri-business sector.**

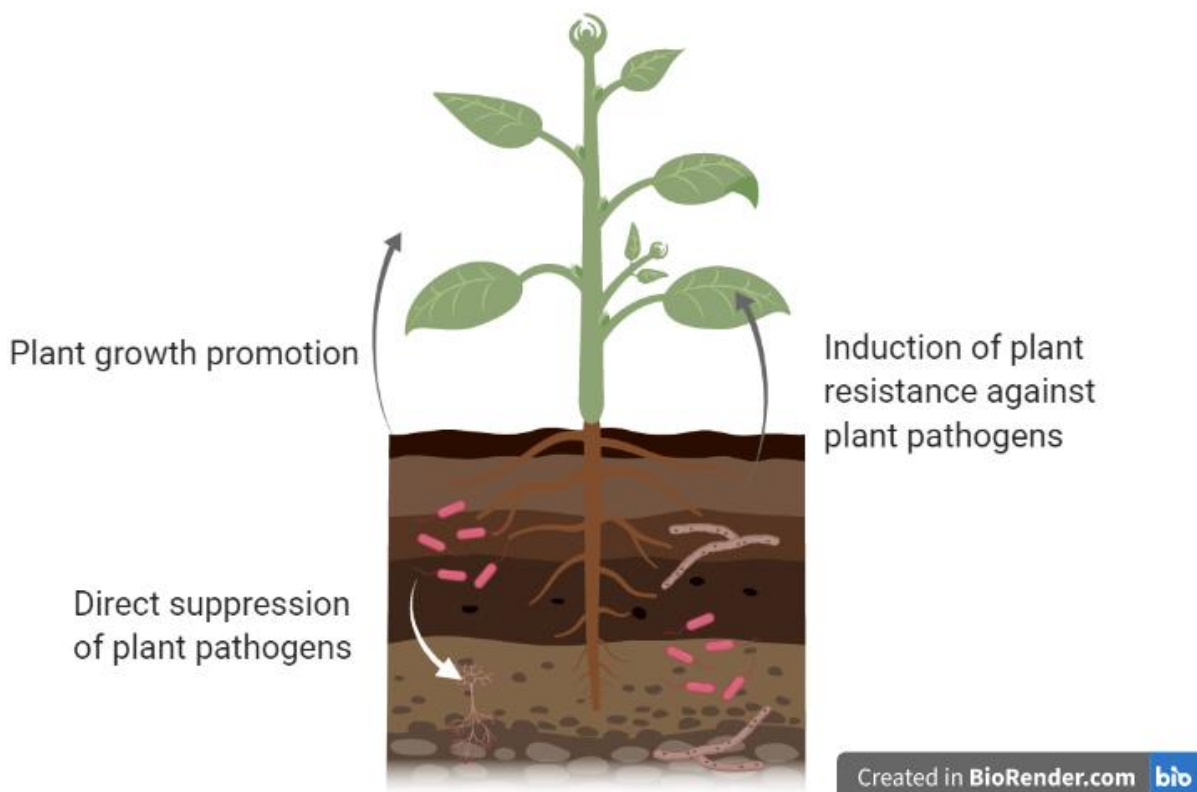
27 **Introduction**

28 By 2100, the United Nations projects that the global population will grow by around 4 billion,
29 which may require agricultural production to double or triple to keep pace with population
30 increases (United Nations, 2017; Rohr et al., 2019). To date, agricultural practice has relied
31 on the application of synthetic chemical inputs to optimise crop yields, including synthetic
32 pesticides, which reduce crop losses by targeting plant pathogens, and synthetic fertilizers,
33 applied to increase crop biomass. Synthetic pesticides play a critical role in mitigating crop
34 damage by pathogens, which are responsible for annual crop losses of 17-30% for the five
35 major crops (Savary et al., 2019). The development of synthetic pesticides is in itself
36 unsustainable, estimated to cost \$250 million to bring a single active ingredient to market, with
37 an estimated success rate of 1 in 140,000 synthesised compounds (Lamberth et al., 2013).
38 Moreover, the over-application of pesticides can lead to the development of pesticide
39 resistance, rendering them less effective. The production and application of inorganic nitrogen
40 fertilizer has resulted in crop production being the largest cause of human alteration to the
41 global nitrogen cycle (Smil, 1999). The Haber-Bosch process is used to produce inorganic
42 nitrogen fertilizer, through the conversion of hydrogen and nitrogen into ammonia. However,
43 the process is energy intensive, occurring at high temperatures and pressure, generating a
44 carbon footprint contributing ~1.2% of overall global anthropogenic CO₂ emissions (Nørskov
45 and Chen, 2016). Furthermore, the application of inorganic nitrogen to soils leads to enhanced
46 microbial production of nitrous oxide (N₂O), the potent greenhouse gas, through soil microbial
47 nitrification and de-nitrification. As such, concentrations of N₂O have substantially increased
48 in the atmosphere since 1960 as a direct result of fertilizer applications (Davidson, 2009). With
49 projected increases in crop demand, agricultural expansion could result in approximately 10-
50 fold increases in pesticide use, and 2.7-fold increases in fertilizer application (Rohr et al.,
51 2019). Concerted efforts should therefore be made to develop more sustainable control

52 methods to reduce over-reliance on synthetic fertilizer and pesticides, through shifts in
53 agronomic practice (Fisher et al., 2018; Tester et al., 2010). Whilst genetically modified crops
54 demonstrating enhanced disease resistance show potential to reduce pathogen damage and
55 could potentially reduce the requirement for pesticide inputs, the regulatory frameworks
56 required to commercialise the crops are lengthy and cumbersome (Kanchiswamy et al., 2015).
57 Therefore, it is an opportune time to explore alternative control strategies to chemical control
58 or genetic modification.

59 One alternative solution to chemical inputs is through the addition of antagonistic, beneficial,
60 microorganisms, due to their ability to antagonise pathogenic soil microbes, and enhance plant
61 biomass. Soil microorganisms produce a wide spectrum of secondary metabolites enabling
62 them to compete with neighbouring microorganisms, which they have likely evolved to
63 compete for the same resources within soil (Brakhage and Schroeckh, 2011; Garbeva and
64 Weisskopf, 2020). For example, bacteria from the genus of soil-dwelling *Streptomyces* spp.
65 produce a diverse range of secondary metabolites, which have been exploited for human
66 medicine, with approximately 80% of antibiotics currently being sourced from the genus (de
67 Lima Procópio et al., 2012). The structural diversity of secondary metabolites explains their
68 broad spectrum of activities, including mediating communication intra- and inter-specifically,
69 defence against competitors, nutrient acquisition, and symbiotic interactions (Spiteller, 2015;
70 Macheleidt et al., 2016). Whilst most research on microbial secondary metabolites focusses
71 on non-volatile compounds, increasing attention is being paid to microbial volatile organic
72 compounds (mVOCs). VOCs are a class of secondary metabolites with a low molecular weight
73 (< 300 Da), high vapour pressure, and low boiling points, which tend to be lipophilic in nature
74 (Schulz-Bohm et al., 2017). Their ability to diffuse through gas and water-filled pores within
75 the heterogenous soil matrix make them suitable for both short and long-distance signalling
76 (Maffei et al., 2011). Under competitive soil conditions, due to the presence of other competing
77 organisms, VOCs are important for antibiosis and signalling for symbiotic interactions (Efmert
78 et al., 2012). The capability of mVOCs to suppress neighbouring pathogens and signal to

79 plants demonstrates their potential to be exploited as alternatives to chemical fertilizers and
80 pesticides, which could provide a more sustainable solution, as well as having negligible
81 hazardous effects on animals and the environment (Tilocca et al., 2020). This review focuses
82 on the role of mVOCs in maintaining plant health, through the direct suppression of plant
83 pathogens, the induction of plant resistance against pathogens, and the promotion of plant
84 growth (Figure 1), highlighting their potential as alternative solutions to synthetic pesticides
85 and fertilizers.



86

87 Figure 1 | Overview of the biological activities of microbial Volatile Organic Compounds
88 (mVOCs).

89

90

91

92

93 **Role of volatiles in the suppression of plant pathogens.**

94 Several studies demonstrate mVOCs can inhibit a range of plant pathogens, highlighting their
95 suitability as a potential sustainable alternative to pesticides. One of the first examples
96 demonstrating an inhibitory role for mVOCs against plant pathogens were those produced by
97 *Pseudomonas* species isolated from soybean and canola, in the inhibition of *Sclerotinia*
98 *sclerotiorum*; a fungal pathogen with a broad host range of over 400 plant species (Fernando
99 et al., 2005). Of 23 VOCs identified from *Pseudomonas* species, six significantly reduced
100 mycelial growth of *S. sclerotiorum*. Similarly, VOC production by two strains of *Bacillus*
101 endophytes significantly reduced the weight and number of the vegetative, long-term survival
102 structures (sclerotia) of *S. sclerotiorum* (Massawe et al., 2018). VOCs from *Burkholderia*
103 *ambifaria* (Groenhagen et al., 2013) and a range of other rhizobacterial isolates (Velivelli et
104 al., 2015) have demonstrated the ability to inhibit growth of the ubiquitous soil-borne pathogen
105 *Rhizoctonia solani*. MVOCs can also display inhibitory activity against bacterial pathogens.
106 Exposure of *Clavibacter michiganensis*, the causal agent of bacterial ring rot of potato, to
107 VOCs from *Bacillus subtilis* led to significant inhibition of pathogen growth, with benzaldehyde,
108 nonanal, benzothiozole and acetophenone specifically demonstrating inhibitory activities
109 (Rajer et al., 2017). *Bacillus* VOCs also inhibited the growth of *Xanthomonas oryzae*, the
110 causal agent of bacterial leaf blight of rice, with decyl acetate and 3,5,5-trimethylhexanol
111 specifically inhibiting pathogen growth (Xie et al., 2018). As well as inhibition against fungal
112 and bacterial pathogens, mVOCs can display inhibitory activity against pathogenic oomycetes.
113 Exposure of *Phytophthora capsici* to *Bacillus* and *Acinetobacter* VOCs significantly reduced
114 mycelial growth of the oomycete, with 3-methylbutanol, isovaleraldehyde, isovaleric acid, 2-
115 ethylhexanol and 2-heptanone showing specific inhibitory roles (Syed-Ab-Rahman et al.,
116 2019). VOCs produced by *Nodulisporium* also demonstrated anti-oomycete activity against
117 several *Pythium* species, although the causal VOCs involved in this inhibition were not
118 individually assayed (Sánchez-Fernández et al., 2016). Taken together, mVOCs can display
119 inhibitory activity against a range of fungal, bacterial and oomycete pathogens, which could

120 have biotechnological potential as alternatives to pesticides. A summary of the individual
121 VOCs involved in pathogen suppression from the studies discussed is provided in Table 1.

122 Due to the presence of a chiral centre, 1-octen-3-ol has two optical isomers; (*R*)-(-)-1-octen-
123 3-ol and (*S*)-(+)-1-octen-3-ol. Interestingly, when these optical isomers were investigated for
124 inhibitory roles against the fruit spoilage pathogen *Penicillium chrysogenum*, (*R*)-(-)-1-octen-
125 3-ol inhibited spore germination of five out of seven isolates, whereas (*S*)-(+)-1-octen-3-ol
126 inhibited spore germination of only two isolates, suggesting the different enantiomers display
127 differences in inhibitory activities (Yin et al., 2019). Furthermore, (*R*)-(-)-1-octen-3-ol
128 modulated the transcription of a greater number of genes in *Penicillium chrysogenum*. This
129 highlights an important consideration in the specificity of mVOCs for target pathogens,
130 providing a potential avenue for future research in the investigation of the bioactivity of chiral
131 VOCs, as well as providing chemical structural information for the development of active
132 substances to replace pesticides.

133 Whilst the role of mVOCs in the suppression of plant pathogens is well established, the
134 molecular mechanisms involved in their inhibitory activities is receiving increasing attention.
135 When exposed to *Bacillus* VOCs, the tomato wilt pathogen *Ralstonia solanacearum* showed
136 a reduction in the expression of a range of virulence factor genes, including those related to
137 chemotaxis, type 3 and type 4 secretion systems, and extracellular polysaccharides, as well
138 as increasing the expression of a global virulence factor (Tahir et al., 2017a). Specifically,
139 benzaldehyde, 1,2-benzisothiazol-3(2H)-one and 1,3-butadiene, which were identified
140 *Bacillus* VOCs, were involved in the modulation of virulence factor expression of the pathogen.
141 Similarly, expression of genes involved in virulence and biofilm formation in *Xanthomonas*
142 *oryzae* were also downregulated upon exposure to *Bacillus* VOCs (Xie et al., 2018). VOCs
143 produced by *Streptomyces alboflavus* inhibited production of aflatoxin from the fungal
144 pathogen *Aspergillus flavus*, through the downregulation of several genes involved in aflatoxin
145 biosynthesis (Yang et al., 2019). Exposure of *Sclerotinia sclerotiorum* to VOCs produced by
146 *Trichoderma* species led to the upregulation of four glutathione S-transferase genes, which

147 are involved in the detoxification of antifungal secondary metabolites, which may contribute to
148 the virulence of *Sclerotinia sclerotiorum* (Ojaghian et al., 2019). Sphingolipid metabolic
149 processes, vesicle formation and trafficking, and membrane localisation were all disrupted
150 upon exposure of *Botrytis cinerea* to the *Streptomyces*-derived VOC caryolan-1-ol (Cho et al.,
151 2017). These findings suggest inhibitory VOCs can mediate virulence factor expression of
152 target pathogens, as well as suppress metabolic processes, including the biosynthesis of
153 toxins. Whilst the modes of action underpinning pathogen suppression by mVOCs are
154 receiving increasing attention, a greater understanding of their molecular targets across a
155 broader range of pathogenic microorganisms is critical prior to their deployment into open
156 fields.

157

158

159

160

161

162

163

164

165

166

167

168

169

170 **Table 1 | Summary of mVOC producing stains, their active VOCs and their target**
 171 **pathogens.**

VOC producing strain	Active VOCs	Target pathogen	Reference
<i>Pseudomonas fluorescens</i>	Benzothiazole	<i>Sclerotinia sclerotiorum</i>	Fernando et al., 2005
<i>Pseudomonas chloroaphis</i>	Cyclohexanol		
<i>Pseudomonas aurantiaca</i>	n-Decanal		
	Dimethyl trisulfide		
	2-Ethyl 1-hexanol		
	Nonanal		
<i>Burkholderia ambifaria</i>	2-Undecanone	<i>Rhizoctonia solani</i>	Groenhagen et al., 2013
	4-Octanone	<i>Alternaria alternata</i>	
	Dimethyl trisulfide		
	S-Methyl methanethiosulfonate		
	2-Propylacetophenone		
	Dimethyl disulfide		
Rhizobacterial isolates	2,4-Di-tert-butylphenol	<i>Rhizoctonia solani</i>	Velivelli et al., 2015
	2-Hexen-1-ol		
<i>Nodulisporium</i> sp. GS4dl1a	Not identified	<i>Pythium aphanidermatum</i>	Sánchez-Fernández et al., 2016
<i>Bacillus amyloliquefaciens</i> FZB42,	Benzaldehyde	<i>Ralstonia solanacearum</i>	Tahir et al., 2017a
<i>Bacillus artrophaeus</i> LSSC22	1,2-Benzisothiazol-3(2H)-one		
	1,3-Butadiene		
<i>Bacillus subtilis</i> FA26	Benzaldehyde	<i>Clavibacter michiganensis</i>	Rajer et al., 2017
	Nonanal		
	Benzothiazole		
	Acetophenone		
<i>Streptomyces</i> spp.	Caryolan-1-ol	<i>Botrytis cinerea</i>	Cho et al., 2017
<i>Bacillus</i> spp. (VM10, VM11, VM42)	2-Undecanone	<i>Sclerotinia sclerotiorum</i>	Massawe et al., 2018
	1,3-Butadiene		
	Benzothiazole		
	N,N-Dimethyldodecylamine		
<i>Bacillus</i> strain D13	Decyl alcohol	<i>Xanthomonas oryzae</i>	Xie et al., 2018
	3,5,5-Trimethylhexanol		
<i>Bacillus amyloliquefaciens</i> UQ154	3-Methylbutanol	<i>Phytophthora capsici</i>	Syed-Ab-Rahman et al., 2019
<i>Bacillus velezensis</i> UQ156	Isovaleraldehyde		
<i>Acinetobacter</i> spp. UQ202	Isovaleric acid		
	2-Ethylhexanol		
	2-Heptanone		
<i>Penicillium expansum</i>	(R)-(-)-1-Octen-3-ol	<i>Penicillium chrysogenum</i>	Yin et al., 2019
<i>Penicillium solitum</i>			
<i>Penicillium paneum</i>			
<i>Streptomyces alboflavus</i>	Not identified	<i>Aspergillus flavus</i>	Yang et al., 2019
<i>Trichoderma</i> spp.	Not identified	<i>Sclerotinia sclerotiorum</i>	Ojaghian et al., 2019

172

173 **Role of volatiles in induced resistance**

174 As well as directly inhibiting plant pathogens, mVOCs can also induce plant resistance to
175 pathogens, reducing their susceptibility to plant diseases. This was first observed by Ryu and
176 colleagues (2004), who exposed *Arabidopsis thaliana* seedlings to *Bacillus subtilis* VOCs,
177 which reduced the severity of symptoms by the soft-rot causing bacterial pathogen *Erwinia*
178 *carotovora*. Seedlings exposed to VOCs produced by strains deficient in 2,3-butanediol and
179 acetoin biosynthesis developed greater disease symptoms relative to wild-type strain VOCs,
180 suggesting a specific role for these VOCs in induced systemic resistance. These findings have
181 been extended under greenhouse conditions, where exposure of cucumber to 2,3-butanediol
182 led to enhanced resistance against the bacterial pathogen *Pseudomonas syringae* (Song et
183 al., 2019b). Interestingly, specificity in the ability of the different isomers of 2,3-butanediol to
184 induce plant resistance have also been observed, with (2*R*, 3*R*)-butanediol inducing
185 resistance of tobacco against *Erwinia carotovorus*, whereas (2*S*, 3*S*)-butanediol was
186 ineffective at inducing resistance (Han et al., 2006). Whilst most work on mVOCs in induced
187 resistance has focussed on 2,3-butanediol and acetoin, 3-pentanol and 2-butanone have also
188 been shown to induce resistance of cucumber against *Pseudomonas syringae*, and albuterol
189 and 1,3-butadiene play a role in the induction of resistance of tobacco against *Ralstonia*
190 *solanacearum* (Song and Ryu., 2013; Tahir et al., 2017b). As stomata can act as entry points
191 for bacterial invasion, mVOCs may induce stomatal closure to reduce pathogen
192 internalisation. This was investigated by Wu and colleagues (2018), who demonstrated that
193 exposure of *A. thaliana* and tobacco to 2,3-butanediol and acetoin induced stomatal closure,
194 although the influence of stomatal closure on pathogen establishment was not determined.

195 Fungal VOCs have also demonstrated a role in inducing plant resistance against pathogens.
196 *A. thaliana* seedlings exposed to *Trichoderma virens* VOCs demonstrated significantly
197 reduced disease symptoms when inoculated with *Botrytis cinerea*, and symptoms were
198 greater in seedlings exposed to *Trichoderma virens* VOCs deficient in sesquiterpene
199 production, suggesting induced resistance could be attributed to the production of

200 sesquiterpenes (Contreras-Cornejo et al., 2014). Exposure of *A. thaliana* seedlings to 6-
201 pentyl-2H-pyran-2-one, a VOC commonly produced by a range of *Trichoderma* species (Jeleń
202 et al., 2014), demonstrated significant reductions in disease symptoms when subsequently
203 inoculated with the fungal pathogens *Botrytis cinerea* and *Alternaria brassicicola* (Kottb et al.,
204 2015). 1-Octen-3-ol, another commonly reported fungal-derived VOC, elicited *A. thaliana*
205 defence responses against *Botrytis cinerea*, although as this was tested as a racemic mixture,
206 the role of the two optical isomers of 1-octen-3-ol in induced resistance cannot be discerned
207 (Kishimoto et al., 2007). More recently, VOC production from archaea (*Nitrosocosmicus*
208 *oleophilus*), which have received little attention relative to bacteria and fungi, have also been
209 shown to induce systemic resistance of *A. thaliana* against *Pseudomonas syringae* and
210 *Pectobacterium carotovorum*; a necrotrophic bacterium responsible for soft-rot of a range of
211 vegetables (Song et al., 2019a). This suggests the biotechnological potential for mVOCs in
212 sustainable agriculture is not limited to bacteria and fungi, and archaea may provide a new
213 avenue for future research. A summary of the individual VOCs involved in induced resistance
214 from the studies discussed is provided in Table 2.

215 Several studies indicate mVOCs induce resistance of plants against pathogens through the
216 modulation of plant hormones, which may be specifically elicited by different VOCs. Induced
217 systemic resistance of *A. thaliana* against *Erwinia carotovora*s by *Bacillus subtilis* GB03
218 VOCs was dependent on ethylene biosynthesis, although induced resistance by *Bacillus*
219 *amyloliquefaciens* IN937A was independent of ethylene signalling, suggesting different VOCs
220 present in the blends may utilise alternative pathways to induce resistance (Ryu et al., 2004).
221 Contrastingly, resistance of cucumber to *Pseudomonas syringae* exposed to *Bacillus subtilis*
222 GB03 VOCs involved the activation of a jasmonic acid marker gene, *CsLOX*, but not the
223 ethylene marker gene, *CsETR*, suggesting a role for jasmonic acid but not ethylene signalling
224 (Song et al., 2019b). Discrepancies in these findings may relate to differences in plant species
225 under investigation, which may utilise different defence pathways in VOC perception, or
226 redundancy in salicylic acid, jasmonic acid and ethylene signalling pathways in induced

227 resistance (Ryu et al., 2004). A role for jasmonic acid signalling has also been observed in 3-
228 pentanol and 2-butanone induced resistance, where expression of *CsLOX* was significantly
229 upregulated upon VOC exposure, whereas expression of salicylic acid and ethylene marker
230 genes were not induced (Song et al., 2013). Similarly, *A. thaliana* mutants exposed to 3-
231 pentanol confirmed 3-pentanol mediated immune response involved jasmonic acid and
232 salicylic acid signalling pathways, as well as the Non-Pathogenesis Related 1 (*NPR-1*) gene,
233 but that ethylene signalling genes were not involved (Song et al., 2015). Tridecane, produced
234 by *Paenibacillus polymyxa* E681, was involved in the induced resistance of *A. thaliana* against
235 *Pseudomonas syringae*, through the modulation of salicylic acid, jasmonic acid, and ethylene
236 marker genes (Lee et al., 2012). The *Bacillus* VOCs albuterol and 1,3- propanediol induced
237 systemic resistance of tobacco against *Ralstonia solanacearum* by inducing expression of
238 resistance (*RRS1*) and pathogenesis related proteins (*Pr1a* and *Pr1b*) (Tahir et al., 2017b).
239 Interestingly, 1,3-propanediol induced greater expression of the *RRS1* gene relative to
240 albuterol, whereas albuterol induced greater expression of PR genes, suggesting specificity
241 in the mechanisms of induced resistance for the VOCs. This is supported by findings from
242 Naznin and colleagues (2014), who demonstrated VOCs from cultures of *Cladosporium* and
243 *Ampleomyces* induced resistance of *A. thaliana* against *Pseudomonas syringae*, from which
244 m-cresol and methyl benzoate were the dominant VOCs from each species, respectively.
245 When individually assayed, m-cresol elicited salicylic acid and jasmonic acid signalling
246 pathways in *A. thaliana*, whereas methyl benzoate induced jasmonic acid signalling with partial
247 salicylic acid signals, although neither VOC elicited ethylene signalling. Expression of genes
248 involved in salicylic acid signalling are also induced in tomato plants exposed to dimethyl
249 disulfide, inducing resistance against *Sclerotinia minor* (Tyagi et al., 2020). Interestingly, as
250 well as directly suppressing growth of *Ralstonia solanacearum*, benzaldehyde, 1,2-
251 benzisothiazol-3(2H)-one and 1,3-butadiene elicited induced systemic resistance in tobacco,
252 through induction in the transcriptional expression of defence related genes, demonstrating
253 potential multi-functional roles of mVOCs (Tahir et al., 2017a).

254 **Table 2 | Summary of mVOC producing stains, their active VOCs, the plants displaying**
 255 **induced resistance upon exposure, and the target pathogens.**

VOC producing strain	Active VOCs	Target pathogen/plant species	Reference
<i>Bacillus subtilis</i> GB03	2,3-Butanediol	<i>Erwinia carotovora/A. thaliana</i>	Ryu et al., 2004
<i>Bacillus amyloliquefaciens</i> IN937a	Acetoin		
<i>Pseudomonas chlororaphis</i> O6	(2R, 3R)-Butanediol	<i>Erwinia carotovora/Nicotiana benthamiana</i>	Han et al., 2006
N.A.	1-Octen-3-ol	<i>Botrytis cinerea/A. thaliana</i>	Kishimoto et al., 2006
<i>Talaromyces wortmannii</i> FS2	β -Caryophyllene	<i>Colletotrichum higginsianum /Brassica campestris</i>	Yamagiwa et al., 2011
<i>Paenibacillus polymyxa</i> E681	Tridecane	<i>Pseudomonas syringae/A. thaliana</i>	Lee et al., 2012
<i>Bacillus</i>	3-Pentanol	<i>Pseudomonas syringae/Cucumis sativus</i>	Song and Ryu, 2013
	2-Butanone		Song et al., 2015
<i>Trichoderma virens</i>	Terpenes	<i>Botrytis cinerea/A. thaliana</i>	Contreras-Cornejo et al., 2014
<i>Cladosporium</i> sp. D-c-4	M-Cresol	<i>Pseudomonas syringae/A. thaliana</i>	Naznin et al., 2014
<i>Ampleomyces</i> sp. F-a-3	Methyl benzoate		
<i>Trichoderma asperellum</i>	6-Pentyl-2H-pyran-2-one	<i>Botrytis cinerea, Alternaria brassicicola/A. thaliana</i>	Kottb et al., 2015
<i>Bacillus amyloliquefaciens</i> FZB42,	Benzaldehyde	<i>Ralstonia solanacearum/Nicotiana benthamiana</i>	Tahir et al., 2017a
<i>Bacillus artrophaeus</i> LSSC22	1,2-Benzisothiazol-3(2H)-one 1,3-Butadiene		
<i>Bacillus subtilis</i> SYST2	Albuterol	<i>Ralstonia solanacearum/Nicotiana benthamiana</i>	Tahir et al., 2017b
	1,3-Propanediol		
<i>Bacillus amyloliquefaciens</i> FZB42	2,3-Butanediol	<i>A. thaliana/Nicotiana benthamiana</i>	Wu et al., 2018
	Acetoin		
<i>Nitrosocosmicus oleophilus</i> MY3	Not identified	<i>Pectobacterium carotovorum, Pseudomonas syringae/A. thaliana</i>	Song et al., 2019a
<i>Bacillus subtilis</i> GB03	2,3-Butanediol	<i>Pseudomonas syringae/Cucumis sativa</i>	Song et al., 2019b
	Acetoin		
N.A.	Dimethyl disulfide	<i>Sclerotinia minor/Tomato</i>	Tyagi et al., 2020

256

257

258 **Role of volatiles in plant growth promotion**

259 MVOCs also have potential to enhance plant growth, enabling them to potentially be exploited
 260 as a new category of fertilizer, previously described as ‘gaseous fertilizer’ (Sharifi and Ryu,
 261 2018). The role of mVOCs in promoting plant growth has been recognised for over a decade
 262 and was first reported by Ryu and colleagues (2003). *A. thaliana* seedlings exposed to VOCs
 263 of *Bacillus subtilis* GB03 and *Bacillus amyloliquefaciens* IN937a had significantly greater total
 264 leaf area relative to controls, suggesting airborne signals from the strains were responsible for
 265 the enhanced plant growth. Seedlings treated with exogenous 2,3-butanediol and acetoin, the

266 most abundantly produced *Bacillus* VOCs, demonstrated significant enhancements in leaf
267 area, highlighting a specific role for the VOCs produced by these *Bacillus* strains in growth
268 promotion. Since this, VOCs from several species of *Bacillus* have shown a role in plant growth
269 promotion. VOCs from a different strain of *Bacillus subtilis* (SYS2) also promoted growth of
270 tomato, for which albuterol and 1,3-propanediol played a specific role (Tahir et al., 2017c),
271 suggesting different strains of the same species of *Bacillus* can deploy different VOCs to
272 enhance plant growth. 2-Pentylfuran, produced by cultures of *Bacillus megaterium*,
273 demonstrated dose dependent growth promotion of *A. thaliana*, with 1.5-fold increases in plant
274 biomass observed at a 10 µg dose (Zou et al., 2010). As well as *Bacillus* spp., VOCs produced
275 by other rhizobacteria can enhance plant growth, including *Proteus vulgaris*, which enhanced
276 plant growth of Chinese cabbage, for which indole demonstrated a role (Yu and Lee, 2013).
277 Groenhagen and colleagues (2013) also observed significant increases in *A. thaliana* biomass
278 when exposed to a range of VOCs, with dimethyl disulfide, the most abundantly produced
279 VOC across a range of *Burkholderia ambifaria* strains, demonstrating the greatest plant growth
280 promoting effects between doses of 1 ng and 1 mg.

281 Several fungal VOCs have also demonstrated a role in plant growth promotion, with 6-pentyl-
282 2H-pyran-2-one from *Trichoderma* spp. shown specifically to influence plant growth. *A.*
283 *thaliana* seedlings exposed to 6-pentyl-2H-pyran-2-one demonstrated a reduction in fresh
284 plant weight, but also a reduction in disease symptoms when inoculated with certain fungal
285 pathogens (Kottb et al., 2015). Contrastingly, Garnica-Vergara et al. (2016) showed the
286 application of 6-pentyl-2H-pyran-2-one led to increased biomass and root branching of *A.*
287 *thaliana* between 50-175 µM, although at the highest tested doses, a phytotoxic effect was
288 observed. Discrepancies in the findings between these studies are likely due to differences in
289 the doses of 6-pentyl-2H-pyran-2-one used in each study, whereby those used by Kottb and
290 colleagues were orders of magnitude greater than those used by Garnica-Vergara. Whilst 6-
291 pentyl-2H-pyran-2-one is the most well-studied *Trichoderma* VOC, evidence suggests other
292 VOCs may also be involved in plant growth promotion. Exposure of *A. thaliana* to VOCs from

293 a range of *Trichoderma* species showed 6-pentyl-2H-pyran-2-one production was reported
294 from certain strains which did not promote plant growth, and was not produced by certain
295 strains which did, suggesting other VOCs could contribute to the growth promotion observed.
296 (Lee et al., 2016). This is supported by findings from Estrada-Rivera and colleagues (2019),
297 who showed that 2-heptanol, 3-octanol and 2-heptanone produced by *Trichoderma atroviride*
298 can also promote plant growth of *A. thaliana*. VOCs from other fungal species have also
299 demonstrated roles in plant growth promotion, including *Fusarium oxysporum*, which
300 significantly enhanced lettuce biomass, increasing the expression of expansin genes in leaves
301 and roots (Minerdi et al., 2011). Seedlings exposed to the *Fusarium oxysporum*-derived
302 sesquiterpene β -caryophyllene showed increased growth promotion, suggesting a specific
303 role for the VOC in the plant growth promotion observed. Interestingly, β -caryophyllene
304 enhanced the biomass of *Brassica campestris*, as well as inducing resistance against
305 *Colletotrichum higginsianum* (Yamagiwa et al., 2011). A summary of the individual VOCs
306 involved in plant growth promotion from the studies discussed is provided in Table 3.

307 Several studies indicate mVOCs may promote plant growth through modulating plant hormone
308 responses. The *cytokinin- and ethylene- insensitive 2 (ein-2)* and *Arabidopsis cytokinin*
309 *receptor-deficient 1 (cre-1)* mutants exposed to *Bacillus subtilis* GB03 VOCs did not display
310 increases in plant biomass, suggesting a role for cytokinin signalling pathways plant growth
311 promotion (Ryu et al., 2003). *ein-2* also demonstrated a role in the growth promotion of *A.*
312 *thaliana* by the VOC 6-pentyl-2H-pyran-2-one, as well as auxin transport proteins (Garnica-
313 Vergara et al., 2016). Exposure of *A. thaliana* to 1-decene, a plant growth promoting
314 *Trichoderma* VOC, led to the differential expression of 123 genes, 17 of which were up-
315 regulated and several of which were auxin related (Lee et al., 2019). Similarly, dimethyl
316 disulfide altered the root system architecture of *A. thaliana*, which showed significant
317 reductions in primary root length upon exposure, but increases in the number of lateral roots
318 per plant, and number of root hairs per cm of root (Tyagi et al., 2019). These root architecture
319 changes were dependent on canonical auxin signalling pathways, with mutants deficient in

320 auxin responsive genes and transcription factors not exhibiting lateral root development or
 321 growth enhancements.

322

323

324

325

326

327

328

329

330

331 **Table 3 | Summary of mVOC producing stains, their active VOCs and the plants**
 332 **displaying enhanced growth promotion upon exposure.**

VOC producing strain	Active VOCs	Plant species	Reference
<i>Bacillus subtilis</i> GB03	2,3 Butanediol	<i>A. thaliana</i>	Ryu et al., 2003
<i>Bacillus amyloliquefaciens</i> IN937a	Acetoin		
<i>Bacillus megaterium</i>	2-Pentylfuran	<i>A. thaliana</i>	Zou et al., 2010
<i>Fusarium oxysporum</i>	β-Caryophyllene	Lettuce (<i>Lactuca sativa</i>)	Minerdi et al., 2011
<i>Proteus vulgaris</i>	Indole	Chinese cabbage (<i>Brassica rapa</i>)	Yu and Lee, 2013
<i>Burkholderia ambifaria</i>	Dimethyl disulfide Acetophenone 3-Hexanone	<i>A. thaliana</i>	Groenhagen et al., 2013
<i>Trichoderma virens</i>	6-Pentyl-2H-pyran-2-one	<i>A. thaliana</i>	Garnica-Vergara et al., 2016
<i>Bacillus subtilis</i> SYST2	Albuterol 1,3-Propanediol	Tomato (<i>Solanum lycopersicum</i>)	Tahir et al., 2017c
<i>Trichoderma</i> spp.	1-Decene	<i>A. thaliana</i>	Lee et al., 2019
N.A.	Dimethyl disulfide	<i>A. thaliana</i>	Tyagi et al., 2019
<i>Trichoderma atroviride</i>	6-Pentyl-2H-pyran-2-one 2-Heptanol 3-Octanol	<i>A. thaliana</i>	Estrada-Rivera et al., 2019

333

334

335

336

337

338

339

340

341

342

343

344 Field applications of VOCs

345 For mVOCs to serve as an alternative to synthetic pesticides and fertilizers, it is important to
346 determine the efficacy of active VOCs under open-field conditions. Dimethyl disulfide is a VOC
347 produced by bacteria including *Bacillus cereus*, which can suppress soil borne pathogens and
348 nematodes, and elicit systemic resistance against *Botrytis cinerea* and *Cochliobolus*
349 *heterostrophus* (Huang et al., 2012). Dimethyl disulfide has been successfully
350 commercialised as an alternative to pesticides as the soil fumigant PALADIN®, which has been
351 patented (Paladin Technical EPA Reg. No. 55050-3), highlighting the potential of mVOCs to
352 serve as alternatives to chemical inputs (de Boer et al., 2019). Performance of other VOCs
353 demonstrating a role in induced plant resistance under laboratory conditions are also
354 demonstrating promise in the field. Field trials with 2,3-butanediol induced resistance of
355 cucumber to viruses (Kong et al., 2018) and maize to the northern corn leaf blight fungus

356 *Setosphaeria turcica* (D'Alessandro et al., 2014). As well as 2,3-butanediol, cucumber plants
357 exposed to 3-pentanol and 2-butanone showed reduced disease symptoms against the
358 *Pseudomonas syringae* under open field conditions (Song and Ryu, 2013). These studies
359 demonstrate promise in the performance of mVOCs in the field, and future work should
360 investigate the efficacy of bioactive VOCs identified from lab-based studies under field
361 conditions.

362 **Conclusions and future outlook**

363 The biological activities of mVOCs highlight their potential to act as alternatives to
364 unsustainable agricultural chemical inputs, to feed a growing population. Much work
365 investigating mVOCs focusses on the model plant species *A. thaliana* and *Nicotiana*
366 *benthamiana* (Table 2, 3), and future research should focus on the protective and growth
367 stimulating effects of mVOCs on crop and vegetable species. Similarly, characterisation of
368 mVOCs has been performed on limited range of microbial species. In the case of VOCs from
369 bacteria, *Bacillus* spp., in particular 2,3-butanediol and acetoin, have been the focus of several
370 studies, and for fungi, *Trichoderma* species has attracted the most attention, specifically 6-
371 pentyl-2H-pyran-2-one (Table 2, 3). Current estimates indicate that <10% of mVOCs have
372 been ascribed a function (Lemfack et al., 2018), suggesting enormous potential for identifying
373 other mVOCs with biotechnological applications. Most studies reported here investigate VOC
374 production from axenic cultures of microbes, although growing bodies of evidence suggest
375 interspecific interactions between microorganisms can enhance production of VOCs which
376 have demonstrated inhibitory activity against pathogens (Tyc et al., 2014; Tyc et al., 2017).
377 This could enable identification of biologically relevant VOCs involved in the suppression of
378 pathogenic microorganisms. Whilst several studies also investigate the role of mVOCs on a
379 single biological activity, there are likely overlaps in the roles of these VOCs. For example, 6-
380 pentyl-2H-pyran-2-one has demonstrated roles in pathogen suppression (e.g. Jeleń et al.,
381 2014), plant growth promotion (Garnica-Vergara et al., 2016), and induced resistance (Kottb
382 et al., 2015), suggesting biological activities should not be considered in isolation. Moreover,

383 whilst many studies demonstrate VOCs have suppressive effects on plant pathogens, it is
384 important to determine the effect of these inhibitory VOCs on plant development. For example,
385 inhibitory mVOCs produced by *Streptomyces yanglinensis* 3-10 against *Aspergillus* were
386 tested to determine their effects on plant development, and showed that VOCs did not inhibit
387 peanut seedling germination, suggesting promise for use under field conditions (Lyu et al.,
388 2020). Whilst investigation of the efficacy of VOCs under field conditions have demonstrated
389 promise, a wider range of VOCs require testing at this scale. More research on methods of
390 application of mVOCs onto fields is also required, for example, the effectiveness of drench
391 versus spraying application (Garbeva and Weiskopf, 2020). The potential for plant production
392 of active VOCs for the biological control of fungal pathogens through companion cropping
393 systems is another potential form of delivery. Bean cultivars resistant to *Colletotrichum*
394 *lindemuthianum*, the causal agent of black spot disease, enhanced resistance of susceptible
395 cultivars to the pathogen when exposed to VOCs from resistant cultivars (Quintana-Rodriguez
396 et al., 2015). These findings could be translated in the field for the control of plant pathogens,
397 through companion cropping systems, using VOCs from disease-resistant cultivars to deliver
398 VOCs to neighbouring crops to enhance disease resistance against fungal pathogens. In
399 conclusion, studies reviewed here demonstrate mVOCs can be exploited to serve as
400 sustainable alternatives to agricultural chemical inputs, which can potentially reduce our
401 overreliance on the current unsustainable methods at a time when population growth, and
402 demand, is likely to substantially increase.

403 **Acknowledgements**

404 GT's PhD studentship was funded by a Biotechnology and Biological Sciences Research
405 Council (BBSRC) South West doctoral training partnership award (project no. 1622285).

406

407

408

409

410

411

412

413

414

415

416

417

418

419 **References**

420 Brakhage, A.A. and Schroeckh, V. (2011) Fungal secondary metabolites - Strategies to
421 activate silent gene clusters. *Fungal Genet Biol* **48**: 15–22.

422 Cho, G., Kim, J., Park, C.G., Nislow, C., Weller, D.M., and Kwak, Y.S. (2017) Caryolan-1-ol,
423 an antifungal volatile produced by *Streptomyces* spp., inhibits the endomembrane
424 system of fungi. *Open Biol* **7**:170075.

425 Contreras-Cornejo, H.A., Macías-Rodríguez, L., Herrera-Estrella, A., and López-Bucio, J.
426 (2014) The 4-phosphopantetheinyl transferase of *Trichoderma virens* plays a role in

427 plant protection against *Botrytis cinerea* through volatile organic compound emission.
428 *Plant Soil* **379**: 261–274.

429 D'Alessandro, M., Erb, M., Ton, J., Brandenburg, A., Karlen, D., Zopfi, J., and Turlings,
430 T.C.J. (2014) Volatiles produced by soil-borne endophytic bacteria increase plant
431 pathogen resistance and affect tritrophic interactions. *Plant, Cell Environ* **37**: 813–826.

432 Davidson, E.A. (2009) The contribution of manure and fertilizer nitrogen to atmospheric
433 nitrous oxide since 1860. *Nat Geosci* **2**: 659–662.

434 De Boer, W., Li, X., Meisner, A., and Garbeva, P. (2019) Pathogen suppression by microbial
435 volatile organic compounds in soils. *FEMS Microbiol Ecol* **95**.

436 de Lima Procópio, R.E., da Silva, I.R., Martins, M.K., de Azevedo, J.L., and de Araújo, J.M.
437 (2012) Antibiotics produced by *Streptomyces*. *Brazilian J Infect Dis* **16**: 466–471.

438 Effmert, U., Kalderás, J., Warnke, R., and Piechulla, B. (2012) Volatile Mediated Interactions
439 Between Bacteria and Fungi in the Soil. *J Chem Ecol* **38**: 665–703.

440 Estrada-Rivera, M., Rebolledo-Prudencio, O.G., Pérez-Robles, D.A., Rocha-Medina, M.D.C.,
441 González-López, M.D.C., and Casas-Flores, S. (2019) *Trichoderma histone*
442 deacetylase HDA-2 modulates multiple responses in arabidopsis. *Plant Physiol* **179**:
443 1343–1361.

444 Fernando, W.G.D., Ramarathnam, R., Krishnamoorthy, A.S., and Savchuk, S.C. (2005)
445 Identification and use of potential bacterial organic antifungal volatiles in biocontrol. *Soil*
446 *Biol Biochem* **37**: 955–964.

447 Fisher, M.C., Hawkins, N.J., Sanglard, D., and Gurr, S.J. (2018) Worldwide emergence of
448 resistance to antifungal drugs challenges human health and food security. *Science (80-*
449 *)* **360**: 739–742.

- 450 Garbeva, P. and Weiskopf, L. (2020) Airborne medicine: bacterial volatiles and their
451 influence on plant health. *New Phytol* **226**: 32–43.
- 452 Garnica-Vergara, A., Barrera-Ortiz, S., Muñoz-Parra, E., Raya-González, J., Méndez-Bravo,
453 A., Macías-Rodríguez, L., et al. (2016) The volatile 6-pentyl-2H-pyran-2-one from
454 *Trichoderma atroviride* regulates *Arabidopsis thaliana* root morphogenesis via auxin
455 signaling and ETHYLENE INSENSITIVE 2 functioning. *New Phytol* **209**: 1496–1512.
- 456 Groenhagen, U., Baumgartner, R., Bailly, A., Gardiner, A., Eberl, L., Schulz, S., and
457 Weiskopf, L. (2013) Production of Bioactive Volatiles by Different Burkholderia
458 ambifaria Strains. *J Chem Ecol* **39**: 892–906.
- 459 Han, S.H., Lee, S.J., Moon, J.H., Park, K.H., Yang, K.Y., Cho, B.H., et al. (2006) GacS-
460 dependent production of 2R, 3R-butanediol by *Pseudomonas chlororaphis* O6 is a
461 major determinant for eliciting systemic resistance against *Erwinia carotovora* but not
462 against *Pseudomonas syringae* pv. *tabaci* in tobacco. *Mol Plant-Microbe Interact* **19**:
463 924–930.
- 464 Huang, C.J., Tsay, J.F., Chang, S.Y., Yang, H.P., Wu, W.S., and Chen, C.Y. (2012)
465 Dimethyl disulfide is an induced systemic resistance elicitor produced by *Bacillus*
466 *cereus* C1L. *Pest Manag Sci* **68**: 1306–1310.
- 467 Jeleń, H., Błaszczuk, L., Chelkowski, J., Rogowicz, K., and Strakowska, J. (2014) Formation
468 of 6-n-pentyl-2H-pyran-2-one (6-PAP) and other volatiles by different *Trichoderma*
469 species. *Mycol Prog* **13**: 589–600.
- 470 Kanchiswamy, C.N., Malnoy, M., and Maffei, M.E. (2015) Bioprospecting bacterial and
471 fungal volatiles for sustainable agriculture. *Trends Plant Sci* **20**: 206–211.
- 472 Kishimoto, K., Matsui, K., Ozawa, R., and Takabayashi, J. (2007) Volatile 1-octen-3-ol
473 induces a defensive response in *Arabidopsis thaliana*. *J Gen Plant Pathol* **73**: 35–37.

474 Kong, H.G., Shin, T.S., Kim, T.H., and Ryu, C.M. (2018) Stereoisomers of the bacterial
475 volatile compound 2,3-butanediol differently elicit systemic defense responses of
476 pepper against multiple viruses in the field. *Front Plant Sci* **9**: 90.

477 Kottb, M., Gigolashvili, T., Großkinsky, D.K., and Piechulla, B. (2015) Trichoderma volatiles
478 effecting Arabidopsis: From inhibition to protection against phytopathogenic fungi. *Front*
479 *Microbiol* **6**: 995.

480 Lamberth, C., Jeanmart, S., Luksch, T., and Plant, A. (2013) Current challenges and trends
481 in the discovery of agrochemicals. *Science (80-)* **341**: 742–746.

482 Lee, B., Farag, M.A., Park, H.B., Kloepper, J.W., Lee, S.H., and Ryu, C.M. (2012) Induced
483 Resistance by a Long-Chain Bacterial Volatile: Elicitation of Plant Systemic Defense by
484 a C13 Volatile Produced by *Paenibacillus polymyxa*. *PLoS One* **7**: e48744.

485 Lee, S., Behringer, G., Hung, R., and Bennett, J. (2019) Effects of fungal volatile organic
486 compounds on *Arabidopsis thaliana* growth and gene expression. *Fungal Ecol* **37**: 1–9.

487 Lee, S., Yap, M., Behringer, G., Hung, R., and Bennett, J.W. (2016) Volatile organic
488 compounds emitted by *Trichoderma* species mediate plant growth. *Fungal Biol*
489 *Biotechnol* **3**: 7.

490 Lemfack, M.C., Gohlke, B.O., Toguem, S.M.T., Preissner, S., Piechulla, B., and Preissner,
491 R. (2018) MVOC 2.0: A database of microbial volatiles. *Nucleic Acids Res* **46**: D1261–
492 D1265.

493 Lyu, A., Yang, L., Wu, M., Zhang, J., and Li, G. (2020) High Efficacy of the Volatile Organic
494 Compounds of *Streptomyces yanglinensis* 3-10 in Suppression of *Aspergillus*
495 Contamination on Peanut Kernels. *Front Microbiol* **11**: 142.

496 Macheleidt, J., Mattern, D.J., Fischer, J., Netzker, T., Weber, J., Schroeckh, V., et al. (2016)
497 Regulation and Role of Fungal Secondary Metabolites. *Annu Rev Genet* **50**: 371–392.

498 Maffei, M.E., Gertsch, J., and Appendino, G. (2011) Plant volatiles: Production, function and
499 pharmacology. *Nat Prod Rep* **28**: 1359–1380.

500 Massawe, V.C., Hanif, A., Farzand, A., Mburu, D.K., Ochola, S.O., Wu, L., et al. (2018)
501 Volatile compounds of endophytic *Bacillus* spp. have biocontrol activity against
502 *Sclerotinia sclerotiorum*. *Phytopathology* **108**: 1373–1385.

503 Minerdi, D., Bossi, S., Maffei, M.E., Gullino, M.L., and Garibaldi, A. (2011) *Fusarium*
504 *oxysporum* and its bacterial consortium promote lettuce growth and expansin A5 gene
505 expression through microbial volatile organic compound (MVOC) emission. *FEMS*
506 *Microbiol Ecol* **76**: 342–351.

507 Naznin, H.A., Kiyohara, D., Kimura, M., Miyazawa, M., Shimizu, M., and Hyakumachi, M.
508 (2014) Systemic resistance induced by volatile organic compounds emitted by plant
509 growth-promoting fungi in *Arabidopsis thaliana*. *PLoS One* **9**: e86882.

510 Nørskov, J., Chen, J. (2016) DOE Roundtable Report: Sustainable Ammonia Synthesis. *US*
511 *Dep Energy*.

512 Ojaghian, S., Wang, L., Xie, G.L., and Zhang, J.Z. (2019) Effect of volatiles produced by
513 *Trichoderma* spp. on expression of glutathione transferase genes in *Sclerotinia*
514 *sclerotiorum*. *Biol Control* **136**: 103999.

515 Quintana-Rodriguez, E., Morales-Vargas, A.T., Molina-Torres, J., Ádame-Alvarez, R.M.,
516 Acosta-Gallegos, J.A., and Heil, M. (2015) Plant volatiles cause direct, induced and
517 associational resistance in common bean to the fungal pathogen *Colletotrichum*
518 *lindemuthianum*. *J Ecol* **103**: 250–260.

519 Rajer, F.U., Wu, H., Xie, Y., Xie, S., Raza, W., Tahir, H.A.S., and Gao, X. (2017) Volatile
520 organic compounds produced by a soil-isolate, *Bacillus subtilis* FA26 induce adverse
521 ultra-structural changes to the cells of *Clavibacter michiganensis* ssp. *Sepedonicus*, the
522 causal agent of bacterial ring rot of potato. *Microbiol (United Kingdom)* **163**: 523–530.

523 Rohr, J.R., Barrett, C.B., Civitello, D.J., Craft, M.E., Delius, B., DeLeo, G.A., et al. (2019)
524 Emerging human infectious diseases and the links to global food production. *Nat*
525 *Sustain* **2**: 445–456.

526 Ryu, C.M., Farag, M.A., Hu, C.H., Reddy, M.S., Kloepper, J.W., and Paré, P.W. (2004)
527 Bacterial volatiles induce systemic resistance in *Arabidopsis*. *Plant Physiol* **134**: 1017–
528 1026.

529 Ryu, C.M., Farag, M.A., Hu, C.H., Reddy, M.S., Wei, H.X., Paré, P.W., and Kloepper, J.W.
530 (2003) Bacterial volatiles promote growth in *Arabidopsis*. *Proc Natl Acad Sci U S A* **100**:
531 4927–4932.

532 Sánchez-Fernández, R.E., Diaz, D., Duarte, G., Lappe-Oliveras, P., Sánchez, S., and
533 Macías-Rubalcava, M.L. (2016) Antifungal Volatile Organic Compounds from the
534 Endophyte *Nodulisporium* sp. Strain GS4d2II1a: a Qualitative Change in the
535 Intraspecific and Interspecific Interactions with *Pythium aphanidermatum*. *Microb Ecol*
536 **71**: 347–364.

537 Savary, S., Willocquet, L., Pethybridge, S.J., Esker, P., McRoberts, N., and Nelson, A.
538 (2019) The global burden of pathogens and pests on major food crops. *Nat Ecol Evol* **3**:
539 430–439.

540 Schulz-Bohm, K., Martín-Sánchez, L., and Garbeva, P. (2017) Microbial volatiles: Small
541 molecules with an important role in intra- and inter-kingdom interactions. *Front Microbiol*
542 **8**: 1-10.

- 543 Sharifi, R. and Ryu, C.M. (2018) Revisiting bacterial volatile-mediated plant growth
544 promotion: Lessons from the past and objectives for the future. *Ann Bot* **122**: 349–358.
- 545 Smil, V. (1999) Nitrogen in crop production: An account of global flows. *Global Biogeochem*
546 *Cycles* **13**: 647–662.
- 547 Song, G.C., Choi, H.K., and Ryu, C.M. (2015) Gaseous 3-pentanol primes plant immunity
548 against a bacterial speck pathogen, *Pseudomonas syringae* pv. *Tomato* via salicylic
549 acid and jasmonic acid-dependent signaling pathways in *Arabidopsis*. *Front Plant Sci* **6**:
550 821.
- 551 Song, G.C., Im, H., Jung, J., Lee, S., Jung, M.Y., Rhee, S.K., and Ryu, C.M. (2019a) Plant
552 growth-promoting archaea trigger induced systemic resistance in *Arabidopsis thaliana*
553 against *Pectobacterium carotovorum* and *Pseudomonas syringae*. *Environ Microbiol*
554 **21**: 940–948.
- 555 Song, G.C., Riu, M., and Ryu, C.M. (2019b) Beyond the two compartments Petri-dish:
556 Optimising growth promotion and induced resistance in cucumber exposed to gaseous
557 bacterial volatiles in a miniature greenhouse system. *Plant Methods* **15**:
- 558 Song, G.C. and Ryu, C.M. (2013) Two volatile organic compounds trigger plant self-defense
559 against a bacterial pathogen and a sucking insect in cucumber under open field
560 conditions. *Int J Mol Sci* **14**: 9803–9819.
- 561 Spiteller, P. (2015) Chemical ecology of fungi. *Nat Prod Rep* **32**: 971–993.
- 562 Syed-Ab-Rahman, S.F., Xiao, Y., Carvalhais, L.C., Ferguson, B.J., and Schenk, P.M. (2019)
563 Suppression of *Phytophthora capsici* infection and promotion of tomato growth by soil
564 bacteria. *Rhizosphere* **9**: 72–75.

565 Tahir, H.A.S., Gu, Q., Wu, H., Niu, Y., Huo, R., and Gao, X. (2017a) *Bacillus volatiles*
566 adversely affect the physiology and ultra-structure of *Ralstonia solanacearum* and
567 induce systemic resistance in tobacco against bacterial wilt. *Sci Rep* **7**: 40481.

568 Tahir, H.A.S., Gu, Q., Wu, H., Raza, W., Safdar, A., Huang, Z., et al. (2017b) Effect of
569 volatile compounds produced by *Ralstonia solanacearum* on plant growth promoting
570 and systemic resistance inducing potential of *Bacillus volatiles*. *BMC Plant Biol* **17**: 133.

571 Tahir, H.A.S., Gu, Q., Wu, H., Raza, W., Hanif, A., Wu, L., et al. (2017c) Plant growth
572 promotion by volatile organic compounds produced by *Bacillus subtilis* SYST2. *Front*
573 *Microbiol* **8**: 171.

574 Tester, M. and Langridge, P. (2010) Breeding technologies to increase crop production in a
575 changing world. *Science (80-)* **327**: 818–822.

576 Tilocca, B., Cao, A., and Migheli, Q. (2020) Scent of a Killer: Microbial Volatilome and Its
577 Role in the Biological Control of Plant Pathogens. *Front Microbiol* **11**: 41.

578 Tyagi, S., Kim, K., Cho, M., and Lee, K.J. (2019) Volatile dimethyl disulfide affects root
579 system architecture of *Arabidopsis* via modulation of canonical auxin signaling
580 pathways. *Environ Sustain* **2**: 211–216.

581 Tyagi, S., Lee, K.J., Shukla, P., and Chae, J.C. (2020) Dimethyl disulfide exerts antifungal
582 activity against *Sclerotinia minor* by damaging its membrane and induces systemic
583 resistance in host plants. *Sci Rep* **10**: 1-12.

584 Tyc, O., van den Berg, M., Gerards, S., van Veen, J.A., Raaijmakers, J.M., de Boer, W., and
585 Garbeva, P. (2014) Impact of interspecific interactions on antimicrobial activity among
586 soil bacteria. *Front Microbiol* **6**:1412.

587 Tyc, O., de Jager, V.C.L., van den Berg, M., Gerards, S., Janssens, T.K.S., Zaagman, N., et
588 al. (2017) Exploring bacterial interspecific interactions for discovery of novel
589 antimicrobial compounds. *Microb Biotechnol* **10**: 910-925.

590 United Nations (2017) World Population Prospects The 2017 Revision Key Findings and
591 Advance Tables ESA/P/WP/248.

592 Velivelli, S.L.S., Kromann, P., Lojan, P., Rojas, M., Franco, J., Suarez, J.P., and Prestwich,
593 B.D. (2015) Identification of mVOCs from Andean Rhizobacteria and Field Evaluation of
594 Bacterial and Mycorrhizal Inoculants on Growth of Potato in its Center of Origin. *Microb*
595 *Ecol* **69**: 652–667.

596 Wu, L., Li, X., Ma, L., Borriss, R., Wu, Z., and Gao, X. (2018) Acetoin and 2,3-butanediol
597 from *Bacillus amyloliquefaciens* induce stomatal closure in *Arabidopsis thaliana* and
598 *Nicotiana benthamiana*. *J Exp Bot* **69**: 5625–5635.

599 Xie, S., Zang, H., Jun Wu, H., Uddin Rajer, F., and Gao, X. (2018) Antibacterial effects of
600 volatiles produced by bacillus strain D13 against *xanthomonas oryzae* pv. *oryzae*. *Mol*
601 *Plant Pathol* **19**: 49–58.

602 Yamagiwa, Y., Inagaki, Y., Ichinose, Y., Toyoda, K., Hyakumachi, M., and Shiraishi, T.
603 (2011) *Talaromyces wortmannii* FS2 emits β -caryophyllene, which promotes plant
604 growth and induces resistance. *J Gen Plant Pathol* **77**: 336–341.

605 Yang, M., Lu, L., Pang, J., Hu, Y., Guo, Q., Li, Z., et al. (2019) Biocontrol activity of volatile
606 organic compounds from *Streptomyces alboflavus* TD-1 against *Aspergillus flavus*
607 growth and aflatoxin production. *J Microbiol* **57**: 396–404.

608 Yin, G., Zhang, Y., Fu, M., Hua, S.S.T., Huang, Q., Pennerman, K.K., et al. (2019) Influence
609 of R and S enantiomers of 1-octen-3-ol on gene expression of *Penicillium*
610 *chrysogenum*. *J Ind Microbiol Biotechnol* **46**: 977–991.

- 611 Yu, S.M. and Lee, Y.H. (2013) Plant growth promoting rhizobacterium *Proteus vulgaris*
612 JBL202 stimulates the seedling growth of Chinese cabbage through indole emission.
613 *Plant Soil* **370**: 485–495.
- 614 Zou, C., Li, Z., and Yu, D. (2010) *Bacillus megaterium* strain XTBG34 promotes plant growth
615 by producing 2-pentylfuran. *J Microbiol* **48**: 460–466.
- 616