

1 *Bacillus subtilis* and *Bacillus velezensis* population dynamic and quantification of  
2 spores after inoculation on ornamental plants  
3 Vincent Charron-Lamoureux<sup>1\*</sup>, Maude Thérien<sup>1\*</sup>, Assena Konk<sup>1</sup> and Pascale B.  
4 Beauregard<sup>1§</sup>

5  
6 <sup>1</sup>Centre SÈVE, Département de biologie, Faculté des sciences, Université de  
7 Sherbrooke, Sherbrooke, Canada

8 [Vincent.charron-lamoureux@usherbrooke.ca](mailto:Vincent.charron-lamoureux@usherbrooke.ca),

9 [Maude.therien@usherbrooke.ca](mailto:Maude.therien@usherbrooke.ca),

10 [Assena.konk@usherbrooke.ca](mailto:Assena.konk@usherbrooke.ca),

11 [Pascale.b.beauregard@usherbrooke.ca](mailto:Pascale.b.beauregard@usherbrooke.ca)

12  
13 \*Co-first authors

14

15 § Correspondence:

16 P.B. Beauregard, Département de biologie, Faculté des sciences, Université de  
17 Sherbrooke, 2500 boulevard de l'Université, Sherbrooke (Québec), Canada, J1K

18 2R1. Email: [pascale.b.beauregard@usherbrooke.ca](mailto:pascale.b.beauregard@usherbrooke.ca)

19

## 20 Abstract

21 *Bacillus subtilis* and *Bacillus velezensis* are used in organic agriculture as an  
22 alternative to chemical pesticides to fight against phytopathogen organisms. These  
23 Gram-positive soil-dwelling bacteria are able to resist harsh conditions and survive  
24 by differentiating into endospores. Few studies have examined how bacterial  
25 populations change on plants over time, and if they remain active or enter a  
26 dormant state. Nonetheless, these characteristics are strikingly important to  
27 determine the usage of *B. subtilis* and *B. velezensis* and their efficacy in  
28 environmental conditions. Here, we investigate the population dynamic on plants  
29 of *B. subtilis* NCIB3610 and *B. velezensis* QST713 when applied as spores on  
30 different ornamental plants. We report that on all plants studied (*Echinacea*  
31 *purpurea* cv. Salsa red, *Echinacea purpurea* cv. Fatal attraction and *Lavandula*  
32 *angustifolia* cv. Hidecote blue) spores rapidly germinated and colonized the  
33 rhizoplane, maintaining a relatively low proportion of spores in the population over  
34 time, whereas bacterial population on leaves rapidly declined. Bacteria in the  
35 surrounding soil did not germinate and persisted as spores. Taken together, these  
36 results suggest that only cells found at the rhizosphere remain metabolically active  
37 to allow the formation of a lasting relationship with the plant, making possible  
38 beneficial effects from the inoculated bacteria.

39

40 Key words: *Bacillus* — Plants — Colonization — Population — Spores

## 41 Résumé

42 *Bacillus subtilis* et *Bacillus velezensis* sont utilisées en agriculture biologique  
43 comme alternative aux pesticides chimiques. Ces bactéries Gram-positives vivant  
44 dans le sol sont capables de résister à des conditions difficiles et de survivre en  
45 se différenciant en endospores. Peu d'études ont examiné comment les bactéries  
46 persistent sur les plantes, si elles demeurent actives ou entrent en dormance.  
47 Néanmoins, ces caractéristiques sont importantes pour déterminer leur utilisation  
48 et leur efficacité dans des conditions environnementales. Nous avons étudié la  
49 dynamique de population de *B. subtilis* NCIB3610 et de *B. velezensis* QST713  
50 lorsqu'appliquées comme spores sur différentes plantes ornementales. Nous  
51 montrons que sur toutes les plantes étudiées (*Echinacea purpurea* cv. Salsa red,  
52 *Echinacea purpurea* cv. Fatal attraction et *Lavandula angustifolia* cv. Hidecote  
53 blue) les spores ont rapidement germées et colonisées la rhizoplane, maintenant  
54 une proportion relativement faible de spores dans la population, alors que la  
55 population sur les feuilles a rapidement diminué. Les bactéries présentes dans le  
56 sol environnant n'ont pas germé et ont persisté sous forme de spores. Ces  
57 résultats suggèrent que seules les bactéries trouvées au niveau des racines  
58 restent métaboliquement actives pour permettre la formation d'une relation durable  
59 avec la plante, rendant possible les effets bénéfiques des bactéries inoculées.

60 *Bacillus* — Plantes — Colonisation — Population — Spores

61

## 62 Introduction

63 The agricultural usage of fertilizers and chemical pesticides has an important  
64 impact on both human health and ecosystem balance (Aktar et al. 2009;  
65 Nicolopoulou-Stamati et al. 2016; Carvalho 2017). Thus, there is a need to find  
66 alternative solutions with similar efficacy and reliability to help reduce the heavy  
67 use of chemical products. Plant-growth promoting rhizobacteria (PGPR) provide  
68 positive effects on plants through indirect or direct pathways (Rudrappa et al. 2008;  
69 Berg 2009; Chen et al. 2013; Chowdhury et al. 2015). These bacteria, such as  
70 members of *Azotobacter*, *Pseudomonas*, *Bacillus* and *Klebsiella* genera, can fix  
71 nitrogen, secrete growth-stimulating phytohormones and, importantly, provide  
72 resistance against a wide range of pathogens (Arkhipova et al. 2005; Ahemad and  
73 Kibret 2014).

74

75 The rhizosphere is composed of the zone directly influenced by plant roots, its  
76 exudates and mucilage, and constitutes a nutrient-rich environment hosting a  
77 complex community of microorganisms (Garbeva et al. 2004; Hartmann et al.  
78 2008) which contrast with the surrounding soil further from the plant. Depending  
79 on the plant species, its age and the environmental conditions, root exudates  
80 composition is very dynamic and can influence the rhizosphere microbiome  
81 (Zhalnina et al. 2018). Inversely, the above-ground parts of plants are known to be  
82 a more hostile environment for microorganisms. Leaves surface, known as the  
83 phyllosphere, is particularly poor in nutrients and faces environmental stresses  
84 such as UV radiations, drastic temperature changes and variable access to  
85 moisture caused by wind and precipitations (Yang et al. 2001; Lindow and Brandl  
86 2003; Turner 2013).

87

88 *Bacillus subtilis* and *Bacillus velezensis* are Gram-positive PGPR that can form  
89 endospores, allowing bacteria to survive for long periods in stressful environments  
90 (Fan et al. 2018; Hashem et al. 2019). Their capacity to withstand inhospitable  
91 environments, and thus persist over time, makes *Bacillus* spores ideal for the  
92 formulation of biofertilizers and biofungicides. However, sporulation could also limit

93 the duration of the bacterial beneficial activities provided to plants, since most of  
94 these activities, such as antimicrobial secretion, require an active metabolism.  
95 Currently, there is a lack of knowledge on colonization efficiency and, more  
96 importantly, on the sporulation of *Bacillus* spp. on plants in industrial growing  
97 conditions. In collaboration with a greenhouse grower, we examined the efficacy  
98 of two *Bacillus* species to colonize and to differentiate from spores into  
99 metabolically active bacteria on plants. Three ornamental plant cultivars,  
100 *Echinacea purpurea* (Purple coneflower) cv. Salsa red, *Echinacea purpurea* cv.  
101 Fatal attraction and *Lavandula angustifolia* (English lavender) cv. Hidecote blue,  
102 were chosen as models because of their market value and sensitivity to fungal  
103 pathogens. Those will thereafter be referred to as *E. purpurea* SR or FA and *L.*  
104 *angustifolia*.

105

## 106 Methods

107 For this study, we used *Bacillus subtilis* NCIB3610 (undomesticated strain)  
108 harboring a spectinomycin resistance cassette (Lécuyer et al. 2018) and *Bacillus*  
109 *velezensis* QST713 (Serenade SOIL®, Bayer), already used in Canada as a  
110 biofungicide. Bacteria were routinely grown in Luria-Bertani (LB) medium in Petri  
111 dishes (1% tryptone, 0.5% yeast extract and 0.5% NaCl) at 37 °C, and incubated  
112 in 100 mL of Difco Sporulation Medium (DSM) during 20 h at 37 °C with agitation  
113 to induce sporulation (Nicholson and Setlow 1990). Treatment at 80 °C for 20 min  
114 was used to kill vegetative cells before the spores are resuspended in sterile  
115 distilled water for inoculation. Inoculation was performed on mature plants intended  
116 for sale in garden centers. Of note, we did not observe presence of *B. subtilis* or  
117 *B. velezensis* on leaves, roots, or soil of non-inoculated *E. purpurea* FA. This result  
118 was obtained by evaluating CFU counts on a selective differential medium for  
119 *Bacillus* species (PEMBA), as described further in the text. Since all plants were in  
120 the same non-sterile soil and maintained in the same conditions, we concluded  
121 that there was no or only very few indigenous *Bacillus* on plants before we  
122 inoculated them. For inoculation, 25 mL of a *B. subtilis* spores solution at an  $OD_{600}$   
123 = 0.75 ( $\sim 1.5 \times 10^7$  CFU/mL) were resuspended in deionized water and applied by

124 spraying onto each plant. For *B. velezensis* QST713, 10 to 30 mL ( $\sim 1.5 \times 10^7$   
125 CFU/mL) from the Serenade SOIL® (Bayer) were also vaporized on plants with a  
126 pump sprayer according to the usual procedures of the Plant Select company  
127 (Saint-Paul d'Abbotsford, Québec, Canada). Inoculants are applied by spraying  
128 mostly on aerial parts for timesaving. We collected samples from the phyllosphere  
129 (entire leaves), from the rhizosphere (roots parts with the soil attached) and from  
130 soil, further from the roots and their influence (from the periphery of the pot). Each  
131 sample was weighed and suspended in 5 mL of phosphate-buffered saline (PBS)  
132 for sonication treatment at 1 s start and 1 s pause for 10 s at 30% amplitude, 3  
133 times, to remove attached bacteria and break the aggregates. To evaluate cell  
134 counts, samples were diluted and plated on selective media: LB supplemented  
135 with spectinomycin (100  $\mu\text{g/mL}$ ) with cycloheximide at 5 mg/L for *B. subtilis*  
136 NCIB3610 (SpecR) and PEMBA (0.1% peptone, 1% mannitol, 0.2% NaCl, 0.01%  
137  $\text{MgSO}_4$  0.25%  $\text{Na}_2\text{HPO}_4$ , 0.025%  $\text{KH}_2\text{PO}_4$ , 1% sodium pyruvate, 0.012% bromothymol  
138 blue, 1.5% agar and 2.5% egg Yolk emulsion) with polymyxin B at 12.7 mg/L (prevents  
139 the growth of Gram-negative bacteria) supplemented with cycloheximide at 5 mg/L  
140 (inhibits growth of eukaryotes) for *B. velezensis* QST713. On this medium,  
141 QST713 forms medium-sized, flat, nearly-round with undulate margins, opaque,  
142 cream-colored, dull colonies and does not ferment mannitol nor precipitate egg  
143 yolk, allowing for a fairly precise identification. A similar phenotypical analysis was  
144 used to differentiate NCIB3610 colonies on LB spectinomycin. To evaluate the  
145 proportion of spores, the same samples were then heat-treated at 80 °C for 20 min  
146 to kill vegetative cells before inoculation on plates. We evaluated the proportion of  
147 spores in the population by dividing the number of spores by the total number of  
148 cells. Of note, few bacterial aggregates might persist through the sonication  
149 process, leading to an underestimation of the total cell count, while heat treatment  
150 allows their dissociation.

151

## 152 Results and discussion

153 Following the application of spores on above-ground parts of plants, we evaluated  
154 the population dynamics by CFU counts. At day 1, *B. subtilis* and *B. velezensis*

155 were found on all studied sites (leaves, soil and roots) of the three plants (Fig. 1-  
156 3). CFU counts at day 1 were generally higher on leaves, in concordance with the  
157 aerial spraying application method. However, the bacterial population on leaves  
158 never increased over time, and this for all plants, suggesting that there was no  
159 sustainable colonization on this plant site (Fig. 1A and B). On *E. purpurea* SR and  
160 *L. angustifolia* leaves, we observed a 2-fold log reduction for both *B. subtilis* and  
161 *B. velezensis* during the 15 days, while on leaves of *E. purpurea* FA populations  
162 had a smaller decrease. Bacteria in the population on leaves were mostly spores  
163 for both strains on *Echinacea* cultivars through the experiment, while on *L.*  
164 *angustifolia*, bacterial cells appeared to be mostly vegetative by the end of the  
165 assay (Fig. 1C and D). The decrease in total population and the large proportion  
166 spores on leaves could be explained by two phenomena. Bacteria in the  
167 phyllosphere are submitted to numerous stress factors such as nutrients depletion  
168 and fluctuation of environmental conditions (Lindow and Brandl 2003) which would  
169 explain the high number of spores on *Echinacea* cultivars and the rapid decline of  
170 the population on leaves, including on *L. angustifolia* where cells have germinated.  
171 Also, greenhouses use an aerial water-spray system which likely washes off  
172 bacteria not firmly associated with the leaf surface. Our observations that spores  
173 are predominant on plant leaves are in concordance with previous results  
174 indicating that spores represent approximately 60% of *Bacillus subtilis* UMAF6614  
175 population after 7 days when inoculated on melon leaves (Zerrouh et al. 2014).

176  
177 We also examined the presence of *Bacillus* in the soil not under the direct influence  
178 of the plant. *B. subtilis* is a soil-dwelling bacteria, but this environment contains  
179 certain constraints, especially predators, nutrients limitation and moisture  
180 availability, which do not favor its development and possess very little of the free  
181 nutrients required for its germination (Hinsinger et al. 2009; Moe 2013; Fierer  
182 2017). Accordingly, population of both *Bacillus* species in soil remained high and  
183 stable over time but also appeared to be mostly composed of spores (Fig. 2).

184

185 Finally, we examined colonization as well as the variation in the presence of spores  
186 in the rhizosphere. For *E. purpurea* SR and *L. angustifolia*, population of both  
187 *Bacillus* species on roots remained somewhat stable through time. However, this  
188 was not the case with *E. purpurea* FA since *B. subtilis* population remained stable  
189 from day 1 to 15 but *B. velezensis* CFU count increased by 1-fold log on the root  
190 surface during the first four days (Fig. 3A and B) showing an efficient colonization.  
191 These results suggest that *B. velezensis* QST713 might be more adapted than *B.*  
192 *subtilis* NCIB3610 for the colonization of the rhizosphere, in the case of the studied  
193 plants. This hypothesis was further supported by the evaluation of the proportion  
194 of spores in the *Bacillus* population associated with roots. For all combinations  
195 tested, except for *B. subtilis* on *E. purpurea* FA, germination on roots was efficient  
196 since there were less than 50% of spores at most time points (Fig. 3C and D).  
197 Particularly, *B. velezensis* spores level remained very low throughout the  
198 experiment, vegetative cells sometimes reaching up to 93% of the bacterial  
199 population (Fig. 3D), which demonstrates that this population is metabolically  
200 active. The difference between the metabolic state of both strains could stem from  
201 diverse reasons, such as a better efficacy of *B. velezensis* to use nutrients sources  
202 in the root exudates or presence of germination receptors more specific to  
203 molecules secreted in the rhizosphere by these ornamental plants. Such  
204 discrepancy between bacterial strains was observed in other studies. *B. velezensis*  
205 QST713 and *B. firmus* I-1582 have different colonization efficiency when applied  
206 on corn seeds, since cell counts gradually decreased from  $10^7$  to  $10^5$  CFU/g of root  
207 for QST713 and from  $10^6$  to  $10^4$  CFU/g of root for I-1582 (Mendis et al. 2018). Of  
208 note, molecules excreted in root exudates vary between plant species, which  
209 suggests that a bacteria well adapted to the rhizosphere of one plant could be less  
210 adapted to another plant (Turner 2013).

211

212 This predominance of *B. subtilis* vegetative cells on roots of greenhouse  
213 ornamental plants contrasts with our recent observation that *B. subtilis* total  
214 number of spores rapidly increases following inoculation and germination of  
215 sporulated bacteria on *A. thaliana* roots (Charron-Lamoureux and Beauregard



216 2019). However, seedlings were used for the *A. thaliana* experiment, while mature  
217 plants in greenhouses were used here. The sharp difference in the amount and  
218 composition of nutrients secreted in both experimental set-ups could explain the  
219 difference in the proportions of spores. Furthermore, the *A. thaliana* study was  
220 performed in sterile hydroponic conditions, while here we used non-sterile soil,  
221 which could also contribute to the difference in metabolic activity of the bacteria.  
222 Indeed, it might be of interest to evaluate if other microorganisms from the  
223 rhizosphere can favor the vegetative state of *Bacillus* species over sporulation.

224  
225 Taken together, our results show that, following application on plants, spores of  
226 both *B. subtilis* NCIB3610 and *B. velezensis* QST713 are rapidly differentiating into  
227 metabolically active cells in the rhizosphere, while those on the leaves and in the  
228 soil were more likely to stay as spores or to sporulate again after a short phase of  
229 germination. Thus, plant sites are clearly different in their impact on the proportions  
230 of dormant *Bacillus* in the population. Bacteria colonizing roots are significantly  
231 more active, strongly pointing toward the rhizosphere as being a key site for the  
232 establishment of a durable relationship between *B. subtilis* and/or *B. velezensis*  
233 and plants. These observations challenge the relevance of inoculating *Bacillus*-  
234 based biofertilizers on leaves since these spores do not appear to gain activities  
235 on this plant site. Inoculation directly into the soil, at root crown, or irrigation for the  
236 penetrance of bacteria will favor a better colonization of roots and the germination  
237 of spores into metabolically active cells. Understanding dynamics of the life cycle  
238 of *Bacillus* species used in commercial products in relation with crops for which  
239 they are used will allow us to improve how we use them. Such optimization should  
240 lead to an increase in the efficacy and reliability of various biofungicides and  
241 biopesticides used in organic agriculture.

242

## 243 Acknowledgments

244 The authors thank the company Plant Select (Saint-Paul d'Abbotsford, Québec,  
245 Canada) for the use of their facilities and the supply of plants, as well as Alain Baril  
246 and Stéphanie Théberge (Plant Select) for their support and help in this project.

247 They also thank Alain Lavigueur for the critical reading of the manuscript. This  
248 work was supported by an NSERC Engage grant 522757 - 17.

249 **Conflict of interest**

250 The authors declare no conflict of interest.

## 251 Bibliography

- 252 Ahemad, M., and Kibret, M. 2014. Mechanisms and applications of plant growth  
 253 promoting rhizobacteria: Current perspective. *J. King Saud Univ. - Sci.* **26**(1):  
 254 1–20. King Saud University. doi:10.1016/j.jksus.2013.05.001.
- 255 Aktar, M.W., Sengupta, D., and Chowdhury, A. 2009. Impact of pesticides use in  
 256 agriculture: their benefits and hazards. *Interdisciplinary Toxicology* **2**(1): 1–  
 257 12. doi:10.2478/v10102-009-0001-7.
- 258 Arkhipova, T.N., Veselov, S.U., Melentiev, A.I., Martynenko, E. V., and  
 259 Kudoyarova, G.R. 2005. Ability of bacterium *Bacillus subtilis* to produce  
 260 cytokinins and to influence the growth and endogenous hormone content of  
 261 lettuce plants. *Plant Soil* **272**(1–2): 201–209. doi:10.1007/s11104-004-5047-  
 262 x.
- 263 Berg, G. 2009. Plant-microbe interactions promoting plant growth and health:  
 264 Perspectives for controlled use of microorganisms in agriculture. *Applied*  
 265 *Microbiology Biotechnology* **84**(1): 11–18. doi:10.1007/s00253-009-2092-7.
- 266 Carvalho, F.P. 2017. Pesticides, environment, and food safety. *Food and Energy*  
 267 *Security* **6**(2): 48–60. doi:10.1002/fes3.108.
- 268 Charron-Lamoureux, V., and Beauregard, P. 2019. *Arabidopsis thaliana*  
 269 seedlings influence *Bacillus subtilis* spore formation. *Molecular Plant-*  
 270 *Microbe Interaction* **32**(9): 1188–1195. doi:10.1094/MPMI-10-18-0278-R.
- 271 Chen, Y., Yan, F., Chai, Y., Liu, H., Kolter, R., Losick, R., and Guo, J.H. 2013.  
 272 Biocontrol of tomato wilt disease by *Bacillus subtilis* isolates from natural  
 273 environments depends on conserved genes mediating biofilm formation.  
 274 *Environmental Microbiology* **15**(3): 848–864. doi:10.1111/j.1462-  
 275 2920.2012.02860.x.
- 276 Chowdhury, S.P., Hartmann, A., Gao, X.W., and Borriss, R. 2015. Biocontrol  
 277 mechanism by root-associated *Bacillus amyloliquefaciens* FZB42 - A review.  
 278 *Frontiers in Microbiology* **6**(780). doi:10.3389/fmicb.2015.00780.
- 279 Fan, B., Wang, C., Song, X., Ding, X., Wu, L., Wu, H., Gao, X., and Borriss, R.  
 280 2018. *Bacillus velezensis* FZB42 in 2018: The gram-positive model strain for  
 281 plant growth promotion and biocontrol. *Frontiers in Microbiology* **9**(OCT): 1–

- 282 14. doi:10.3389/fmicb.2018.02491.
- 283 Fierer, N. 2017. Embracing the unknown: Disentangling the complexities of the  
284 soil microbiome. *Nature Review in Microbiology* **15**(10): 579–590. Nature  
285 Publishing Group. doi:10.1038/nrmicro.2017.87.
- 286 Garbeva, P., van Veen, J.A., and van Elsas, J.D. 2004. Microbial diversity in soil:  
287 selection microbial populations by plant and soil type and implications for  
288 disease suppressiveness. *Annual Review Phytopathology* **42**: 243–70.  
289 doi:10.1146/annurev.phyto.42.012604.135455.
- 290 Hartmann, A., Rothballer, M., and Schmid, M. 2008. Lorenz Hiltner, a pioneer in  
291 rhizosphere microbial ecology and soil bacteriology research. *Plant Soil*  
292 **312**(1–2): 7–14. Springer Netherlands. doi:10.1007/s11104-007-9514-z.
- 293 Hashem, A., Tabassum, B., and Fathi Abd\_Allah, E. 2019. *Bacillus subtilis*: A  
294 plant-growth promoting rhizobacterium that also impacts biotic stress. *Saudi*  
295 *Journal of Biological Science* **26**(6): 1291–1297. King Saud University.  
296 doi:10.1016/j.sjbs.2019.05.004.
- 297 Hinsinger, P., Bengough, A.G., Vetterlein, D., and Young, I.M. 2009.  
298 Rhizosphere: Biophysics, Biogeochemistry and Ecological Relevance. *Plant*  
299 *Soil* **321**(1–2): 117–152. doi:10.1007/s11104-008-9885-9.
- 300 Lécuyer, F., Bourassa, J.-S., Gélinas, M., Charron-Lamoureux, V., Burrus, V.,  
301 and Beauregard, P.B. 2018. Biofilm formation drives transfer of the  
302 conjugative element ICEBs1 in *Bacillus subtilis*. *mSphere* **3**(5): e00473-18.
- 303 Lindow, S.E., and Brandl, M.T. 2003. Microbiology of the phyllosphere. *Appl.*  
304 *Environmental Microbiology* **69**(4): 1875–1883. doi:10.1128/AEM.69.4.1875-  
305 1883.2003.
- 306 Mendis, H.C., Thomas, V.P., Schwientek, P., Salamzade, R., Chien, J.T.,  
307 Waidyarathne, P., Kloepper, J., and De La Fuente, L. 2018. Strain-specific  
308 quantification of root colonization by plant growth promoting rhizobacteria  
309 *Bacillus firmus* I-1582 and *Bacillus amyloliquefaciens* QST713 in non-sterile  
310 soil and field conditions. *PLoS One* **13**(2): e0193119.  
311 doi:10.1371/journal.pone.0193119.
- 312 Moe, L.A. 2013. Amino acids in the rhizosphere: From plants to microbes.

- 313 American Journal of Botany **100**(9): 1692–1705. doi:10.3732/ajb.1300033.  
314 Nicholson, W.L., and Setlow, P. 1990. Sporulation, germination and outgrowth. *In*  
315 Molecular Biological Methods for *Bacillus*. John Wiley and Sons Ltd, New  
316 York. pp. 391–431.
- 317 Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., and Hens, L.  
318 2016. Chemical pesticides and human health: The urgent need for a new  
319 concept in agriculture. *Frontiers in public ealth.* **4**: 148. Frontiers Media S.A.  
320 doi:10.3389/fpubh.2016.00148.
- 321 Rudrappa, T., Czymmek, K.J., Pare, P.W., and Bais, H.P. 2008. Root-Secreted  
322 malic acid recruits beneficial soil bacteria. *Plant Physiology* **148**(3): 1547–  
323 1556. doi:10.1104/pp.108.127613.
- 324 Turner. 2013. The Plant Microbiome. *Adv. Bot. Res.* **69**: 279–309.
- 325 Yang, C.H., Crowley, D.E., Borneman, J., and Keen, N.T. 2001. Microbial  
326 phyllosphere populations are more complex than previously realized.  
327 *Proceedings of the National Acadademy of Sciences of the United States of*  
328 *America* **98**(7): 3889–94.
- 329 Zeriuoh, H., de Vicente, A., Pérez-García, A., and Romero, D. 2014. Surfactin  
330 triggers biofilm formation of *Bacillus subtilis* in melon phylloplane and  
331 contributes to the biocontrol activity. *Environmental Microbiology* **16**(7):  
332 2196–2211. doi:10.1111/1462-2920.12271.
- 333 Zhalnina, K., Louie, K.B., Hao, Z., Mansoori, N., Nunes da Rocha, U., Shi, S.,  
334 Cho, H., Karaoz, U. *et al.* 2018. Dynamic root exudate chemistry and  
335 microbial substrate preferences drive patterns in rhizosphere microbial  
336 community assembly. *Nature Microbiology* **3**(4): 470–480.  
337  
338

339 Figures legends

340

341 **Fig. 1.** Dynamics of population (CFU/g) and proportion of spores (%) of *B.*  
 342 *subtilis* and *B. velezensis* on *E. purpurea* SR (gray circle), FA (dark-gray square)  
 343 and *L. angustifolia* (black triangle) on leaves. **A) Total cells of *B. subtilis* on**  
 344 **leaves.** Statistical analysis showed a significant difference between *E.*  
 345 *purpurea* SR and the two other cultivars at day 1 and 4, and between *L.*  
 346 *angustifolia* and the other cultivars at day 15. **B) Total cells of *B. velezensis* on**  
 347 **leaves. C) Proportion of spores in *B. subtilis* population on leaves.** At day  
 348 15, statistical analysis demonstrated a difference between *L.*  
 349 *angustifolia* compared to the other plant species. **D) Proportion of spores in *B.***  
 350 ***velezensis* population on leaves.** Statistical analysis revealed significant  
 351 differences between *E. purpurea* FA and *L. angustifolia* at day 2, between *E.*  
 352 *purpurea* SR and *E. purpurea* FA at day 4, between *L. angustifolia* and the two  
 353 other plants at day 7, and between *L. angustifolia* and the two *Echinacea* plants at  
 354 day 15. For all panels, two biological replicates composed of 4 technical replicates  
 355 were combined. Error bars represent the standard error of the mean. Statistical  
 356 significance was assayed using One-way ANOVA, followed by Tukey's test (\* = P  
 357 < 0.05, \*\* = P < 0.01).

358

359 **Fig. 2.** Dynamics of population (CFU/g) and proportion of spores (%) of *B. subtilis*  
 360 and *B. velezensis* on *E. purpurea* SR (gray circle), cv. FA (dark-gray square) and  
 361 *L. angustifolia* (black triangle) in soil. **A) Total cells of *B. subtilis* in the soil.**  
 362 Statistical analysis showed significant differences between *E. purpurea* FA and the  
 363 two other cultivars at day 1 and 4. **B) Total cells of *B. velezensis* in the soil.** At  
 364 day 4, there was a statistical difference between *E. purpurea* SR and *L. angustifolia*  
 365 as well as between *E. purpurea* SR compared to the other plant species at day 7.  
 366 **C) Proportion of spores in *B. subtilis* population in soil.** Significant differences  
 367 were observed between *E. purpurea* FA and *L. angustifolia* at day 1 and between  
 368 *L. angustifolia* and *E. purpurea* SR at day 4. **D) Proportion of spores in *B.***  
 369 ***velezensis* population in the soil.** Statistical analysis revealed a significant

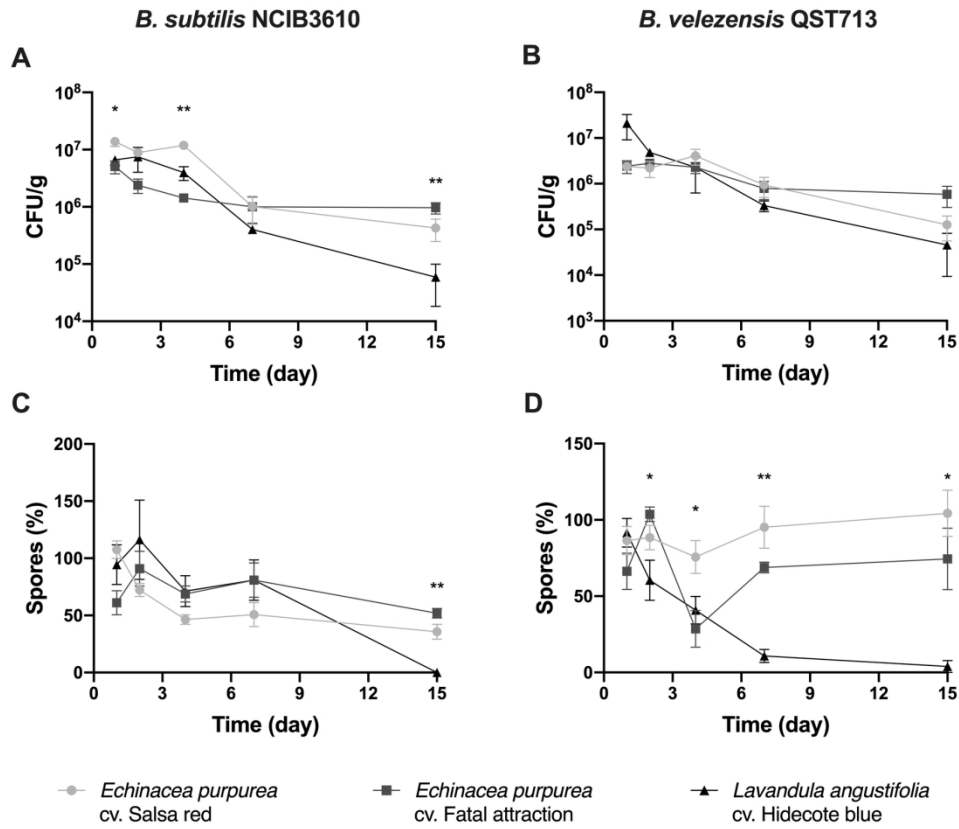
370 difference between *E. purpurea* FA and *L. angustifolia* at day 7. For all panels, two  
371 biological replicates composed of 4 technical replicates were combined. Error bars  
372 represent the standard error of the mean. Statistical significance was assayed  
373 using One-way ANOVA, followed by Tukey's test (\* =  $P < 0.05$ , \*\* =  $P < 0.01$ , \*\*\*  
374 =  $P < 0.001$ , \*\*\*\* =  $P < 0.0001$ ).

375

376 **Fig. 3.** Dynamics of population (CFU/g) and proportion of spores (%) of *B. subtilis*  
377 and *B. velezensis* on *E. purpurea* SR (gray circle), FA (dark-gray square) and *L.*  
378 *angustifolia* (black triangle) on roots. **A) Total cells of *B. subtilis* on roots.**  
379 Statistical analysis showed significant differences between all plants at day 7, and  
380 between *E. purpurea* FA, and the two other species at day 15. **B) Total cells of**  
381 ***B. velezensis* on roots.** At day 1, a statistical analysis demonstrated the  
382 difference between *E. purpurea* FA and *L. angustifolia*. Statistical analysis was  
383 evaluated using a t test. Letter "a" denotes a significant increase in total cells  
384 between day 1 and 2. **C) Proportion of spores in *B. subtilis* population on**  
385 **roots.** Significant differences were observed between *E. purpurea* FA and the  
386 other plants at day 1 and 2, and between *E. purpurea* FA and *L. angustifolia* at day  
387 7. **D) Proportion of spores in *B. velezensis* population on roots.** Statistical  
388 analysis revealed significant differences between *E. purpurea* FA and all other  
389 plants at day 1 and at day 7, between *E. purpurea* SR and *L. angustifolia*. For all  
390 panels, two biological replicates composed of 4 technical replicates were  
391 combined. Error bars represent the standard error of the mean. Statistical  
392 significance was assayed using One-way ANOVA, followed by Tukey's test (\* =  $P$   
393 < 0.05, \*\* =  $P < 0.01$ , \*\*\*\* =  $P < 0.0001$ ).

394

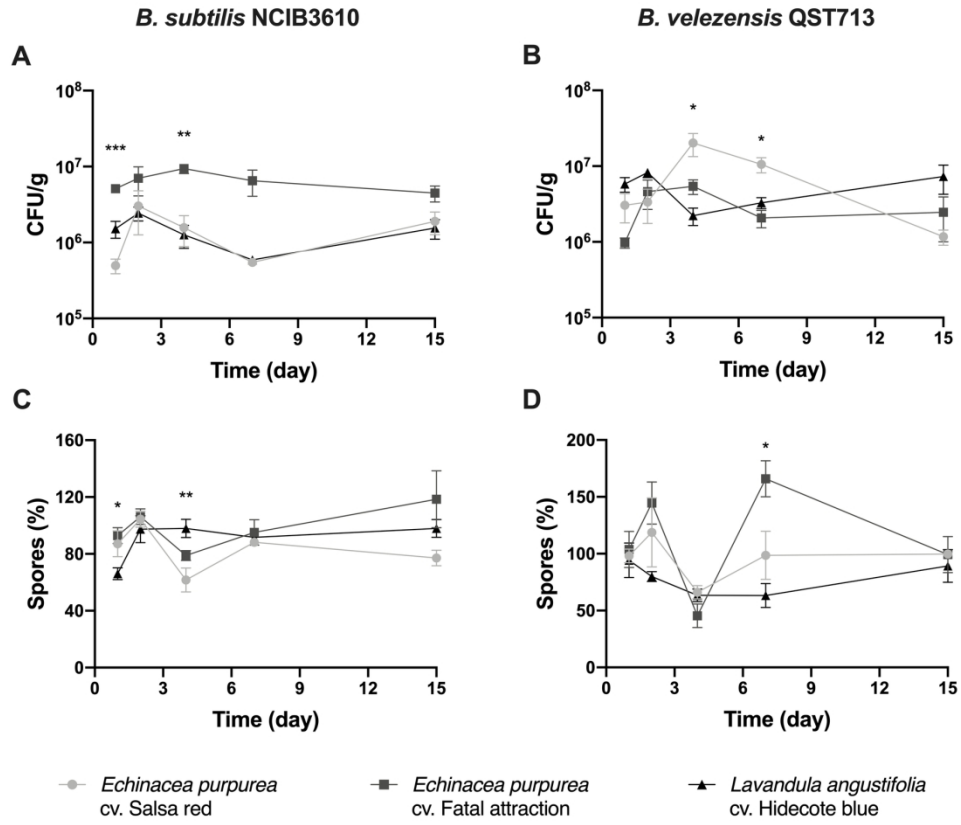
## LEAVES



82x72mm (600 x 600 DPI)

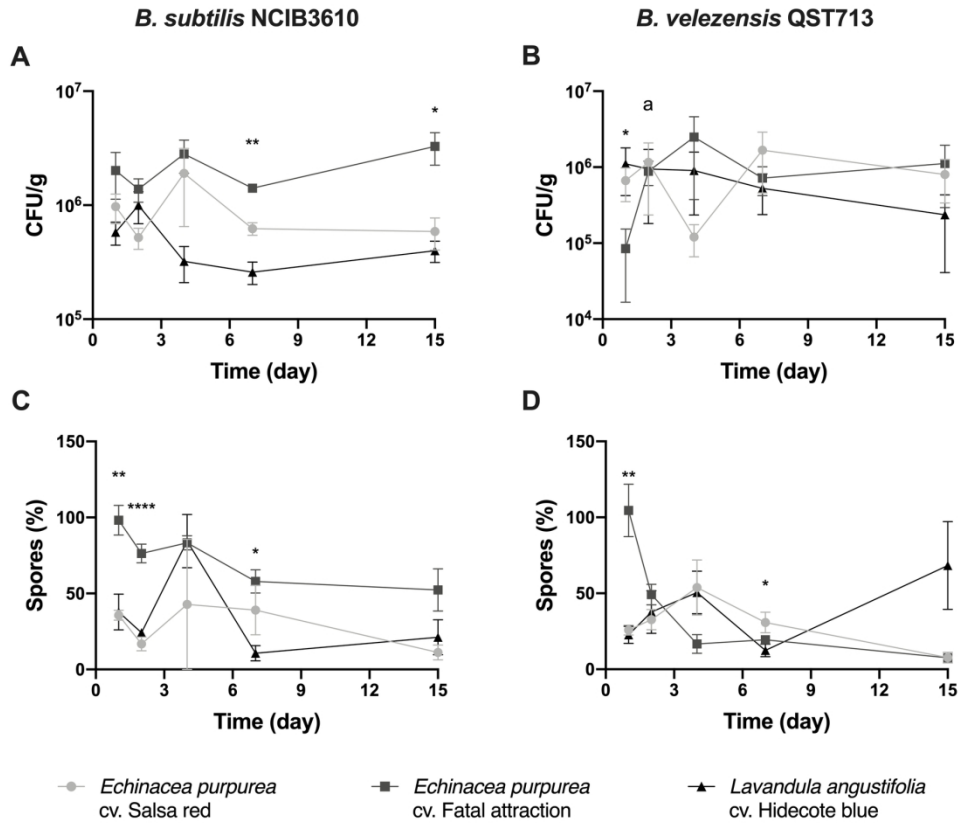


SOIL



82x72mm (600 x 600 DPI)

ROOTS



82x72mm (600 x 600 DPI)