Trehalose increases tomato drought tolerance, induces defenses, and increases resistance to bacterial wilt disease

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Contributions: AMM and CA designed the experiments and wrote the text with contributions from VM, AIP, and ZG. AMM carried out most of the experiments and created most of the figures. VM performed the meta-transcriptomic analysis. ZG performed the xylem sap metabolite analysis. SPA advised and assisted with LI-COR experiments and created the trehalose stomatal conductance, photosynthesis, transpiration, and water efficiency graphs. CJD assisted with trehalose physiology, ABA treatment, and *Xanthomonas* experiments. CDH advised on xylem sap collection. AIP, MK, CDH, and KM provided feedback and edited the manuscript.

Abstract

Ralstonia solanacearum causes bacterial wilt disease, leading to severe crop losses. Xylem sap from *R. solanacearum*-infected tomato is enriched in the disaccharide trehalose. Water-stressed plants also accumulate trehalose, which increases drought tolerance via abscisic acid (ABA) signaling. Because *R. solanacearum*-infected plants suffer reduced water flow, we hypothesized that bacterial wilt physiologically mimics drought stress, which trehalose could mitigate. We found that *R. solanacearum*-infected plants differentially expressed drought-associated genes, including those involved in ABA and trehalose metabolism, and had more ABA in xylem sap. Consistent with this, treating tomato roots with ABA reduced both stomatal conductance and stem colonization by *R. solanacearum*. Treating roots with trehalose increased xylem sap ABA and reduced plant water use by lowering stomatal conductance and temporarily improving water use efficiency. Trehalose treatment also upregulated expression of salicylic acid (SA)-dependent tomato defense genes; increased xylem sap levels of SA and other antimicrobial compounds; and increased bacterial wilt resistance of SA-insensitive *NahG* tomato plants. Additionally, trehalose treatment increased xylem concentrations of jasmonic acid and related oxylipins. Finally, trehalose-treated plants were substantially more resistant to bacterial wilt disease. Together, these data show that exogenous trehalose reduced both water stress and bacterial wilt disease and triggered systemic disease resistance, possibly through a Damage Associated Molecular Pattern (DAMP) response pathway. This suite of responses revealed unexpected linkages between plant responses to biotic and abiotic stress and suggested that *R. solanacearum*-infected plants increase trehalose to improve water use efficiency and increase wilt disease resistance. The pathogen may degrade trehalose to counter these efforts. Together, these results suggest that treating tomatoes with exogenous trehalose could be a practical strategy for bacterial wilt management.

Keywords: *Ralstonia solanacearum,* bacterial wilt disease, drought tolerance, trehalose, plant hormones, xylem water physiology

58 **Introduction**

59 *Ralstonia solanacearum* is a vascular plant pathogen that causes bacterial wilt disease in over
60 200 species of plants worldwide (Denny 2006). It is widely studied because it is economically damaging
61 to food, fiber, and feed production (Elphinstone 2005, Mansfield, Genin et al. 2012). *R. solanacearum* is
62 also notoriously difficult to eradicate because it persists long-term in soil, water, and latently infected
63 hosts (Álvarez, Bosca et al. 2010).

64 Most *R. solanacearum* strains infect plants through the soil (Elphinstone 2005). *R. solanacearum*
65 chemo- or aero-taxes towards roots, evades root border cell traps, enters roots at points of lateral root
66 emergence, moves to the root cortex, and eventually migrates to the xylem, all with the help of secreted
67 proteins such as hydrolytic enzymes and Type III secreted effectors (Vasse, Frey et al. 1995, Tans-
68 Kersten, Huang et al. 2001, Liu, Zhang et al. 2005, Yao and Allen 2006, Yao and Allen 2007, Digonnet,
69 Martinez et al. 2012, Deslandes and Genin 2014, Tran, MacIntyre et al. 2016). Once in xylem vessels, *R.*
70 *solanacearum* forms biofilm matrices composed of extracellular DNA and polysaccharide (EPS) and
71 proliferates to $>10^9$ CFU/g of stem tissue (Orgambide, Montrozier et al. 1991, Mori, Inoue et al. 2016,
72 Tran, MacIntyre et al. 2016). *R. solanacearum* also conditions the xylem environment to favor bacterial
73 growth (Lowe-Power, Hendrich et al. 2018). The large amounts of bacteria and EPS clog xylem vessels
74 and disrupt water flow (Ingel, Caldwell et al. 2021). If the plant cannot contain bacterial growth and
75 spread, it will wilt and eventually die.

76 Researchers long assumed that *R. solanacearum* and other vascular pathogens cause drought-
77 like physiological symptoms in host plants and that severity of the disease is affected by environmental
78 water availability (Abd El-Rahim, Fahmy et al. 1998, McElrone, Sherald et al. 2003, Daugherty, Lopes et
79 al. 2010). *R. solanacearum* dies more quickly in drought-stressed chickpea plants, and *R. solanacearum*
80 infection and water stress induce similar osmolyte biosynthesis and expression of genes related to the
81 plant signaling molecule abscisic acid (ABA), a well-characterized marker of water stress (Sinha, Gupta et
82 al. 2016, Sinha, Gupta et al. 2017). Further, stomatal conductance decreases in *R. solanacearum* infected
83 plants (Lowe-Power, Hendrich et al. 2018). These studies suggest but do not prove that plant response
84 to bacterial wilt mimics the drought stress response.

85 During water and osmotic stress, many plants, including tomatoes, accumulate trehalose and
86 trehalose-6-phosphate (T6P), which contribute to tolerance of these stresses (Romero, Bellés et al.
87 1997, Garg, Kim et al. 2002, Cortina and Culiáñez-Macià 2005, Lyu, Min et al. 2012, Khater, Dawood et

88 al. 2018). Xylem sap from *R. solanacearum*-infected tomato plants contains about 20-fold more
89 trehalose than healthy plants (Lowe-Power, Hendrich et al. 2018). This non-reducing disaccharide
90 composed of glucose and UDP-glucose is best known for its role as a compatible solute. Trehalose
91 synthesis helps *R. solanacearum* tolerate osmotic stress and contributes to pathogen fitness and
92 virulence (MacIntyre, Barth et al. 2019). However, the tomato host produces the trehalose in xylem sap
93 during infection; *R. solanacearum* does not export its trehalose in culture and it aggressively expresses
94 the secreted trehalase TreA *in planta* (Jacobs, Babujee et al. 2012).

95 The roles of trehalose and T6P as nutritional signaling metabolites in plants have been reviewed
96 (Lunn 2007, Paul 2007, Iordachescu and Imai 2008, Paul, Primavesi et al. 2008, Lunn, Delorge et al.
97 2014). In *Arabidopsis,* most trehalose metabolism-related proteins have predicted functions in
98 development or signaling (Vandesteene, Ramon et al. 2010, Vandesteene, Lopez-Galvis et al. 2012). The
99 current paradigm is that T6P, and by extension trehalose, are direct indicators of sucrose levels that help
100 plants maintain sucrose-to-starch ratios through SnRK1 signaling (Schluepmann, Pellny et al. 2003,
101 Kolbe, Tiessen et al. 2005, Lunn, Feil et al. 2006, Schluepmann, Berke et al. 2012). Trehalose also likely
102 improves drought tolerance in plants by two mechanisms: by regulating carbohydrate levels during
103 stress conditions, possibly via the SnRK1 kinase; and by altering ABA signaling and stomatal conductance
104 (Garg, Kim et al. 2002, Paul, Gonzalez-Uriarte et al. 2018, Yu, Zhao et al. 2019). Members of another
105 kinase family, SnRK2, also integrate with ABA signaling, and trehalose metabolism may connect to this
106 network via the TOR regulator (Signorelli, Tarkowski et al. 2019, Chen, Li et al. 2020).

107 Some studies suggest that trehalose may mediate host-microbe interactions and contribute to
108 plant defense. For example, treating *Arabidopsis* seedlings in liquid culture with 30 mM exogenous
109 trehalose triggered the expression of defense genes in ethylene and methyl-jasmonate pathways (Bae,
110 Herman et al. 2005). Infusing tobacco leaves with a 10-50 mM trehalose solution increased resistance to
111 tobacco mosaic virus and triggered a transcriptomic response suggestive of biotic and abiotic stress
112 tolerance (Shi, Sun et al. 2019). A *Xanthomonas citri* trehalose synthesis mutant had reduced fitness and
113 infusing citrus leaves with a trehalose solution induced defense gene expression, suggesting that
114 trehalose might simultaneously be a virulence factor and an indicator of pathogen attack (Piazza, Zimaro
115 et al. 2015). In another study, spraying tomato leaves with the trehalase inhibitor Validamycin A
116 increased resistance to bacterial wilt and *Fusarium* wilt and activated systemic acquired resistance
117 against a wide range of other pathogens in *Arabidopsis* (Ishikawa, Fujimori et al. 1996, Ishikawa,
118 Shirouzu et al. 2005, Bian, Duan et al. 2020). Additionally, silencing some tomato trehalose-6-phosphate

119 synthase genes decreased resistance to *Botrytis cinerea* and *P. syringae* pv. *tomato* DC3000 and affected
120 expression of JA, ET, and SA signaling responsive, defense related genes (Zhang, Hong et al. 2016).

121 We investigated the relationship between bacterial wilt and drought stress in tomatoes and
122 explored the role of trehalose in this interaction. We tested the following hypotheses: 1) *R.*
123 *solanacearum infection mimics abiotic stress triggered by disrupted water flow;* and 2) *Exogenous*
124 *trehalose inhibits bacterial wilt development in tomatoes by enhancing plant water use efficiency and*
125 *inducing defense response*s. To test H_o1, we used meta-transcriptomic analyses to show that tomato
126 genes involved in trehalose metabolism and water stress responses are differentially expressed during
127 *R. solanacearum* infection in susceptible tomato hosts. Using ABA treatment to close stomates, we
128 found that *R. solanacearum* depends on stomatal conductance and water flow for effective host
129 colonization. We also discovered that *R. solanacearum* infection increases xylem sap ABA levels.

130 Testing H_o2 revealed that treating plants with exogenous trehalose improved water use
131 efficiency in drought-stressed tomatoes, decreased short-term stomatal conductance and transpiration,
132 and increased ABA concentration in xylem sap. Trehalose-treated tomato plants were more resistant to
133 bacterial wilt. This protective effect appeared to be mediated by up-regulation of salicylic acid (SA)- and
134 jasmonic acid (JA)-related defense genes and by increased levels of jasmonates and other defense
135 compounds in xylem sap.

136

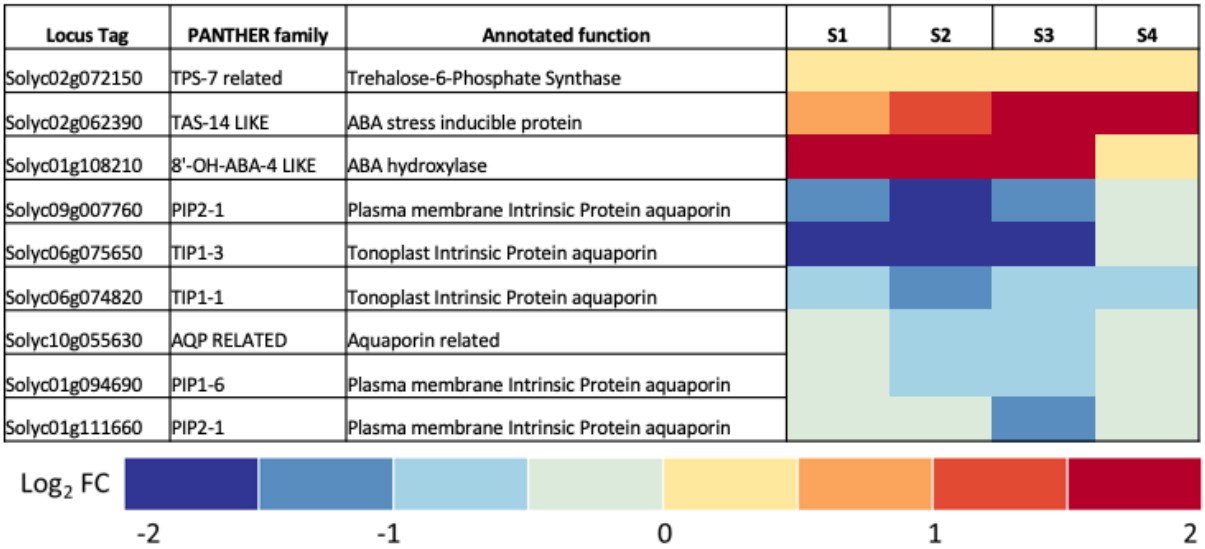
137 **Results**

138 **Bacterial wilt disease altered expression of tomato trehalose and water stress genes**

139 To investigate the relationship between bacterial wilt and drought stress, we measured
140 expression of all tomato genes annotated as related to trehalose metabolism, ABA, or water stress
141 responses. Candidate genes included those known or predicted to encode synthesis and degradation of
142 trehalose, including trehalose-6-phosphate phosphatase (TPP), trehalose-6-phosphate synthase (TPS),
143 and trehalase (Leyman, Van Dijck et al. 2001, Vandesteene, Ramon et al. 2010, Vandesteene, Lopez-
144 Galvis et al. 2012). Genes encoding water stress-associated proteins included: 1) those involved in
145 biosynthesis, regulation, and response to ABA, a known indicator of water stress (Song, Huang et al.
146 2016, Zhou, Yu et al. 2019); 2) late embryogenesis/dehydrins (LEAs), which accumulate in water-stressed
147 plants (Cao and Li 2015); and 3) aquaporins, channel proteins that allow living cells to move water and
148 small solutes more efficiently (Kapilan, Vaziri et al. 2018).

149 We used four datasets that profiled transcriptomes of two wilt-susceptible tomato plants
150 (WV700 and Bonny Best) responding to infection by *R. solanacearum* strain K60 or GMI1000 (French,
151 Kim et al. 2018). After normalizing the datasets to allow comparisons across these experiments, we
152 measured the expression of these candidate genes in healthy plants relative to their levels in *R.*
153 *solanacearum*-infected plants.

154 The datasets came from diverse experiments involving different plant genotypes, tissues, and *R.*
155 *solanacearum* strains. Despite considerable experiment-associated variation across datasets, this
156 analysis revealed that *R. solanacearum*-infected tomato plants significantly changed the expression of
157 several water stress physiology genes (Fig. 1). Nine genes meeting the annotation criteria were
158 significantly differentially expressed across all datasets. Notably, the trehalose-6-phosphate synthase
159 gene *SlTPS7* was upregulated in all of the susceptible tomatoes in response to *Rs,* suggesting this may be
160 the main T6P protein responsible for elevated trehalose levels in xylem sap during infection. All infected
161 plants upregulated an ABA hydroxylase and an ABA- and environmental stress-inducible dehydrin
162 protein TAS14 and downregulated many diverse aquaporins, consistent with transcript changes
163 observed in plants undergoing abiotic water stress. These results led us to directly measure
164 physiological and metabolic indicators of drought stress in *R. solanacearum*-infected plants.



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166 **Figure 1. *R. solanacearum* infection consistently changes expression of nine tomato genes involved in**
167 **trehalose metabolism and water stress physiology.** Meta-analysis of transcriptomic data from four
168 different experiments measuring gene expression of tomato plants responding to infection by *R.*
169 *solanacearum* identified nine genes of interest that were commonly differentially expressed across all

170 data sets. The heatmaps show relative expression (as $\log_2$ fold change) compared to mock-inoculated
171 control plants of all tomato genes that were both: 1) Annotated with the terms *trehalose*, *drought*, or
172 *abscisic acid* / *ABA*; and 2) Identified by the meta-transcriptomic analysis as significantly differentially
173 expressed across four different tomato RNAseq studies. The four studies were: **S1**, roots of wilt-
174 susceptible West Virginia 700 tomato plants (WV) sampled 24 h after infection with *R. solanacearum*
175 strain K60; **S2**, roots of WV sampled 48 h after infection with *R. solanacearum* strain K60; **S3**, seedling
176 roots of wilt-susceptible cv. Bonny Best (BB) sampled 24 h after infection with *R. solanacearum* strain
177 GMI1000; **S4**, mid-stems of BB sampled 72 h after inoculation with *R. solanacearum* strain GMI1000.
178 Tomato locus tags, gene names, and PANTHER families are from NCBI and the Sol Genomics Network,
179 accessed in 03/2020. Transcriptomic data were normalized across experiments to allow comparisons as
180 described in Methods.

181

182 **Bacterial wilt disease increased ABA concentration in xylem sap**

183 Plants can use ABA to mitigate drought stress. This plant hormone, synthesized locally in guard
184 cells or the vasculature, closes stomates to reduce water loss through transpiration (Raghavendra,
185 Gonugunta et al. 2010). To test the hypothesis that tomato plants produce ABA in response to bacterial
186 wilt, we used LCMS/MS to compare ABA levels in xylem sap of healthy plants and *R. solanacearum*-
187 inoculated plants showing early wilt symptoms. Xylem sap from the *R. solanacearum*-infected plants
188 contained 40% more ABA than sap from mock-inoculated plants (Fig. 2A).

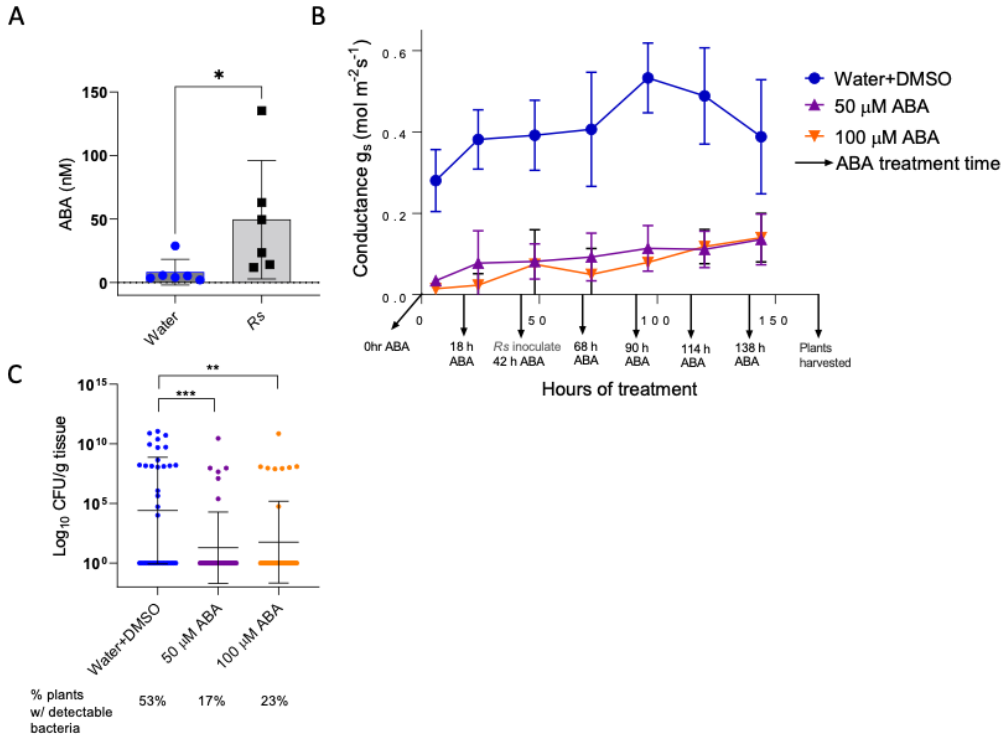


Figure 2. ABA concentration increased in *R. solanacearum*-infected plants, and ABA treatment reduced stomatal conductance, which correlated with reduced *R. solanacearum* stem colonization. A) ABA concentration in xylem sap harvested from healthy and *R. solanacearum*-infected Bonny Best tomato plants showing early wilt symptoms (1-25% of leaf area wilted) (T-test, **P*=.03). Bars show mean and standard deviation of xylem sap ABA levels, measured by GC-MS, from six plants per treatment. **B)** Stomatal conductance in tomato leaves as measured with LI-COR instrument. Conductance of plants watered with 50 or 100 uM of ABA differed from that of mock-inoculated controls at all time points (2-way ANOVA Tukey's multiple comparisons, *P*<.05). Data represent four biological replicate experiments, each with three plants analyzed per treatment per time point. Bars represent standard deviation. **C)** *R. solanacearum* population sizes as quantified in tomato plant stems five days after soil-soak inoculation with *R. solanacearum* strain GMI1000. *R. solanacearum* colonization was lower in ABA-treated plants at 5 dpi, which corresponds to 168 h of ABA treatment (Kruskal Wallis, 50 uM ABA, ****P*<.001; 100 uM ABA, ***P*<.01). The proportion of ABA-treated plants containing detectable *R. solanacearum* cells (shown below X-axis labels) was also lower (Chi-square, *P*=.0016). Dots represent *R. solanacearum* population sizes in individual plants. Black bars represent the geometric mean and standard deviation of the data. Plants with undetectable *R. solanacearum* populations were given a value of 1 for statistical analysis. Data represent three biological replicate experiments, each with 12 plants per treatment (36 total plants/treatment).

208

209 ***R. solanacearum* depends on host stomatal conductance to establish infection**

210 We previously observed that *R. solanacearum*-infected tomatoes had reduced stomatal
211 conductance (Lowe-Power, Khokhani et al. 2018). To test the hypothesis that the increased ABA levels
212 and lowered stomatal conductance in infected plants also affect bacterial wilt disease development, we
213 measured the effects of exogenous ABA treatment on stomatal conductance, transpiration rates, and *R.*
214 *solanacearum* colonization of susceptible tomato plant stems. We watered tomato plants daily with 50
215 mL of either 100 µM ABA, 50 µM ABA, or water as control and measured stomatal conductance four to
216 six hours later. After 48 h of ABA treatment, plants were drench-inoculated by adding a suspension of *R.*
217 *solanacearum* strain GMI1000 to the ABA treatment solutions, which we determined were neither
218 nutritious nor cytotoxic to *R. solanacearum* (Supp. Fig. 1). Treatment with ABA reliably reduced stomatal
219 conductance in the plants over the seven days of the experiment; 100 µM and 50 µM ABA treatment
220 had similar effects (Fig. 2B).

221

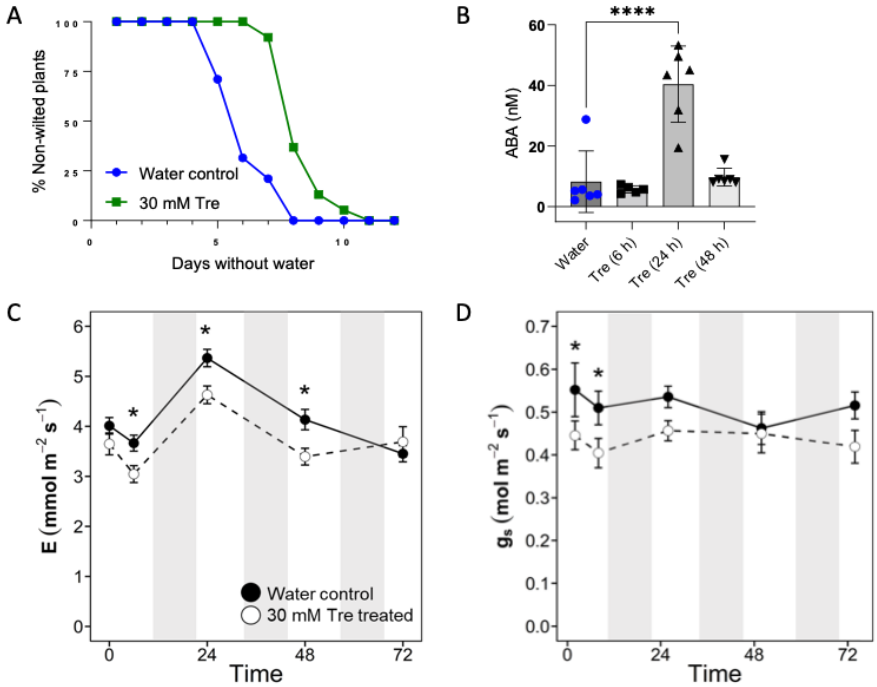
222 ABA treatments continued for four days after plants were inoculated with *R. solanacearum*. At
223 five dpi we measured *R. solanacearum* population sizes in the stems. Stems of ABA-treated plants were
224 less frequently colonized by *R. solanacearum* than control plants (50 µM ABA = 17% plants colonized;
225 100 µM = 23% plants colonized; water control = 53% plants colonized) and they contained lower *R.*
226 *solanacearum* population sizes than untreated plants (Fig. 2C). The correlation between ABA treatment,
227 reduced stomatal conductance, and lower *R. solanacearum* colonization in tomato stems suggests that
228 plant water movement assists *R. solanacearum* infection either at the roots or in the stem and that
229 reducing host water flow reduces or slows *R. solanacearum* spread in the plant.

230 **Trehalose treatment transiently improved water use efficiency in tomatoes**

231 Tomato plants become more drought tolerant following exposure to exogenous trehalose via
232 ABA signaling and stomatal closure (Yu, Zhao et al. 2019). Because ABA and trehalose concentrations
233 increased in xylem sap from *R. solanacearum*-infected plants and pathogen spread depends on host
234 xylem water transport, we asked whether exogenous trehalose could also protect plants from bacterial
235 wilt.

236 We first confirmed that trehalose increases tomato drought tolerance under our experimental
237 conditions. Two-week-old wilt-susceptible Bonny Best tomato plants were treated with 50 mL of 30 mM
238 trehalose solution or water and then were not watered for the rest of the experiment. Trehalose-
239 treated plants remained turgid for three days after the water-treated control plants began wilting,

240 showing that trehalose treatment delayed wilting from lack of water under our conditions (Fig. 3A).
241 Quantitative mass spectrometry analysis of tomato xylem sap revealed that ABA content was 5-fold
242 higher in trehalose-treated plants at 24 h (Fig. 3B). However, at 6 and 48 h after treatment, ABA levels
243 were no different from those of water-treated control plants. Gravimetric assays quantifying water loss
244 in trehalose-treated plants compared to water controls confirmed that trehalose-treated plants lose
245 water at a slower rate due to transpiration than controls (data not shown).



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247 **Figure 3. Trehalose treatment delayed tomato wilting due to drought, likely by transiently**
248 **increasing ABA content in xylem sap and by decreasing stomatal conductance and transpiration. A)**
249 Curves show how long it took plants to wilt following a single treatment with either water (blue) or
250 30 mM trehalose (green) and then no water for two weeks (*P*=.01, T-test of areas under the curve).
251 Data shown represent four biological replicates totaling 38 plants per treatment. **B)** ABA
252 concentration in trehalose treated plants (one-way ANOVA Fisher's LSD multiple comparisons to
253 water-treated control: 6 h tre *P*=.63; 24 h tre *P*<.0001; 48 h tre *P*=.76). Water controls were collected
254 concurrently with the treated samples and averaged. Data shown represent xylem sap samples from
255 six plants. **C)** Transpiration and **D)** stomatal conductance were measured with LI-COR. Asterisks
256 indicate *P*>.05, mixed-model ANOVA, Tukey's HSD. Data shown represent 20 plants/treatment.
257 Measurements were taken 10 min after treatment with trehalose or water, then each day after that
258 in the morning two hours after growth chamber lights went on. Shaded gray vertical bars on the

259 graphs represent night periods. Bars on the data points represent the standard deviation. All data
260 were calculated from the same LICOR time points.

261 Next, we assessed stomatal conductance, transpiration, and photosynthesis of trehalose-treated plants.
262 We measured tomato leaf responses with a LI-COR portable photosynthesis machine at 10 min, 6, 24,
263 48, and 72 h after treatment with 30mM trehalose or water. Transpiration in trehalose-treated plants
264 was lower than in controls at 6, 24, and 48 h (Fig. 3B). Relative to untreated plants, trehalose treatment
265 detectably reduced stomatal conductance after just 10 min, an effect that persisted for at least 6 h. By
266 24 h after treatment, stomatal conductance of treated plants was indistinguishable from that of water
267 controls (Fig. 3C). Water use efficiency, the relationship between water loss and plant productivity
268 (photosynthesis/transpiration), was temporarily improved at 6 h by trehalose treatment (Supp. Fig. 2A).
269 Trehalose treatment did not negatively affect photosynthesis (Supp. Fig. 2B).
270 We found that tomato plant growth was not significantly altered by trehalose treatment and the
271 leaf relative water content was the same in trehalose-treated and water-treated plants at 48h (Supp.
272 Fig. 2C). Further, 1-3 days of watering with trehalose solution did not affect tomato seedling root growth
273 *in vitro* relative to water controls (Supp. Fig. 2D). Chlorophyll content, measured by SPAD meter, was
274 transiently lower in trehalose treated plants 2-10 days after treatment, although it eventually returned
275 to the same levels as in untreated plants (Supp. Fig. 2E). Together, these diverse physiological and
276 biochemical analyses showed that treating tomato plants with exogenous trehalose temporarily
277 increased their drought tolerance, likely because trehalose increased ABA levels and ABA signaling
278 closes stomates.

279 **Trehalose is a potent inhibitor of bacterial wilt disease**

280 After finding that bacterial wilt impacts host water stress physiology and that exogenous
281 trehalose reduces stomatal conductance in tomatoes, we wondered if the decreased water movement
282 in trehalose-treated plants would slow bacterial wilt disease development. We therefore tested the
283 hypothesis that trehalose makes plants more wilt resistant. Forty-eight hours after plants had been
284 treated with 30 mM trehalose solution or water, they were drench inoculated with a suspension of *R.*
285 *solanacearum* bacteria. Bacterial wilt incidence and severity was reduced by 25-75% across biological
286 replicates in the trehalose-treated plants, demonstrating that exogenous trehalose increases tomato
287 resistance to bacterial wilt (Fig. 4A).

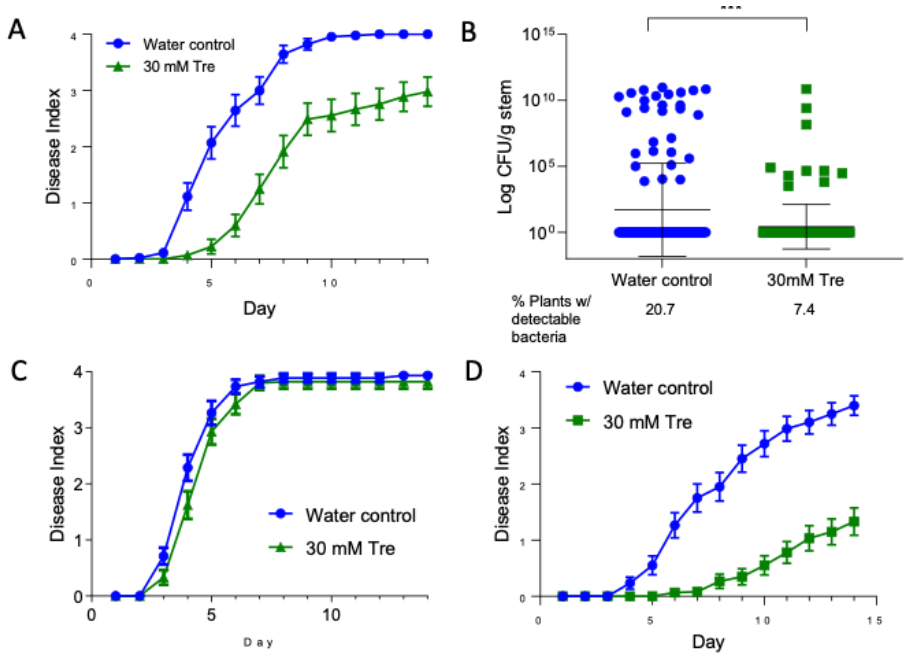


Figure 4. Treating plants with trehalose at the root delayed bacterial wilt symptoms and reduced *R. solanacearum* stem colonization, but only following soil inoculation. A) Wilt disease progress of cv. Bonny Best tomato plants treated with trehalose, then after 48 h soil soak inoculated with *R. solanacearum* GMI1000. Disease symptoms were rated on a 0-4 disease index scale where 0=no wilt, and 4=76-100% wilted or dead. Trehalose treatment delayed appearance of wilt symptoms (*P*=.0082, t-test comparing areas under the curves or AUC). Data represent three replicate experiments, each with 15 plants/treatment. Bars represent standard error. **B)** Bacterial population sizes were smaller in stems of trehalose-treated plants at 5-7 days after inoculation (Mann-Whitney, *P*=.0009). Data represent three biological replicates with 15 plants/day sampled/treatment; each dot represents the *R. solanacearum* population size in one plant (including plants without detectable *R. solanacearum*). Black bars represent geometric mean and standard deviation of the data. The percentage of plants that contained any detectable *R. solanacearum* cells is shown below the X-axis; the limit of detection is ~10^2 cells. Relative to controls, fewer trehalose-treated plants contained detectable *R. solanacearum* populations (Chi-square, *P*=.0016). C**)** Disease progress of plants treated with trehalose (green) or water (blue), then 48 h later inoculated by introducing *R. solanacearum* directly into the xylem through a cut leaf petiole. Trehalose treatment did not affect wilt disease development in petiole-inoculated plants (*P*=.2067, T-test of AUC). Data represent three replicate experiments, each containing 15 plants/treatment. Bars represent standard error. **D)** Disease progress of plants treated with 30 mM trehalose or water, then 120 h later inoculated with *R. solanacearum* the soil drench

308 method (*P*=.0003, T-test of AUC) Data represent four replicate experiments, each containing 15
309 plants/treatment. Bars represent standard error of the mean.
310
311 Since *R. solanacearum* can infect plants without causing visible symptoms, the absence of wilt in
312 trehalose treated plants could indicate either the lack of disease or latent infection. To distinguish
313 between these possibilities, we measured *R. solanacearum* colonization of tomato midstems at 5, 6, and
314 7 dpi. Water-treated control plants were three times more likely to contain detectable *R. solanacearum*
315 cells than trehalose-treated plants (20.7% compared to 7.4%), and control plants contained significantly
316 higher bacterial population sizes (Fig. 4B). This showed that trehalose treatment did not increase the
317 frequency of latent infections but rather that *R. solanacearum* was less likely to colonize stems of
318 trehalose treated plants. The reduced colonization frequency at this relatively early time point is
319 consistent with the observed trehalose-associated delay in disease development.
320 We tested the protective limits of trehalose treatment by varying inoculation sites, treatment
321 methods, and pathogens. Interestingly, inoculating *R. solanacearum* cells directly into the xylem through
322 a cut petiole overwhelmed any protective benefit of trehalose treatment (Fig. 4C). This result suggests
323 that *R. solanacearum* must overcome a trehalose-enhanced checkpoint at or in tomato roots to colonize
324 plant stems. However, the protective effect of root drenching is enduring; trehalose treatment reduced
325 bacterial wilt incidence even when plants were inoculated five days later (Fig. 4D).
326 *R. solanacearum* produces a trehalase enzyme and can use trehalose as a carbon source, so we
327 wondered if treating tomato roots with trehalose nutritionally enriched the soil environment, thereby
328 de-incentivizing *R. solanacearum* root invasion. We tested this hypothesis by inoculating trehalose-
329 treated tomato plants with either wild-type *R. solanacearum* or a Δ*treA* trehalase mutant that cannot
330 grow on trehalose (MacIntyre, Barth et al. 2019). Trehalose treatment similarly reduced disease caused
331 by wild type and Δ*treA R. solanacearum*, so bacterial catabolism of trehalose cannot explain trehalose
332 treatment's protective effect (Suppl. Fig. 3A).
333 Spraying trehalose solution onto leaves and then inoculating *R. solanacearum* by soil-soak 48 h
334 later also did not affect bacterial wilt progress (Supp. Fig. 3B). To explore the effects of trehalose
335 treatment on another bacterial pathogen, we dip-inoculated tomato leaves with the tomato leaf spot
336 pathogen *Xanthomonas gardneri* 72 h after plant roots were treated with trehalose. Trehalose had no
337 significant effect on leafspot disease severity or *X. gardneri* colonization of leaves sampled 3, 6, and 9
338 dpi (Supp. Fig. 3C). These experiments demonstrated that trehalose needs to be applied at the roots to
339 protect against bacterial wilt and that trehalose might increase resistance only to *R. solanacearum.*

340 **Exogenous trehalose increases the expression of tomato defense genes, and SA signaling may mediate**
341 **its protective effect**

342 Although trehalose treatment of roots decreased plant transpiration and stomatal conductance
343 temporarily (6-48 h), this treatment protected plants from bacterial wilt for at least five days (Fig. 4D),
344 suggesting that trehalose induced plant defense responses. We tested this by quantifying the effect of
345 trehalose treatment on defense gene expression, bacterial wilt resistance of defense hormone-
346 insensitive tomato plants, and levels of hormones and defense compounds.

347 To measure the effect of trehalose on defense gene expression, we extracted RNA from tomato
348 stem sections 6 or 48 h after roots were treated with 30 mM trehalose solution or water. We measured
349 expression of two JA-responsive genes, *PIN2* and *LOXA* (Peña-Cortés, Fisahn et al. 1995, Zhao, Thilmony
350 et al. 2003). *PIN2* was significantly downregulated 6 h after trehalose treatment (Fig. 5A), suggesting JA
351 levels may have been affected by trehalose treatment, while *LOXA* expression did not change after
352 trehalose treatment. G*LUA*, *PR1A*, and *PR1B* are tomato pathogenesis-related (PR) genes induced by SA
353 and by pathogen challenge (Joosten and De Wit 1989, Tornero, Gadea et al. 1997, Block, Schmelz et al.
354 2005). Both *GLUA* and *PR1A* were expressed 10-fold more in trehalose-treated plants compared to
355 water controls 6 h after treatment (Fig. 5A) and their expression increased to over 100-fold at 48 h (Fig.
356 5B). *PR1B* and *OSM,* which respond to the defense hormone ethylene (ET), were also significantly
357 upregulated 48 h after trehalose treatment (Tornero, Gadea et al. 1997, Smart, Myers et al. 2003). The
358 ET synthesis gene *ACO5* was also 10-fold more highly expressed compared to water controls 6 and 48 h
359 after trehalose treatment (Fig. 5A+B) (Jacobs, Milling et al. 2013). Transcripts of the ABA-responsive
360 genes *RD22* and *DHN_TAS* were slightly but significantly upregulated 48 h after trehalose treatment,
361 possibly in response to the increased levels of ABA in xylem sap shown in Fig. 5B (Yamaguchi-Shinozaki
362 and Shinozaki 1993, Shinozaki, Yamaguchi-Shinozaki et al. 2003, Kissoudis, Seifi et al. 2016). Together,
363 these qRT-PCR experiments revealed that tomato plants respond to trehalose by increasing expression
364 of genes in JA, SA, ET, and ABA hormonal pathways involved in, among other things, biotic and abiotic
365 responses.

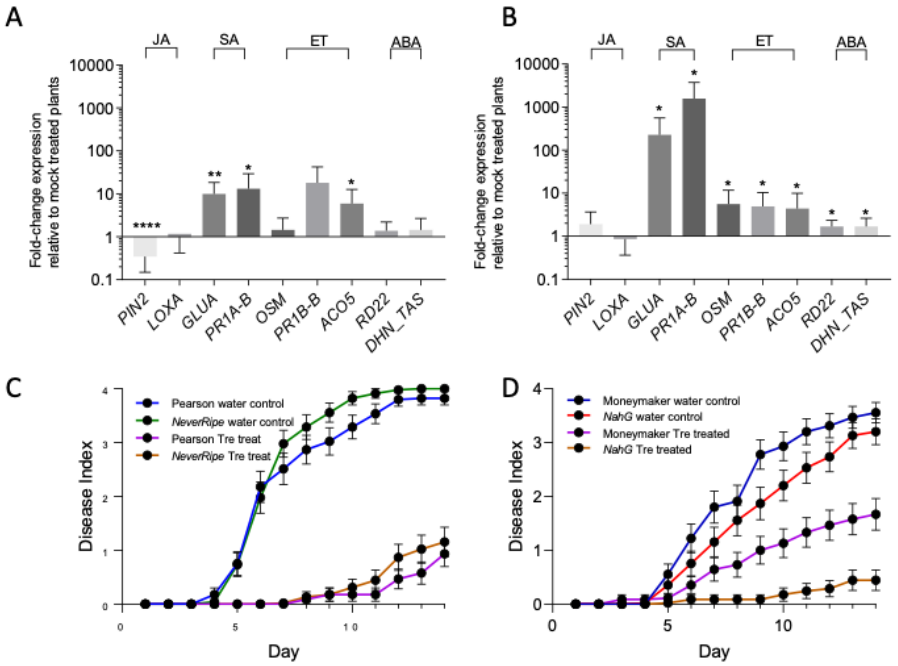


Figure 5. Exogenous trehalose treatment affects expression of hormone-responsive tomato defense genes, and trehalose-mediated disease resistance is partially regulated by salicylic acid signaling. Effects of trehalose treatment on expression of diverse defense genes in Bonny Best tomato stems, determined by qRT-PCR at **A)** 6 h and **B)** 48 h after trehalose treatment at the roots (one-sample t-test to a hypothetical mean of 1.0 where 1.0 = no difference in expression; asterisks: *, *P*=.01-.05; **, *P*=.001-.01; ***, *P*=.0001-.001; ****, *P*<.0001). Expression levels are shown as fold-change relative to those in mock (water)-treated plants. Data represent three biological replicates per time point, each containing five plants per treatment. Bars represent standard deviation. **C)** Wilt disease progress following *R. solanacearum* inoculation of water- or trehalose-treated *NeverRipe* ET-insensitive tomatoes and their parent cultivar 'Pearson' (one-way ANOVA of Area Under the Curve or AUC; 'Pearson' H_20 vs. *NeverRipe* H_20, *P*=.91; 'Pearson' tre vs. *NeverRipe* tre, *P*=.98). **D)** Wilt disease progress following *R. solanacearum* inoculation of water- or trehalose-treated SA-insensitive *NahG* over-expressing plants and their parent cv. Moneymaker (one-way ANOVA of AUC, Moneymaker H_20 control vs. *NahG* H_20 control, *P*=.74; Moneymaker tre vs. *NahG* tre, *P*=.04). For **C)** and **D**) plants were treated at the roots with trehalose, then inoculated with *R. solanacearum* via soil drenching 48 h later. Data represent three biological replicates, each containing 15 plants/treatment (45 plants total). Bars represent standard error.

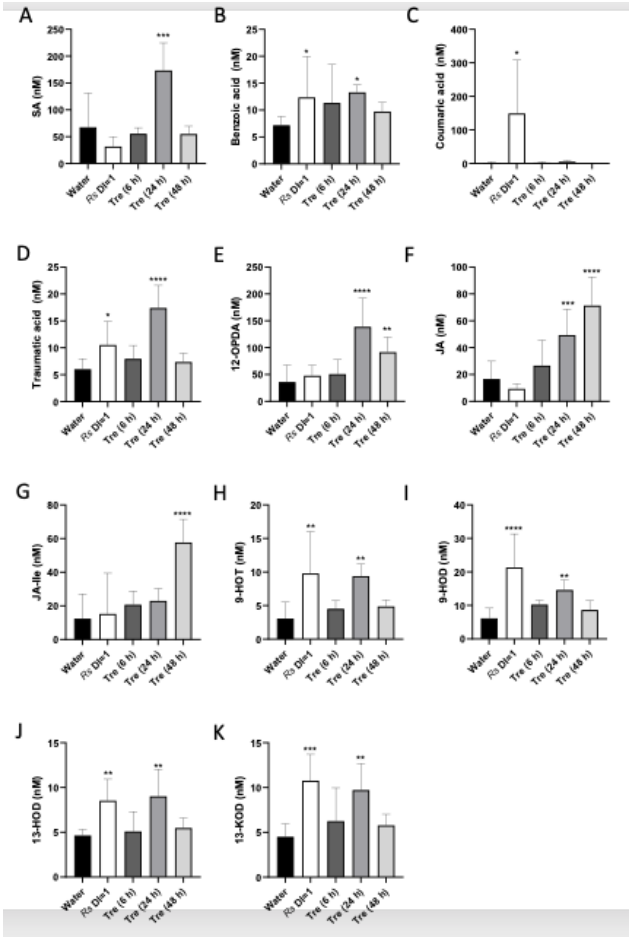
384 The finding that SA- and ET-responsive tomato genes were upregulated 6 and 48 h after
385 trehalose treatment suggested that ET perception and SA responses might be necessary for trehalose-
386 mediated increase in bacterial wilt resistance. We tested this hypothesis by treating with trehalose the
387 roots of ET-insensitive *NeverRipe* mutant tomatoes (cv. Pearson) and SA-degrading *NahG* transgenic
388 tomatoes (cv. Moneymaker). After 48 h, all plants were soil drench inoculated with *R. solanacearum*. As
389 observed for cv. Bonny Best, trehalose treatment increased bacterial wilt resistance of Pearson and
390 Moneymaker plants. Trehalose also increased bacterial wilt resistance of both the *NeverRipe* and *NahG*
391 plants compared to untreated controls (Fig. 5C and 5D). However, trehalose-mediated bacterial wilt
392 resistance does not require ET perception since bacterial wilt developed similarly on trehalose-treated
393 *NeverRipe* and the parental Pearson genotype ($P<.98$). In contrast, trehalose-treated *NahG* tomatoes
394 were significantly more resistant to bacterial wilt than trehalose-treated Moneymaker parental line
395 ($P<.04$). These results suggest that either a lack of downstream SA responses or overaccumulation of SA
396 degradation products contributes to the trehalose-mediated increase in bacterial wilt resistance.
397 Alternatively, trehalose may improve resistance downstream of SA responses.
398 More than half of the *NahG* tomato plants displayed a transient marginal leaf necrosis
399 phenotype following trehalose treatment (data not shown), which could have been due to increased
400 ROS production. To test this, we infused leaves from four-week-old wild type Bonny Best, Moneymaker,
401 or *NahG* tomato plants with water, *R. solanacearum* cells, or 30 mM trehalose solution and observed
402 ROS production with DAB staining. Infusing leaves with trehalose did not trigger excess ROS production
403 (Supp. Fig. 4), suggesting that another physiological effect caused the transient marginal necrosis in the
404 *NahG* plants.

405 **Trehalose treatment promoted accumulation of hormones and defense compounds in xylem sap**

406 To better understand the mechanisms of trehalose-mediated wilt resistance, we measured
407 levels of a suite of plant hormones, oxylipins, and defense-related compounds in xylem sap from
408 infected and trehalose-treated Bonny Best tomatoes. We harvested sap from tomatoes displaying a
409 disease index of 1 (DI=1, partially wilted, late-stage infection) and from uninoculated plants at 6, 24, and
410 48 h after trehalose treatment. Using targeted quantitative mass spectrometry, we measured
411 metabolites from the phenylpropanoid biosynthesis pathway, several oxylipins from the 9-lipoxygenase
412 and 13-lipoxygenase pathways, and other selected defense metabolites (Supplemental Table 2).

413 Sap from *R. solanacearum*-infected plants and trehalose-treated plants contained different
414 levels of targeted metabolites compared to sap from healthy tomatoes. Sap collected 24 h after
415 trehalose treatment had the highest number of enriched compounds. These were consistent with a

416 shock or wound response even though plants were not wounded (Vallad and Goodman 2004).
417 Surprisingly, trehalose treatment, but not *R. solanacearum* infection, increased SA concentration at 24 h
418 (Fig. 6A). This result could mean that trehalose plays a role in SA signaling and suggested that SA might
419 not have a significant role in xylem sap at late-stage disease. However, benzoic acid levels did increase in
420 xylem sap from both trehalose treated and *R. solanacearum* infected plants (Fig. 6B). Benzoic acid is a
421 potent antimicrobial compound produced through the phenylpropanoid pathway that can be a direct
422 precursor to SA (Chen, Zheng et al. 2009, Widhalm and Dudareva 2015). Interestingly, coumaric acid,
423 another phenylpropanoid defense compound that *R. solanacearum* can degrade, was significantly
424 increased in sap from *R. solanacearum*-infected plants but not in sap from trehalose treated plants (Fig.
425 6C) (Lowe, Ailloud et al. 2015). Levels of traumatic acid, which results from the oxidation of traumatin
426 produced by wounded plants, were higher in sap from both *R. solanacearum*-infected plants and
427 trehalose-treated plants at 24 h (Fig. 6D) (Zimmerman and Coudron 1979).



428

429 **Figure 6. Trehalose treatment increased metabolites related to defense and wounding in tomato**
430 **xylem sap**. LC-MS/MS determined concentrations of hormones, oxylipins, and other metabolites in

431 xylem sap collected from cv. Bonny Best tomato plants that were mock-inoculated (H_2O), inoculated
432 with *R. solanacearum* GMI1000 when wilt symptoms first appeared (*Rs* DI=1), or had been treated at
433 the roots with 30mM trehalose (Tre (6h), etc.) Differences from water-treated control plants were
434 determined by one-way ANOVA Fisher's LSD multiple comparison test: *, *P*=.01-.05; **, *P*=.001-.01;
435 ***, *P*=.0001-.001; ****, *P*<.0001. Data shown represent the means of samples from 4-6 individual
436 plants. Bars indicate mean, vertical lines show standard deviation. **A)** SA=salicylic acid; **B)** BA=benzoic
437 acid; **C)** COUMA=coumaric acid; **D)** TA= Traumatic acid; **E)** 12-OPDA= 12-oxo-8(*Z*),13(*Z*)-phytodienoic
438 acid; **F)** JA=jasmonic acid; **G)** JA-Ile=jasmonic acid isoleucine; **H)** 9-HOT= 9(*S*)-hydroxy-
439 10(*E*),12(*Z*),15(*Z*)-octadecatrienoic acid; **I)** 9-HOD= 9(*S*)-hydroxy-10(*E*),12(*Z*)-octadecadienoic acid; **J)**
440 13-HOD= 9(*S*)-hydroxy-10(*E*),12(*Z*)-octadecadienoic acid; and **K)** 13-KOD= 13-oxo-9(*Z*),11(*E*)-
441 octadecadienoic acid.

442 The chemical signatures exclusively present in trehalose treated plants were the persistent
443 increases in JA, the JA precursor 12-OPDA, and JA-Ile, the biologically active form of JA (Fig. 6E-G). This
444 accumulation of jasmonates, together with the upregulation of JA and ET-pathway defense genes,
445 suggested that trehalose treatment induces a systemic defense response that contributes to its
446 protective effect (Vallad and Goodman 2004, Choudhary, Prakash et al. 2007).

447 Curiously, several defense-related oxylipins from the reductase and lipoxygenase branches of
448 the LOX pathway were enriched in sap from *R. solanacearum*-infected plants and 24 h post-trehalose
449 treatment plants (Fig. 6H-K). These oxylipins include 9(*S*)-hydroxy-10(*E*),12(*Z*),15(*Z*)-octadecatrienoic
450 acid (9-HOT, Fig. 6H); 9(*S*)-hydroxy-10(*E*),12(*Z*)-octadecadienoic acid (9-HOD, Fig. 6I); 13(S)-hydroxy-
451 9(Z),11(E)-octadecatrienoic acid (13-HOD, Fig. 6J); and 13-oxo-9(*Z*),11(*E*)-octadecadienoic acid (13-KOD,
452 Fig. 6K). All these oxylipins have antimicrobial and signaling activity in defense against pathogens (Prost,
453 Dhondt et al. 2005, Vicente, Cascón et al. 2012, Marcos, Izquierdo et al. 2015). Induction of these
454 oxylipins in this study suggests involvement in resistance to bacterial wilt. Altogether, trehalose
455 treatment had an unexpectedly large impact on xylem levels of defense metabolites, particularly
456 jasmonates and SA, which have often been considered antagonistic signals.

457

458 **Discussion**

459 To survive drought, plants must respond to and overcome multiple adverse physiological events
460 (Shinozaki, Yamaguchi-Shinozaki et al. 2003, Osakabe, Osakabe et al. 2014). Our stringent meta-analysis

461 of four different transcriptomic studies found that plants infected with *R. solanacearum* have a gene
462 expression signature like that of water-stressed plants, suggesting that bacterial wilt disease
463 physiologically mimics abiotic drought stress. The consistency of this response across multiple tomato
464 cultivars suggest that this is a robust, replicable phenomenon.

465 Infected tomatoes slightly upregulated TPS7, (Zhang, Hong et al. 2016), suggesting this gene
466 could be responsible for the observed increased trehalose synthesis during bacterial wilt. This gene was
467 also upregulated 36 h after infection with *P. syringae and* silencing this gene (annotated as *SlTPS3* in
468 that study) decreased tomato trehalose content in response to the fungal pathogen *Botrytis cinerea* and
469 also decreased plant resistance to *B. cinerea* (Zhang, Hong et al. 2016).

470 In response to *R. solanacearum* infection, tomatoes downregulated multiple aquaporins,
471 suggesting that plants may try to alter water flow during bacterial wilt. In particular, SlTIP2;2
472 (Solyc03g120470), an aquaporin that improved transpiration and yield when overexpressed in tomato
473 plants, was notably downregulated in our meta-transcriptomic analysis (Sade, Vinocur et al. 2009).
474 Accordingly, *Arabidopsis* PIP family aquaporin proteins were generally downregulated during drought
475 stress (Alexandersson, Fraysse et al. 2005). Tomato aquaporins are encoded by diverse families and
476 channel other solutes in addition to water, so it would not be expected that all aquaporins play the
477 same role during bacterial wilt (Reuscher, Akiyama et al. 2013, Patel and Mishra 2021).

478 An ABA-inducible dehydrin and an ABA hydroxylase gene were strongly upregulated in infected
479 plants. In *Arabidopsis* and tomato, CYP707A ABA hydroxylases, which catalyze the first step in ABA
480 degradation, are involved in dehydration and rehydration (Kushiro, Okamoto et al. 2004, Saito, Hirai et
481 al. 2004, Umezawa, Okamoto et al. 2006, Ji, Kai et al. 2014). This suggests both that tomatoes tightly
482 regulate ABA catabolism during bacterial wilt and that bacterial wilt causes water stress. Expression of
483 TAS14, a dehydrin inducible by environmental stress and ABA, was highly upregulated in infected plants,
484 further evidence that bacterial wilt causes drought stress (Godoy, Pardo et al. 1990). *Arabidopsis* ABA
485 synthesis, signaling, and responsive genes were similarly affected during late-stage bacterial wilt
486 infection (Hu, Barlet et al. 2008). Elevated ABA levels are usually associated with drought stress;
487 together with the observation that *R. solanacearum* infection lowers plant stomatal conductance, this
488 indicates that bacterial wilt disease significantly disrupts tomato water physiology. Further
489 characterization of ABA-responsive networks in tomatoes could identify targets for breeding more
490 hydraulically efficient tomatoes that are also more resistant to bacterial wilt.

491 Our data suggest that there might be an indirect defensive component to ABA concentration
492 and signaling. The levels of ABA in xylem sap increased in response to both *R. solanacearum* infection
493 and trehalose treatment, and our transcriptomic data suggest tomato plants degrade ABA during
494 disease via an ABA hydroxylase. Although it would be interesting to test the effects of trehalose and *R.*
495 *solanacearum* on tomato ABA synthesis mutants, existing ABA mutants (*flacca, notabilis, sitiens)* share a
496 wilting phenotype due to ethylene overproduction and lack of control over stomatal closure. This
497 physiological wilting interferes with assessing pathogen-caused wilting symptoms (Tal 1966, Thompson,
498 Thorne et al. 2004, Kaiser, Morales et al. 2020). However, *Arabidopsis* studies also indicate that ABA is
499 important for tomato resistance to wilt disease. Deleting several cellulose synthase (*irx*) genes increased
500 *Arabidopsis* resistance to bacterial wilt in an ABA-dependent manner and ABA synthesis mutants were
501 more susceptible to *R. solanacearum* (Hernández-Blanco, Feng et al. 2007). Further, ABA perception
502 mutants are also hyper-susceptible to bacterial wilt (Zhao, Wang et al. 2019).
503 Treating tomato roots with ABA reduced both stomatal conductance and *R. solanacearum*
504 colonization of stems, suggesting that *R. solanacearum* spread depends, at least in part, on active
505 hydraulic conductance and a negative pressure gradient in host xylem. It has long been assumed that *R.*
506 *solanacearum* uses the plant's own water transport to spread within its host, but this is now
507 demonstrated experimentally. When this pathogen blocks host plant water flow, it decreases its own
508 spread and pathogenic fitness. Interestingly, some wilt-resistant tomato varieties thrive even when they
509 are latently infected. This supports the theory that resistant hosts can, to some extent, locally limit
510 vascular bacterial growth enough to prevent occlusion of xylem vessels (Beattie 2011). We speculate
511 that ancestors of *R. solanacearum* were once endophytes, but lost the ability to moderate their
512 population growth in susceptible hosts, possibly as a result of evolutionary selection pressures imposed
513 by agriculture, as has been proposed for *Xylella fastidiosa* (Beattie and Lindow 1995).

514 In our system, exogenous trehalose temporarily improved water conservation in uninfected
515 tomatoes and delayed abiotic wilting of drought-stressed plants. This was likely because trehalose
516 treatment temporarily reduced stomatal conductance and transpiration and increased water use
517 efficiency. Trehalose increased ABA content in xylem sap 24 h post-treatment, and disease symptoms
518 often occurred after ABA concentration peaked, consistent with a potential signaling role for ABA.
519 These results confirm and extend findings that trehalose, ABA signaling, and water use interact in
520 complex ways. Additional studies are needed to connect ABA signaling and concentration in xylem sap
521 to downstream effects on disease resistance.

522 Other *Arabidopsis* and tomato trehalose/water stress studies have confirmed some of the
523 phenotypes we observed. The *Arabidopsis TPS1* gene, which is responsible for maintaining T6P levels,
524 responds to ABA and helps regulate stomatal aperture (Avonce, Leyman et al. 2004, Gomez, Gilday et al.
525 2010). At least two *Arabidopsis* TPP proteins (AtTPPI and AtTPPE) that are controlled by ABA-responsive
526 transcription factors contribute to trehalose production and mitigate drought stress (Lin, Yang et al.
527 2019, Lin, Wang et al. 2020, Wang, Chen et al. 2020). Yu and co-workers found that applying trehalose
528 to tomato roots improved water conservation, decreased ABA biosynthesis gene expression, increased
529 expression of ABA-responsive genes, kept stomates closed five days after trehalose treatment without
530 water, and increased ABA levels in leaves after 24 h (Yu, Zhao et al. 2019). Our results contrast in some
531 ways with this paper. We found that ABA content temporarily increased in xylem sap 24 h after
532 trehalose treatment, while stomatal conductance was reduced for less than 24 h. These differences may
533 be explained by methodological variation: Yu and co-workers did not include non-drought stressed
534 tomato controls, they measured ABA in leaves rather than xylem, and they measured stomatal aperture
535 rather than conductance. We observed a lag between the stomatal conductance response in leaves and
536 ABA concentration in stem xylem sap, leading us to hypothesize that ABA concentration in leaves would
537 be a more biologically relevant marker for behavior of leaf stomates. It would be interesting to
538 determine if either exogenous trehalose or ABA treatment changes trehalose concentration in tomato
539 xylem sap.

540 In our system, trehalose protected plants against disease when applied via soil drenching, but
541 not when applied as a spray. Spraying wheat leaves with trehalose did increase plant growth, improve
542 water stress tolerance, and provide resistance to powdery mildew, a leaf disease (Reignault, Cogan et al.
543 2000, Renard-Merlier, Randoux et al. 2007, Smeekens 2017). Multiple applications of trehalose to leaves
544 via spray also increased tobacco growth under nitrogen limiting conditions (Lin, Zhang et al. 2017). We
545 found no studies demonstrating that spraying tomato leaves with trehalose promotes resistance to
546 pathogens, although leaf infiltration provides resistance to aphid predation (Singh and Shah 2012).
547 These findings suggest that all plants do not respond to exogenous trehalose in the leaves in the same
548 way. Additionally, the receptors for sugar-mediated DAMP response in tomatoes are unknown; grafting
549 experiments may offer insights into whether these are primarily expressed in the roots.

550 The plant hormone SA, which mediates resistance to *R. solanacearum* in many systems,
551 is also necessary for induction of plant innate immunity and activation of systemic acquired resistance
552 (Deslandes, Olivier et al. 2002, Hirsch, Deslandes et al. 2002, Chen, Lin et al. 2009, An and Mou 2011,

553 Boatwright and Pajerowska-Mukhtar 2013, Jacobs, Milling et al. 2013).. We found SA-responsive tomato
554 defense genes were highly upregulated 6 and 48 h after trehalose treatment. These same genes are
555 upregulated in diseased tomato plants following *R. solanacearum* soil soak infection, suggesting
556 trehalose treatment activates SA-mediated resistance pathways that could affect *R. solanacearum*
557 infection (Milling, Babujee et al. 2011). Expression of the SA-responsive marker gene PR1b is affected in
558 several tomato trehalose metabolism knockdown mutants (decreased in *SlTPS4,* increased in *SLTPS5* and
559 *SlTPP2*) after *P. syringae* pv. *tomato* challenge (Zhang, Hong et al. 2016), further suggesting interplay
560 between trehalose metabolism and resistance to bacterial pathogens.

561 Trehalose treatment increased wilt resistance of SA-degrading *NahG* tomato plants, but not of
562 the parental Moneymaker cultivar. There are several possible explanations for this result. First,
563 trehalose treatment could have increased SA levels so much that overproduction of catechol, the
564 breakdown product of SA, contributed to resistance (Retig and Chet 1974). Second, the excess ET and JA
565 in *NahG* plants could promote resistance to *R. solanacearum*. After infection with *P. syringae* DC3000
566 *NahG* tomatoes produce more ET and JA, both of which can contribute to disease resistance (Spoel,
567 Koornneef et al. 2003, López-Gresa, Lisón et al. 2016). ET is involved in *R. solanacearum*-host
568 interactions and ET-responsive defense genes were upregulated at 6 and 48 h post trehalose treatment.
569 Interestingly, ET and JA signaling-responsive defense-related genes were downregulated in tomatoes
570 with silenced *SlTPS3*, *4*, and *7* (Zhang, Hong et al. 2016). However, ET-insensitive *NeverRipe* tomatoes
571 were neither more nor less resistant to *R. solanacearum* than wild type after trehalose treatment.
572 Treatment with a biocontrol strain of the oomycete *Pythium* enhanced resistance to *R. solanacearum* in
573 *NahG* tomatoes, but not in a JA mutant (Hase, Takahashi et al. 2008). Since the ET defense pathway
574 appeared less relevant in our study, it may be useful to determine if JA enhances *R. solanacearum*
575 resistance in tomatoes. Our experiments with tomato defense hormone mutants also demonstrated
576 that trehalose treatment significantly delays disease incidence relative to water-treated control plants in
577 multiple tomato genotypes.

578 Plants use hormones to fine-tune their growth, development, and defense, and xylem vessels
579 can act as signal highways for long-distance transport of auxin, ABA, JA, and SA (Jackson 1997, Park, Lee
580 et al. 2017). We observed increased SA concentration in xylem sap of trehalose-treated plants but not in
581 sap from *R. solanacearum*-infected plants. This was surprising because SA signaling and concentration
582 are integral to plant response to bacterial wilt (Jacobs, Milling et al. 2013). However, *R. solanacearum*
583 actively degrades SA, which is toxic to the pathogen (Lowe-Power, Jacobs et al. 2016), so SA
584 concentrations might be higher in root xylem than in stem xylem. For instance, an *Arabidopsis* mutant

585 impaired in secondary wall deposition had higher SA concentrations in the root and increased resistance
586 to *R. solanacearum* infection, possibly through vascular immunity (Denancé, Ranocha et al. 2013). Taken
587 together, the increased SA levels in xylem sap, the increased expression of SA-dependent defense genes,
588 and the trehalose-dependent *NahG* resistance phenotype suggest that the protective effect of trehalose
589 requires systemic resistance induced via SA.

590 Unexpectedly, jasmonate levels were elevated in trehalose-treated plants but not in *R.*
591 *solanacearum*-infected plants. As discussed earlier, there is some evidence that JA can play a role in *R.*
592 *solanacearum* defense. Genes related to ET, JA, and auxin signaling were upregulated in resistant LS-89
593 tomato seedlings one day after stem inoculation with *R. solanacearum* (Ishihara, Mitsuhara et al. 2012).
594 The higher concentration of jasmonates in trehalose-treated xylem sap may have contributed to
595 resistance. This hypothesis could be tested using JA-deficient tomato mutants.

596 SA- and JA-mediated plant defense responses are often antagonistic (Glazebrook 2005, Derksen,
597 Rampitsch et al. 2013). Our gene expression analysis followed this pattern; at 6 h post trehalose
598 treatment JA-responsive *pin2* was downregulated, while SA-responsive *PR1b* and *gluA* were
599 upregulated. Roots of wilt resistant Hawaii7996 tomatoes displayed a similar gene expression profile 24
600 h after *R. solanacearum* infection (French, Kim et al. 2018). Despite the antagonism suggested by the
601 gene expression data, the concentrations of SA, JA, and the JA precursor 12-OPDA all increased in
602 trehalose-treated plants at 24 h. In maize, levels of OPDA and JA are irrelevant for the classic SA/JA-Ile
603 antagonism and 12-OPDA and SA are co-regulated (Wang, Borrego et al. 2020). Infusing maize xylem
604 sap with 12-OPDA increased resistance to the fungal pathogen *Colletotrichum graminicola* while infusing
605 similar concentrations of JA increased susceptibility. Disease experiments with JA-insensitive tomato
606 mutants could determine if 12-OPDA or JA contribute to resistance and define their relationship to SA
607 signaling.

608 JA signaling also induces leaf senescence, and chlorosis is associated with increased JA
609 production (He, Fukushige et al. 2002). Consistent with this, trehalose treatment significantly reduced
610 chlorophyll content 2-10 days post-treatment, which correlated with the increased JA production in
611 trehalose-treated plants. Similarly, applying 5 mM trehalose to soybean roots did not affect JA
612 concentration, but it did decrease chlorophyll content at ten days (Asaf, Khan et al. 2017).

613 Trehalose treatment and *R. solanacearum* infection both increased xylem sap levels of several
614 antimicrobial compounds, notably phenolics. *R. solanacearum* requires at least two multi-drug efflux
615 pumps and a hydroxycinnamic acid degradation pathway for full virulence and fitness, showing that

616 antimicrobials, including phenolics, protect tomato plants from bacterial wilt (Brown, Swanson et al.
617 2007, Lowe, Ailloud et al. 2015). Plants have specialized cells that store and release phenolics into the
618 xylem as part of their defense response (Beckman 2000), and our data indicate that trehalose treatment
619 and *R. solanacearum* infection both induce this release.

620 Levels of the oxylipins 9-HOT, 9-HOD, 13-HOD, and 13-KOD increased in sap from both *R.*
621 *solanacearum*-infected plants and trehalose-treated plants 24 h after treatment. These metabolites,
622 which accumulate in wounded maize, bean, and *Arabidopsis*, can be potent inducers of defense gene
623 response (Alméras, Stolz et al. 2003, Walters, Cowley et al. 2006, He, Borrego et al. 2020). Further, these
624 oxylipins have direct antimicrobial activity against fungal and bacterial pathogens (Prost, Dhondt et al.
625 2005). The data generated by this study deserve further analysis since little is known about oxylipin
626 signatures during bacterial wilt or if oxylipins are toxic to *R. solanacearum*. However, 9-LOX-derived
627 oxylipins, including 9-HOT and 9-HOD, increase in tobacco leaves inoculated with *R. solanacearum*
628 GMI100, which triggers an intense defensive hypersensitive response (HR) on tobacco (Montillet, Agnel
629 et al. 2002). We speculate that the presence of related oxylipins in our study may indicate vascular HR. It
630 would be interesting to see if defense compound concentration changed in the xylem sap of resistant
631 tomatoes challenged with *R. solanacearum.*

632 We propose that tomato plants activated induced systemic resistance because they recognized
633 trehalose as an endogenous (self-derived) damage associated molecular pattern or DAMP (Tanaka and
634 Heil 2021). DAMPs are products released during pathogen invasion or wounding that alert the plant to
635 an attack (Ranf, Eschen-Lippold et al. 2011, De Lorenzo, Ferrari et al. 2018, Hou, Liu et al. 2019). A DAMP
636 can be almost anything that is usually intracellular like DNA, sugars, or NAD (indicating cellular leakage),
637 or products of lignin and cellulose degradation (indicating mechanical or enzymatic disruption of plant
638 cell walls). Plant systemic resistance response to DAMPs, mechanical wounding, or pathogen challenge
639 typically involves accumulation of SA, JA, and defense oxylipins, and increased expression of SA, JA, and
640 ET-responsive defense genes (Vallad and Goodman 2004, Choudhary, Prakash et al. 2007, Savatin,
641 Gramegna et al. 2014, Wang, Borrego et al. 2020). Consistent with this definition, we found that treating
642 tomato roots with trehalose increased SA, JA, and defense oxylipins, and upregulated SA, JA, and ET
643 defense genes. Our finding adds to the growing evidence that sugars, specifically trehalose, contribute
644 to plant innate immunity (Bolouri Moghaddam and Van den Ende 2012).

645 Could treating roots with compatible solutes like trehalose in the field provide temporary
646 resilience until disease or drought pressure have passed? Other chemical inducers of plant defense

647 responses can reduce bacterial wilt. Acibenzolar-S-methyl (Actigard) and DL-3-aminobutyric acid (BABA)
648 both lowered wilt disease incidence in greenhouse and field by promoting defense gene expression and
649 defense compound production, respectively (Jakab, Cottier et al. 2001, Pradhanang, Ji et al. 2005,
650 Hassan and Abo-Elyousr 2013). Additionally, growing tomatoes hydroponically with SA and JA increased
651 defense enzyme production and reduced bacterial wilt incidence (Mandal, Kar et al. 2013). Finally,
652 exogenous L-histidine induced resistance to bacterial wilt in an ET-dependent manner in *Arabidopsis* and
653 tomato (Seo, Nakaho et al. 2016). Further studies are needed to know if treating tomatoes with
654 exogenous trehalose could be a practical strategy for bacterial wilt management.

655 *R. solanacearum* GMI1000 produces and secretes abundant amounts of a trehalase (TreA)
656 during tomato infection. Trehalose is not an important nutrient for the pathogen but the ability to
657 degrade trehalose contributes to bacterial fitness and virulence by an unknown mechanism (Jacobs,
658 Babujee et al. 2012, Hamilton, Steidl et al. 2021). Interestingly, treating plants with the trehalase
659 inhibitor Validamycin A increased bacterial wilt resistance (Ishikawa, Fujimori et al. 1996). We previously
660 showed that the trehalose in xylem sap of *R. solanacearum*-infected tomato plants is produced by the
661 plant (MacIntyre, Barth et al. 2019). Could *R. solanacearum* secrete trehalase to counter the effects of
662 plant-produced trehalose?

663 Breaking down plant-produced trehalose could benefit *R. solanacearum* in several ways. First,
664 the pathogen may degrade xylem sap trehalose in order to reduce host plant defenses by removing this
665 potential DAMP. Second, as discussed above, trehalose reduces tomato transpiration, and transpiration
666 helps *R. solanacearum* spread and colonize host xylem. *R. solanacearum* may degrade trehalose to
667 maintain this disseminating sap flow by preventing trehalose-mediated stomatal closing. Third, *R.*
668 *solanacearum* could degrade trehalose to interfere with the trehalose-T6P signaling that plants use to
669 fine-tune their sucrose to starch ratios. Trehalose degradation could thus indirectly increase the
670 amount of available sucrose, which is a key carbon source fueling *R. solanacearum* growth in xylem
671 (Jacobs, Babujee et al. 2012, Hamilton, Steidl et al. 2021). The fact that the *R. solanacearum* effector
672 RipTPS is a functional trehalose-6-phosphate synthase is consistent with the hypothesis that this
673 pathogen benefits from manipulating host plant trehalose metabolism (Poueymiro et al., 2014).
674 Additional experiments are needed to test these non-exclusive hypotheses about how trehalase benefits
675 *R. solanacearum*.

676

677 **Conclusion**

678 This study argues that trehalose is present in xylem sap of *R. solanacearum*-infected tomatoes
679 as a response to wilt disease-mediated water stress. Both trehalose levels and the expression of water
680 stress genes changed in response to *R. solanacearum* infection. Further, ABA content, an indicator of
681 drought status, increased in xylem sap of diseased plants. We confirmed the link between trehalose and
682 ABA signaling and drought stress in plants by demonstrating that trehalose treated plants temporarily
683 reduce stomatal conductance, contain more ABA in their xylem sap, and are more drought tolerant.

684 When applied to plant roots, trehalose reduced bacterial wilt incidence and reduced host xylem
685 conductance, which aids bacterial colonization. SA-responsive defense genes were upregulated in
686 trehalose-treated plants, SA non-responsive *NahG* plants were more resistant to *R. solanacearum* after
687 trehalose treatment, and SA concentration transiently spiked in xylem sap from treated plants. JA, other
688 oxylipins, and many antimicrobial compounds also increased in xylem sap of trehalose-treated plants.
689 Taken together, these results suggest that plants perceive trehalose as a DAMP and respond with
690 systemic disease resistance. In turn, *R. solanacearum* degrades trehalose as a counter-defense.

691

692 **Methods**

693 **Total RNA extraction and RNA-seq sample preparation |** RNA extraction and RNA-seq sample
694 preparation for the transcriptomic studies were performed as described (Khokhani, Lowe-Power et al.
695 2017, French, Kim et al. 2018).

696 **RNA-seq data analysis |** Illumina HiSeq2500 paired-end 100-bp; Illumina HiSeq2500 paired-end 150 bp
697 and Illumina HiSeq2000 paired-end 100-bp RNA sequencing were performed on the 18, 6, and 12
698 samples from three independent studies (two previously unpublished [Studies A+B], and [Study C]
699 French, Kim et al. 2018). RNA-sequencing data were analyzed through the same analysis pipeline. Read
700 quality was controlled using FastQC version 0.11.9 (Andrews). Reads were trimmed to remove low-
701 quality sequences and the Illumina sequencing adaptors (TruSeq3-PE-2) using Trimmomatic version 0.39
702 (Bolger, Lohse et al. 2014). After trimming and mapping, a total of 930,004,276, 135,532,392, and
703 103,831,164 paired-end reads were generated for Studies A, B, and C, respectively. Reads for each
704 sample were mapped to the ITAG4.0 *S. lycopersicum* reference genome using STAR version 2.7.4.a
705 (Dobin, Davis et al. 2012). Library type was set to strand-specific (reverse) for Study A and Study B, and
706 set to no-stranded in Study C. Gene expression was measured as the total number of reads for each
707 sample uniquely mapped to the reference using HTSeq version 0.11.1 and samtools version 1.10 (Li,

708 Handsaker et al. 2009, Anders, Pyl et al. 2014). Each sample averaged about 12, 9, and 4 million uniquely
709 aligned reads for Studies A, B, and C, respectively. "EdgeR" package version 3.28.0 was used to filter for
710 low counts such that at least three of the samples in each study had at least three counts per million,
711 resulting in a total of 19123, 18193, and 18917 genes remaining for Studies A, B, and C, respectively
712 (Robinson, McCarthy et al. 2009).

713 Differential gene expression within each study was conducted using the DESeq2 package
714 Bioconductor version 3.11 and a Benjamini-Hochberg false discovery rate multiple testing correction of
715 *P*=.05 (Benjamini and Hochberg 1995, Love, Huber et al. 2014). Meta-transcriptomic analysis was
716 performed using the metaRNASeq package version 1.0, and differential gene expression within the
717 meta-analysis was determined with the DESeq2 results, Fisher, and inverse normal techniques with a
718 *P*=.05, as described (Rau, Marot et al. 2014). Differentially expressed genes were filtered using a log2
719 fold change >|0.585| (corresponds to a fold change of |1.5|).

720 Gene Ontology analysis was performed using the ShinyGO analysis tool version 0.61, agriGO
721 version 2.0, and PANTHER GO analysis tool version 14 (Tian, Liu et al. 2017, Mi, Muruganujan et al. 2018,
722 Ge, Jung et al. 2019). *Arabidopsis thaliana* homologs of S. *lycopersicum* genes coding for transcription
723 factors were identified using Phytozome platform version 12.1 (Goodstein, Shu et al. 2011). Heatmaps
724 were visualized with R software version 3.6.1 package "pheatmap" version 1.0.12 (R , Kolde 2019).
725 Boxplots were visualized using package "ggplot2" (Wickham 2016).

726 Candidate TPS and TPP proteins were identified in the most recent annotations of the tomato
727 (cv. 'Heinz 1706') genome available on NCBI and the Sol Genomics Network (ITAG4.0,
728 https://solgenomics.net/tools/blast/, accessed 03/2020) using BLASTP with standard settings (Sato,
729 Tabata et al. 2012). BLASTP protein sequences were derived from the *E. coli* K-12 *otsA* and *otsB* proteins,
730 as well as *Arabidopsis* trehalose-related proteins (Leyman, Van Dijck et al. 2001, Vandesteene, Ramon et
731 al. 2010, Vandesteene, Lopez-Galvis et al. 2012). Tomato cv. Heinz 1706 genome contained ten TPS
732 genes, eight TPP genes, and one trehalase gene. These genes were previously identified as summarized
733 (Zhang, Hong et al. 2016). Tomato ABA-related, LEA, and aquaporin genes were identified by their
734 PANTHER annotation, and many were previously annotated (Ma, Szostkiewicz et al. 2009, Reuscher,
735 Akiyama et al. 2013).

736 The transcriptomic studies were performed and labeled as follows: S1, roots of wilt-susceptible
737 West Virginia 700 tomato plants (WV) sampled 24 h after infection with *R. solanacearum* strain K60; S2,

738 roots of WV sampled 48 h after infection with *R. solanacearum* strain K60; S3, seedling roots of wilt-
739 susceptible cv. Bonny Best (BB) sampled 24 h after infection with *R. solanacearum* strain GMI1000; S4,
740 mid-stems of BB sampled 72 h after inoculation with *R. solanacearum* strain GMI1000.

741 **Hormone analysis leaf and sap collection |** For the hormone analysis, xylem sap was harvested and
742 collected as described (Lowe-Power, Hendrich et al. 2018). Unwounded three-week-old wilt-susceptible
743 Bonny Best plants were inoculated with wild-type *R. solanacearum* or water via naturalistic soil soak in
744 which 50 mL of $1x10^8$ CFU/mL were poured into a pot containing one plant. Plants were harvested when
745 they reached DI=1 (1-25% of leaf area wilted). Trehalose-treated plants were treated with 50 mL of 30
746 mM trehalose solution or water, then harvested 6, 24, or 48 h later. Xylem sap was harvested by de-
747 topping plants 1 cm above the cotyledon and collecting the exuded sap over 30 minutes. The sap that
748 seeped within the first five minutes was discarded to reduce phloem contamination. Sap was kept on
749 ice. Bacteria were removed from sap via chilled centrifugation, and sap was subsequently flash frozen.
750 Stems were destructively sampled to quantify *R. solanacearum* colonization as CFU/g stem by serial
751 dilution plating. Xylem sap was normalized by sap volume (250-500 µL) and concentration of bacteria in
752 the stem. Each test group contained six technical replicates (individual plants).

753 For metabolite analysis of sap, 95 µL of xylem sap was mixed with 5 µL of 50 µM of internal
754 standard consisting of; d-ABA ([$^2H_6$](+)-*cis*, *trans*-abscisic acid, Olchem, Olomouc, Czech Republic), d-CA
755 (d_7-CA, *trans*-cinnamic acid), d-IAA ([$^2H_5$] indole-3-acetic acid, Olchem), d-JA (2,4,4-d_3; acetyl-2,2-d_2
756 jasmonic acid, CDN Isotopes, Pointe Claire, Quebec, Canada), and d-SA (d_6-SA; Sigma-Aldrich, St Louis,
757 MO, USA). Samples were transferred into autosampler vials for LC-MS/MS analysis. Authentic analytical
758 external standards for each metabolite were used to identify select ions and retention times for each
759 metabolite (Supplemental Table 3). The simultaneous detection of several phytohormones used the
760 following methods with modifications (Müller and Munné-Bosch 2011). We used an Ascentis Express C-
761 18 Column (3 cm × 2.1 mm, 2.7 µm) (Sigma-Aldrich, St. Louis, MO, USA) connected to an API 3200 LC-
762 MS/MS (Sciex, Framingham, MA, USA) using electrospray ionization in negative mode with multiple
763 reaction mentoring. The injection volume was 10 µL and had a 500 µL min^{-1} mobile phase consisting of
764 Solution A (0.2% acetic acid in water) and Solution B (0.2% acetic acid in acetonitrile) with a gradient
765 consisting of (time – %B): 0.5 – 10%, 1.0 – 20%, 21.0 – 70%, 24.6 – 100%, 24.8 – 10%, 29 – stop. After
766 integration with Analyst v1.6.3 (Sciex), the concentration of endogenous metabolites was determined by
767 comparing their peak areas to those of isotopically labeled internal standards using calculated response
768 factors.

769 **Stomatal conductance, transpiration, and photosynthesis measurements with LI-COR |**To assess the
770 effect of trehalose treatment on tomato stomatal conductance (g_s), transpiration (E), and
771 photosynthesis (A_{net}), we used a LI-COR portable photosynthesis system (model LI-6400XT, LI-COR
772 Biosciences, Lincoln, NE, USA) equipped with a CO_2-controller and a red-blue LED chamber (model LI-
773 6400-02b). The ambient CO_2 concentration was set to 400 ppm, flow rate to 300 µmol s^{-1}, and the
774 chamber light source set at an intensity of 1200 µmol m^{-2} s^{-1} for all measurements. Fifteen-day-old
775 Bonny Best plants were treated with 50 mL of trehalose solution or water, then watered every day after
776 for the duration of the experiment. LI-COR measurements were taken at 10min, 6 h, 24 h, 48 h, and 72 h
777 post trehalose treatment. The experiment was repeated twice with ten plants per treatment.

778 ***R. solanacearum* culture conditions |** *R. solanacearum* strains were cultured from water stocks or -80 °C
779 glycerol stocks on TZC plates containing casamino acids, peptone, glucose, and tetrazolium chloride, and
780 incubated at 28 °C for 48 h (Hendrick and Sequeira 1984). *Rs* Δ*treA* cultures were supplemented with 20
781 mg L^{-1} spectinomycin. The kanamycin-resistant variant of wild-type GMI1000 was supplemented with 25
782 mg L^{-1} kanamycin on plates and in liquid culture. Overnight cultures of *R. solanacearum* were grown at
783 28 °C in CPG in a shaking incubator.

784 **ABA treatments |** We assessed the effect of ABA treatment on two-week-old tomato stomatal
785 conductance and subsequent bacterial wilt incidence. ABA was prepared at a stock concentration of
786 50mg/mL in DMSO. The water + DMSO control plants were watered daily with 50 mL of water
787 supplemented with the volume of DMSO used to make the 100 µM ABA solution. The ABA-treated
788 plants were watered daily with 50 mL of 50 or 100 µM ABA solution in water. After 48 h of ABA
789 treatment, plants were soil drenched with 50 mL of a $1x10^8$ CFU/mL of *R. solanacearum* GMI1000 with
790 kanamycin resistance (to aid in recovery from the plant) (Lowe, Ailloud et al. 2015). Bacteria were
791 resuspended at the desired O.D. in water + DMSO or the ABA solutions, so plants were inoculated and
792 ABA treated concurrently. Tomatoes were destructively sampled five days post-infection (after 168 h of
793 ABA treatment) to assess bacterial colonization of stems. Stomatal conductance, transpiration, and
794 photosynthesis measurements were taken for the entirety of the experiment with the LI-COR as
795 described 4-6 h after watering/ABA treating in the morning, which was the length of time it took ABA
796 treatment to affect conductance.

797 We assessed ABA toxicity to *R. solanacearum* by supplementing CPG broth with 50-100 µM ABA
798 or the equivalent volume of DMSO, and growing *R. solanacearum* cells in this media for 48 h. Cells were

799 grown in a shaking 96 well plate at 28°C in a BioTek Synergy HT plate reader, which measured Abs_{600}
800 every half hour. The experiment was performed once with eight technical replicates per treatment.

801 **Plant assays with *R. solanacearum* |** Plant growth conditions, colonization, and disease curve assays
802 were performed as described (Khokhani, Tuan et al. 2018, MacIntyre, Barth et al. 2019). All plant assays
803 were grown on 12 h light/dark cycles at 28 °C with ~60% relative humidity unless stated otherwise. For
804 the disease progress assays with trehalose treatments, fifteen-day old Bonny Best tomatoes were
805 treated with either water (control) or 50 mL of 30 mM trehalose solution. Plants were not watered 24 h
806 post-treatment per standard procedure for soil soak inoculation assays. At 48 h post-treatment plants
807 were soil drenched with 50 mL of $1x10^8$ CFU/mL of wild type *R. solanacearum* GMI1000 (or Δ*treA* for the
808 trehalose treatment experiments with this mutant). For the petiole inoculations, 3-week-old tomatoes
809 were treated with trehalose or water as described above; after 48 h a true leaf was removed, and 2000
810 wild type GMI1000 cells in 10 µL of water were pipetted onto the cut stem. To assess the effect of
811 spraying trehalose on bacterial wilt incidence, fifteen-day-old Bonny Best plants were either treated
812 with 30 mM trehalose solution through the soil as described or leaves were sprayed with trehalose
813 solution (or water) to run-off. For all assays, plants were inoculated with *R. solanacearum* 48 h post
814 trehalose root or leaf treatment.

815 After treatments and inoculations, all plants in disease assays were watered on alternating days
816 post-inoculation with half-strength Hoagland's solution. Disease assay plants were rated daily on a 0-4
817 disease index scale where 0= no symptoms, 1=1-25% of leaf area wilted, and 4=76-100% of leaf area
818 wilted or dead. All disease curves represent four biological replicates, each containing 15
819 plants/treatment.

820 To assess *R. solanacearum* colonization of trehalose treated plants, fifteen-day-old Bonny Best
821 plants were treated with 50 mL 30 mM trehalose solution or water. 48 h post-treatment, plants were
822 inoculated with 50 mL of $1x10^8$ CFU/mL wild type *R. solanacearum* GMI100. At 5, 6, and 7 dpi plant
823 stems were destructively sampled 1 cm above the cotyledon, ground, and serially dilution plated.

824 *NahG* salicylic insensitive tomato seeds ('Moneymaker' background) and *NeverRipe* ET-
825 insensitive seeds ('Pearson' background) were propagated in our lab (Lanahan, Yen et al. 1994, Brading,
826 Hammond-Kosack et al. 2000). Disease assays with trehalose-treated hormone insensitive plants were
827 as described above.

828 **Water conservation |** Two-week-old Bonny Best plants grown as described above were treated with
829 trehalose or water. Water was subsequently withheld and plants were assessed for wilting symptoms,
830 using the same visual index scale as for disease progress assays. The experiment included four biological
831 replicates, each with ten plants per treatment.

832 **QRT-PCR measurement of tomato trehalose and defense gene expression |** JA, SA, and ET defense
833 gene primers were previously characterized in tomato *R. solanacearum* response (Supplemental Table 1)
834 (Milling, Babujee et al. 2011). The *rd22* and *dhn_tas* ABA-responsive primers were previously described
835 (Yamaguchi-Shinozaki and Shinozaki 1993, Kissoudis, Seifi et al. 2016). *ACTIN* and *DNAJ* primers served
836 as our constitutively expressed normalizing genes.

837 To measure defense gene expression, two-week-old Bonny Best tomatoes were treated with 50
838 mL of 30 mM trehalose or water. 100 mg of stem tissue was harvested 6 or 48 h post-treatment, then
839 flash frozen. For all samples, stems were homogenized for a minute in a Powerlyzer (Qiagen) at room
840 temperature in 450 µL of Buffer RLT from the RNAeasy Plant Mini Kit (Qiagen). RNA extraction
841 proceeded as specified in the kit protocol with off-column DNase I treatment using a DNA-free kit
842 (Thermo-Fisher). RNA quality and quantity were assessed with a NanoDrop (Thermo-Fisher) and reverse
843 transcribed into cDNA using Superscript III First-Strand Synthesis SuperMix (Invitrogen). Lack of genomic
844 DNA contamination was confirmed with PCR of no reverse transcriptase controls. Quantitative real-time
845 PCR was performed in 10 µL reactions with EvaGreen Mastermix (Biotium) using kit-specified reaction
846 parameters in a Quant Studio 5 Real-Time PCR machine (ThermoFisher Scientific). Averaged actin and
847 *dnaJ* values were used as the stably expressed normalization genes. Fold change values were calculated
848 using the $\Delta\Delta C_T$ method as described (Milling, Babujee et al. 2011). The data are presented as fold change
849 in gene expression compared to mock-inoculated controls and represent 16 plants per treatment over
850 four biological replicates.

851 **Relative water content |** To assess the water status and osmotic adjustment of tomato plants treated
852 with trehalose, we measured relative water content (RWC) in leaves. This directly measures cellular
853 hydration. Three-week-old Bonny Best tomato plants were treated with 50 mL of water or 30 mM
854 trehalose solution. 48 h post-treatment, leaves were excised at the petiole and weighed for fresh
855 weight. Leaves were then rehydrated by floating on distilled water in closed petri dishes for 4 h, at
856 which time they were blotted, and their turgid weight was assessed. Leaves were then thoroughly dried
857 in a food dehydrator oven for 24 h, and dry weight was evaluated. RWC was calculated as follows: %
858 RWC= [(Leaf fresh weight – dry leaf weight)/(turgid leaf weight-leaf dry weight)] x 100 (Barrs and

859 Weatherley 1962). The data represent two biological replicates with five technical reps (leaf from an
860 individual plant) per treatment.

861 **Root length assays |** Root length assays to quantify trehalose treatment effect on root length were
862 performed as described with modification (Tran, MacIntyre et al. 2016). Tomato seeds were sterilized in
863 10% bleach for 10 min, then 95% ethanol for 10 min, then washed five times with sterile water and
864 germinated in the dark on 1% water agar plates at room temperature. Four-day-old tomato seedlings
865 were transferred to germination pouches (Mega International, Newport, MN) then watered with either
866 10 mL of water or 30 mM trehalose solution and incubated in a 28°C growth chamber. Following the
867 initial trehalose treatment, pouches were kept moist as needed with sterile water and imaged ten days
868 post-treatment. This experiment was performed three times with ten tomato
869 seedlings/pouch/treatment. New root growth was calculated as % new root growth= (new growth/total
870 root length) x 100.

871 **Chlorophyll determination |** To measure the amount of chlorophyll in tomatoes, we used a SPAD-502
872 meter (Konica Minolta, Inc.) (Ling, Huang et al. 2011). Readings were performed on the first true leaves
873 of two-week-old Bonny Best plants treated with 50 mL of a 30 mM trehalose solution or water. The data
874 represent two biological replicates with ten plants per treatment per replicate.

875 **Bacterial leaf spot of tomato inoculations |** *Xanthomonas gardneri* culture conditions, infection, and
876 colonization analysis were as described (Potnis, Soto-Arias et al. 2014). *X. gardneri* strains were cultured
877 on nutrient agar plates (Millipore-Sigma) supplemented with nalidixic acid (50 µg/mL) at 28°C. Inoculum
878 was grown for 48 h in a shaking incubator at 28°C in nutrient broth supplemented with nalidixic acid.
879 The inoculum was adjusted to OD_{600}=0.3 and diluted 1:100 in 400 mL sterile water with 75 µL Silwet
880 (Fisher Scientific). Four-week-old Bonny Best tomato plants that were treated with water or 50 mL of 30
881 mM trehalose solution 72 h previously were dip-inoculated for 30 s in the inoculum. Plants were
882 incubated in high humidity conditions (85-90%) in plastic containers in the growth chamber for 48 h.
883 After 48 h, plants were moved to low humidity conditions during the day (taken out of containers, 45-
884 60%) and high humidity conditions at night on a 12 h cycle. Bacterial spot symptoms appeared 5-6 days
885 post-inoculation. Leaf tissue from individual plants was sampled at 3, 6, and 9 days post-inoculation.
886 Two 2 cm^2 portions of leaf were sampled from each plant, ground in a Powerlyzer (Qiagen) in water, and
887 serially diluted and enumerated on nutrient agar + nalidixic acid. The data represent three biological
888 replicates with 12 technical reps/treatment/timepoint.

889 **DAB staining tomato leaves for ROS production |** Tomato leaves were stained with 3,3'-
890 Diaminobenzidine (DAB, Sigma-Aldrich) as described (Flores-Cruz and Allen 2009). Leaves from 4-5-
891 week-old Bonny Best tomato plants were infused with water, 30 mM of trehalose solution, or $1x10^9$
892 CFU/mL *R. solanacearum* cells by pricking with a needle then flushing the apoplast with the solution.
893 Plants were returned to the growth chamber overnight. At 24 h, leaves were cut and placed in 0.1
894 mg/mL DAB solution prepared at pH 3.8 at room temperature (Thordal-Christensen, Zhang et al. 1997).
895 Containers holding the stain and leaves were wrapped in aluminum foil to exclude light and incubated
896 for 8 h at room temperature with gentle shaking. At 8 h, leaves were bleached by boiling in 95% ethanol
897 for 10 min, then further cleared with overnight incubation in 70% ethanol before imaging. These
898 experiments were performed three times with leaves from four individual tomato plants per treatment.

899 **Statistical Analyses |** Statistical analyses and graphs were generated with GraphPad Prism 8.

900

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902 discussions. Thank you to Dr. Eric Kruger for the use of equipment and Dr. Kim Cowles for *X. gardneri*
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905

906 **Captions for Supplemental Materials**

907 **Supplemental Table 1.** Strains and primers used in this study

908 **Supplemental Table 2.** Concentration of hormones, oxylipins, and phenylpropanoids in tomato xylem
909 sap following various treatments

910 **Supplemental Table 3.** Metabolite key for hormone analysis

911 **Supplemental Figure 1. ABA and DMSO are not toxic to *Rs*.** Growth of Rs cultured in rich CPG broth
912 supplemented with ABA in DMSO, or water with DMSO because ABA stock solutions were dissolved in
913 DMSO (100 µM treatments contained 0.1 µL of either DMSO or ABA stock solution, and 50 µM
914 treatments contained 0.05 µL DMSO or ABA stock solution) (ANOVA Area Under Curve/AUC, Fisher's LSD
915 multiple comparisons to CPG control, 100 µM DMSO, P=.55; 50 µM DMSO, P=.53; 100 µM ABA, P=.67;

916 50 µM ABA, P=.45). Growth was measured spectrophotometrically using a Bio-Tek plate reader; the data
917 represent eight technical reps/treatment. The bars represent the standard error of the mean.

918 **Supplemental Figure 2. Trehalose treatment temporarily improves water efficiency without affecting**
919 **photosynthesis; trehalose treatment does not affect root growth and relative water content of leaves**
920 **but lowers chlorophyll concentration. A)** Water efficiency, measured with a LI-COR as photosynthetic
921 rate/transpiration, in trehalose treated plants compared to water only controls (t-test, *P*<0.5). **B)**
922 Photosynthesis, measured as A_{net}, in trehalose treated plants (Mixed model ANOVA, Tukey's HSD,
923 *P*>.05). The water efficiency and photosynthetic data represent 20 plants/treatment. LI-COR
924 measurements were taken ~10 min after treatment and then in the morning every time point after.
925 Shaded bars on the graphs represent night periods. All data were calculated from the same LICOR time
926 points. The barson the data points represent the standard deviation. **C)** Relative water content of leaves
927 was calculated from the fresh weight, hydrated weight, and dry weight of leaves from water control or
928 trehalose treated BB tomatoes (t-test, *P*=.14). The data represent two biological replicates with five
929 technical replicates (leaves)/treatment). The bars represent the standard deviation. **D)** 'Bonny Best'
930 tomato seedlings were placed in root growth pouches, watered with 10mL of either water or trehalose
931 for one day, then watered as needed for ten days. Root length was measured after ten days (t-test,
932 *P*=.31). The data represent three biological replicates with ten roots/treatment. Percent of new root
933 growth was calculated as %new root growth= (new growth/total root length)*100. The bars represent
934 the standard deviation. **E)** SPAD meter measurements of chlorophyll content in leaves following
935 trehalose treatment (2-way ANOVA Fishers LSD multiple comparison, * = *P*<0.5 or lower). The bars
936 represent the standard deviation.

937 **Supplemental Figure 3. Trehalose treatment does not nutritionally enrich the soil environment for *R.***
938 ***solanacearum*, and its protection is limited to root application. A)** Disease progress curve of plants
939 treated with trehalose or water, then infected with either wild-type *Rs* or a Δ*treA* mutant unable to
940 catabolize trehalose (one-way ANOVA of areas under the curve, WT H_20 vs. WT tre, *P*=.0008, Δ*treA* H_20
941 vs. Δ*treA* tre, *P*=.017). The data represent three bioreps each containing 13-15 plants per treatment. The
942 bars represent the standard error. **B)** Disease development in plants sprayed once with 30 mM trehalose
943 or water, and then soil-soak inoculated with *Rs* 48 h later (ANOVA of AUC, Fisher's LSD multiple
944 comparisons to H_20 soil, H_20 spray, *P*=.67; tre spray, *P*=.63; tre soil, *P*=.63). The data represent three
945 biological replicates each containing fifteen plants per treatment. The bars represent the standard error.
946 **C)** Colonization of tomato leaves by the bacterial leaf spot pathogen *Xanthomonas gardneri*; plants were

947 dip-inoculated with *X. gardneri* 72 h after root treatment with water or 30 mM trehalose (Mann-
948 Whitney, day 3, *P*=.06; day 6, *P*=.61; day 9, *P*=08). The data represent three biological replicates with
949 twelve plants/treatment/timepoint. The graph displays the geometric means; bars indicate the
950 geometric standard deviation of the data.

951 **Supplemental Figure 4. Infusing leaves with trehalose did not trigger ROS production in 'Bonny Best'**
952 **tomato leaves.** DAB staining of 'Bonny best' tomato leaves infused with water, 10^9 CFU/mL *Rs*, or 30
953 mM trehalose solution to assess the effect of trehalose treatment on ROS production. The data
954 represent three biological replicates, with four plants per biological replicate per treatment. Photos are
955 representative samples and images were uniformly sharpened 25% to increase contrast.

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