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

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Abstract

Ralstonia solanacearum causes plant bacterial wilt disease, leading to severe crop losses. Xylem sap from *R. solanacearum*-infected tomato is enriched in host produced trehalose. Water stressed plants accumulate the disaccharide trehalose, which increases drought tolerance via abscisic acid (ABA) signaling networks. Because infected plants have reduced water flow, we hypothesized that bacterial wilt physiologically mimics drought stress, which trehalose could mitigate. Transcriptomic responses of susceptible vs. resistant tomato plants to *R. solanacearum* infection revealed differential expression of drought-associated genes, including those involved in ABA and trehalose metabolism. ABA was enriched in xylem sap from *R. solanacearum*-infected plants. Treating roots with ABA lowered stomatal conductance and reduced *R. solanacearum* stem colonization. Treating roots with trehalose increased ABA in xylem sap and reduced plant water use by reducing stomatal conductance and temporarily improving water use efficiency. Further, trehalose-treated plants were more resistant to bacterial wilt disease. Trehalose treatment also upregulated expression of salicylic acid (SA)-dependent defense genes, increased xylem sap levels of SA and other antimicrobial compounds, and increased wilt resistance of SA-insensitive *NahG* tomato plants. Additionally, trehalose treatment increased xylem concentrations of jasmonic acid and related oxylipins. Together, these data show that exogenous trehalose reduced both water stress and bacterial wilt disease and triggered systemic resistance. This suite of responses revealed unexpected linkages between plant responses to biotic and abiotic stress and suggests that that *R. solanacearum*-infected tomato plants produce more trehalose to improve water use efficiency and increase wilt disease resistance. In turn, *R. solanacearum* degrades trehalose as a counter-defense.

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

52 **Introduction**

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

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

70 Researchers long assumed that *R. solanacearum* and other vascular pathogens cause drought-
71 like physiological symptoms in host plants and that severity of the disease is affected by environmental
72 water availability (Abd El-Rahim, Fahmy et al. 1998, McElrone, Sherald et al. 2003, Daugherty, Lopes et
73 al. 2010). *R. solanacearum* dies more quickly in drought-stressed chickpea plants, and *R. solanacearum*
74 infection and water stress induce similar osmolyte biosynthesis and expression of genes related to the

75 plant signaling molecule abscisic acid (ABA), a well-characterized marker of water stress (Sinha, Gupta et
76 al. 2016, Sinha, Gupta et al. 2017). Further, stomatal conductance decreases in *R. solanacearum* infected
77 plants (Lowe-Power, Hendrich et al. 2018). These studies suggest but do not prove that plant response
78 to BW mimics the drought stress response.

79 During water and osmotic stress, many plants, including tomato, accumulate trehalose and
80 trehalose-6-phosphate (T6P), which contribute to tolerance of these stresses (Romero, Bellés et al.
81 1997, Garg, Kim et al. 2002, Cortina and Culiáñez-Macià 2005, Lyu, Min et al. 2012, Khater, Dawood et
82 al. 2018). Xylem sap from *R. solanacearum*-infected tomato plants contains about 20-fold more
83 trehalose than healthy plants (Lowe-Power, Hendrich et al. 2018). This non-reducing disaccharide
84 composed of glucose and UDP-glucose is best known for its role as a compatible solute. Trehalose
85 synthesis helps *R. solanacearum* tolerate osmotic stress and contributes to pathogen fitness and
86 virulence (MacIntyre, Barth et al. 2019). However, the tomato host produces the trehalose in xylem sap
87 during infection; *R. solanacearum* does not export its trehalose in culture and it aggressively expresses
88 the secreted TreA trehalase *in planta* (Jacobs, Babujee et al. 2012).

89 Some studies suggest that trehalose may mediate host-microbe interactions and contribute to
90 plant defense. For example, treating *Arabidopsis* seedlings in liquid culture with 30 mM exogenous
91 trehalose triggered expression of defense genes in ethylene and methyl-jasmonate pathways (Bae,
92 Herman et al. 2005). Infusing tobacco leaves with a 10-50 mM trehalose solution increased resistance to
93 tobacco mosaic virus and triggered a transcriptomic response suggestive of biotic and abiotic stress
94 tolerance (Shi, Sun et al. 2019). A *Xanthomonas citri* trehalose synthesis mutant has reduced fitness and
95 infusing citrus leaves with a trehalose solution induced defense gene expression, suggesting that
96 trehalose might simultaneously be a virulence factor and an indicator of pathogen attack (Piazza, Zimaro
97 et al. 2015). In another study, spraying leaves with the trehalase inhibitor Validamycin A increased
98 tomato resistance to BW and *Fusarium* wilt and activated systemic acquired resistance against a wide

99 range of other pathogens in *Arabidopsis* (Ishikawa, Fujimori et al. 1996, Ishikawa, Shirouzu et al. 2005,
100 Bian, Duan et al. 2020).

101 We investigated the relationship between BW and drought stress in tomatoes and explored the
102 role of trehalose in this interaction. We tested the following hypotheses: 1) *R. solanacearum infection*
103 *mimics abiotic stress triggered by disrupted water flow;* and 2) *exogenous trehalose inhibits BW*
104 *development in tomato by sustaining plant water use efficiency and inducing defense response*s. To test
105 H_o1, we used meta-transcriptomic analyses to show that tomato genes involved in trehalose metabolism
106 and water stress responses are differentially expressed during *R. solanacearum* infection and between
107 susceptible and resistant tomato hosts. Using ABA treatment to close stomates, we found that *R.*
108 *solanacearum* depends on stomatal conductance and water flow for effective host colonization. We also
109 discovered that *R. solanacearum* infection increases xylem sap ABA levels.

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

116 **Results**

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

118 To investigate the relationship between BW and drought stress, we measured tomato gene
119 expression related to trehalose metabolism and water stress responses. Candidate genes included those
120 known or predicted to encode synthesis and degradation of trehalose, including trehalose-6-phosphate
121 phosphatase (TPP), trehalose-6-phosphate synthase (TPS), and trehalase (Leyman, Van Dijck et al. 2001,
122 Vandesteene, Ramon et al. 2010, Vandesteene, Lopez-Galvis et al. 2012). We also focused on genes
123 encoding water stress-associated proteins such as: 1) those involved in biosynthesis, regulation, and
124 response to ABA, a known indicator of water stress (Song, Huang et al. 2016, Zhou, Yu et al. 2019); 2)
125 late embryogenesis/dehydrins (LEAs), which accumulate in water-stressed plants (Cao and Li 2015); and
126 3) aquaporins, channel proteins that allow living cells to move water and small solutes more efficiently
127 (Kapilan, Vaziri et al. 2018).

128 We used seven datasets that profiled transcriptomes of a wilt-resistant tomato breeding line
129 (Hawaii 7996) and two wilt-susceptible tomato plants (WV700 and Bonny Best) responding to infection
130 by various *R. solanacearum* strains (K60 and GMI1000) (French, Kim et al. 2018). After normalizing the
131 datasets to allow comparisons across these experiments, we measured the expression of these
132 candidate genes in healthy plants relative to their levels in *R. solanacearum*-infected plants.

133 Although the datasets came from diverse plant genotypes, tissues, and *R. solanacearum* strains,
134 this analysis revealed that *R. solanacearum*-infected tomato plants significantly changed expression of
135 multiple genes related to trehalose metabolism and water stress physiology (Fig. 1). Expression trends
136 varied across infected tissue types, cultivar disease resistance, and within gene families. Some genes in
137 these categories were differentially expressed in both resistant and susceptible infected tomato plants.
138 For example, resistant and susceptible plants upregulated an ABA hydroxylase and an ABA and

139 environmental stress-inducible protein TAS14 (Fig. 1C) and downregulated many diverse aquaporins
140 (Fig. 1D). In contrast, with the exception of TPS-7, many tomato trehalose synthase and phosphatase
141 genes (Fig. 1A) and LEA genes (Fig. 1B) were downregulated in resistant tomatoes, suggesting that
142 trehalose and LEA genes could be involved in resistance against *R. solanacearum.* These results led us to
143 directly measure physiological and metabolic indicators of drought stress in *R. solanacearum*-infected
144 plants.

145 **Bacterial wilt disease increases ABA concentration in xylem sap**

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

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

153 We previously observed that *R. solanacearum*-infected tomatoes have reduced stomatal
154 conductance (Lowe-Power, Khokhani et al. 2018). To test the hypothesis that the increased ABA levels
155 and lowered stomatal conductance in infected plants also affects BW development, we measured the
156 effects of exogenous ABA treatment on stomatal conductance, transpiration rates, and *R. solanacearum*
157 colonization of susceptible tomato plant stems. We watered tomato plants daily with 50 mL of either
158 100 µM ABA, 50 µM ABA, or water + DMSO as control and measured stomatal conductance four to six
159 hours later. After 48 h of ABA treatment, plants were drench-inoculated by adding a suspension of *R.*
160 *solanacearum* strain GMI1000 to the ABA treatment solutions, which we determined were not
161 nutritious or cytotoxic to *R. solanacearum* (Supp. Fig. 1). Treatment with ABA reliably reduced stomatal

162 conductance in the plants over the seven days of the experiment; 100 µM and 50 µM ABA treatment
163 had similar effects (Fig. 2B).

164 ABA treatments continued for four days after plants were inoculated with *R. solanacearum*. At
165 five dpi we measured *R. solanacearum* population sizes in the stems. Stems of ABA-treated plants were
166 less frequently colonized by *R. solanacearum* than control plants (50 µM ABA = 17% plants colonized;
167 100 µM = 23% plants colonized; water control = 53% plants colonized) and they contained lower *R.*
168 *solanacearum* population sizes than untreated plants (Fig. 2C). The correlation between ABA treatment,
169 reduced stomatal conductance, and lower *R. solanacearum* colonization in tomato stems suggests that
170 plant water movement assists *R. solanacearum* infection either at the roots or in the stem and that
171 reducing host water flow reduces or slows *R. solanacearum* spread in the plant.

172 **Trehalose treatment transiently improves water use efficiency in tomatoes**
173
174 Tomato plants become more drought tolerant following exposure to exogenous trehalose, via
175 the mechanisms of ABA signaling and stomatal closure (Yu, Zhao et al. 2019). Because ABA and trehalose
176 concentrations increase in xylem sap from *R. solanacearum*-infected plants and pathogen spread
177 depends on host xylem water transport, we asked whether exogenous trehalose could also protect
178 plants from bacterial wilt.

179 We first confirmed that trehalose increases tomato drought tolerance under our experimental
180 conditions. Two-week-old wilt-susceptible Bonny Best tomato plants were treated with 50 mL of 30 mM
181 trehalose solution or water and then did not water them again. Trehalose-treated plants remained
182 turgid for three days after control plants began wilting, showing that trehalose treatment delayed
183 wilting from lack of water under our conditions (Fig. 3A).

184 Next, we assessed stomatal conductance, transpiration, and photosynthesis of trehalose-treated
185 plants. After watering with 30 mM trehalose as above, we measured tomato leaf responses with a LI-
186 COR portable photosynthesis machine at 10 min, 6, 24, 48, and 72 h after treatment. Relative to

187 untreated plants, trehalose treatment detectably reduced stomatal conductance in 10 min, an effect
188 that persisted for at least 6 h. By 24 h after treatment, stomatal conductance of treated plants was
189 indistinguishable from that of water controls (Fig. 3B). However, transpiration in trehalose-treated
190 plants was lower than controls at 6, 24, and 48 h (Fig. 3C). Water use efficiency, the relationship
191 between water loss and plant productivity (photosynthesis/transpiration), was temporarily improved at
192 6 h by trehalose treatment (Supp. Fig. 2A). Quantitative mass spectrometry analysis of tomato xylem sap
193 revealed that ABA content was 5-fold higher in trehalose-treated plants at 24 h (Fig. 3D). However, at 6
194 and 48 h after treatment ABA levels were no different from those of water-treated control plants.
195 Trehalose treatment did not negatively affect photosynthesis (Supp. Fig. 2B), plant growth
196 determined by measuring plant mass (data not shown), relative water content (RWC), or seedling
197 growth. Tomato leaf RWC was the same in trehalose-treated and water-treated plants at 48h (Supp. Fig.
198 2C). Further, 1-3 days of watering with trehalose solution did not affect tomato seedling root growth *in*
199 *vitro* relative to water controls (Supp. Fig. 2D). Chlorophyll content, measured by SPAD meter, was
200 transiently lower in trehalose treated plants 2-10 days after treatment, although chlorophyll content
201 eventually returned to the same levels as in untreated plants (Supp. Fig. 2E). Together, these diverse
202 physiological and biochemical analyses showed that treating tomato plants with exogenous trehalose
203 temporarily increased their drought tolerance, likely because trehalose increased ABA levels and ABA
204 signaling closes stomates.

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

206 Since we found that BW impacts host water stress physiology and that exogenous trehalose
207 reduces stomatal conductance in tomatoes, we wondered if this decreased water movement would
208 affect BW development. We tested the hypothesis that treating plants with trehalose would make them
209 more wilt resistant by watering tomato plants with 30 mM trehalose solution or water as above. After
210 48 h, treated plants were drench inoculated with a suspension of *R. solanacearum* bacteria. Bacterial

211 wilt incidence and severity was reduced by 25-75% across biological replicates in the trehalose-treated
212 plants, demonstrating that exogenous trehalose increases tomato resistance to BW (Fig. 4A). This
213 protective effect was enduring; trehalose treatment reduced BW incidence even when plants were
214 inoculated five days later (data not shown).

215 Since *R. solanacearum* can infect plants without causing visible symptoms, the absence of wilt in
216 trehalose treated plants could indicate either the lack of disease or latent infection. To distinguish
217 between these possibilities we measured *R. solanacearum* colonization of tomato midstems at 5, 6, and
218 7 dpi. Water-treated control plants were three times more likely to contain detectable *R. solanacearum*
219 cells than trehalose-treated plants (20.7% compared to 7.4%), and control plants contained significantly
220 higher bacterial population sizes (Fig. 4B). This showed that trehalose treatment did not increase the
221 frequency of latent infections but rather that *R. solanacearum* was less likely to colonize stems of
222 trehalose treated plants. The reduced colonization frequency at this relatively early time point is
223 consistent with the observed trehalose-associated delay in disease development.

224 We tested the protective limits of trehalose treatment by varying inoculation sites, treatment
225 methods, and pathogens. Interestingly, inoculating *R. solanacearum* cells directly into the xylem through
226 a cut petiole overwhelmed any protective benefit of trehalose treatment (Fig. 4C). This result suggests
227 that *R. solanacearum* must overcome a trehalose-enhanced checkpoint at or in tomato roots in order to
228 colonize plant stems.

229 *R. solanacearum* produces a trehalase enzyme and can use trehalose as a carbon source, so we
230 wondered if treating tomato roots with trehalose nutritionally enriched the soil environment, thereby
231 de-incentivizing *R. solanacearum* root invasion. We tested this hypothesis by inoculating trehalose-
232 treated tomato plants with either wild-type *R. solanacearum* or a Δ*treA* trehalase mutant that cannot
233 grow on trehalose (MacIntyre, Barth et al. 2019). Trehalose treatment similarly reduced disease caused

234 by wild type and Δ*treA R. solanacearum*, so bacterial catabolism of trehalose cannot explain trehalose
235 treatment's protective effect (Suppl. Fig. 3A).

236 Spraying trehalose solution onto leaves and then inoculating *R. solanacearum* by soil-soak 48 h
237 later also did not affect BW progress (Supp. Fig. 3B). To explore the effects of trehalose treatment on
238 another bacterial pathogen, we dip-inoculated tomato leaves with the tomato leaf spot pathogen
239 *Xanthomonas gardneri* 72 h after plant roots were treated with trehalose. Trehalose had no effect on
240 leafspot disease severity or *X. gardneri* colonization of leaves sampled 3, 6, and 9 dpi (Supp. Fig. 3C).
241 These experiments demonstrated that trehalose needs to be applied at the roots to protect against BW
242 and that trehalose might increase resistance only to *R. solanacearum.*

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

245 Although trehalose treatment of roots decreased plant transpiration and stomatal conductance
246 temporarily (6-48 h), this treatment protected plants from BW for at least five days, suggesting that
247 trehalose might also induced plant defense responses. We tested this by quantifying the effect of
248 trehalose treatment on defense gene expression, BW resistance of defense hormone-insensitive tomato
249 plants, and levels of hormones and defense compounds.

250 To measure the effect of trehalose on defense gene expression, we extracted RNA from tomato
251 stem sections 6 or 48 h after roots were treated with 30 mM trehalose solution or water. We measured
252 expression of two JA-responsive genes, *pin2* and *loxA* (Peña-Cortés, Fisahn et al. 1995, Zhao, Thilmony et
253 al. 2003). *Pin2* was significantly downregulated 6 h after trehalose treatment (Fig. 5A), suggesting JA
254 levels may have been affected by trehalose treatment, while *loxA* expression did not change after
255 trehalose treatment. G*luA*, *PR1a*, and *PR1b* are tomato pathogenesis-related (PR) genes induced by SA
256 and by pathogen challenge (Joosten and De Wit 1989, Tornero, Gadea et al. 1997, Block, Schmelz et al.
257 2005). Both *GluA* and *PR1a* were expressed 10-fold more in trehalose-treated plants compared to water

258 controls 6 h after treatment (Fig. 5A) and their expression increased to over 100-fold at 48 h (Fig. 5B).
259 *PR1b* and *osm,* which respond to the defense hormone ethylene (ET), were also significantly
260 upregulated 48 h after trehalose treatment (Tornero, Gadea et al. 1997, Smart, Myers et al. 2003). The
261 ET synthesis gene *ACO5* was also 10-fold more highly expressed compared to water controls 6 and 48 h
262 after trehalose treatment (Fig. 5A+B) (Jacobs, Milling et al. 2013). Transcripts of the ABA-responsive
263 genes *rd22* and *dhn-tas* were slightly but significantly upregulated 48 h after trehalose treatment,
264 possibly in response to the increased levels of ABA in xylem sap shown in Fig. 5B (Yamaguchi-Shinozaki
265 and Shinozaki 1993, Shinozaki, Yamaguchi-Shinozaki et al. 2003, Kissoudis, Seifi et al. 2016). Together,
266 these qRT-PCR experiments revealed that tomato plants respond to trehalose by increasing expression
267 of genes in multiple biotic and abiotic stress signal pathways, including those responding to JA, SA, ET,
268 and ABA.

269 The finding that SA- and ET-responsive tomato genes were upregulated 6 and 48 h after
270 trehalose treatment suggested that ET perception and SA responses might be necessary for trehalose-
271 mediated increase in BW resistance. We tested this hypothesis by treating with trehalose the roots of
272 ET-insensitive *NeverRipe* mutant tomatoes (cv. Pearson) and SA-degrading *NahG* transgenic tomatoes
273 (cv. Moneymaker). After 48 h, all plants were soil drench inoculated with *R. solanacearum*. As observed
274 for cv. Bonny Best, trehalose treatment increased BW resistance of Pearson and Moneymaker plants.
275 Trehalose also increased BW resistance of both the *NeverRipe* and *NahG* plants compared to untreated
276 controls (Fig. 5C and 5D). However, trehalose-mediated BW resistance does not require ET perception
277 since BW developed similarly on trehalose-treated *NeverRipe* and the parental 'Pearson' genotype
278 ($P<.98$). In contrast, trehalose-treated *NahG* tomatoes were significantly more resistant to BW than
279 trehalose-treated Money Maker parental line ($P<.04$). These results suggest that either a lack of
280 downstream SA responses or overaccumulation of SA degradation products contributes to the

281 trehalose-mediated increase in BW resistance. Alternatively, trehalose may improve resistance
282 downstream of SA responses.

283 More than half of the *NahG* tomato plants displayed a transient marginal leaf necrosis
284 phenotype following trehalose treatment (data not shown), which could have been due to increased
285 ROS production. To test this, we infused leaves from four-week-old wild type Bonny Best, Moneymaker,
286 or *NahG* tomato plants with water, *R. solanacearum* cells, or 30 mM trehalose solution and observed
287 ROS production with DAB staining. Infusing leaves with trehalose did not trigger excess ROS production
288 (Supp. Fig. 4), suggesting that another physiological effect caused the transient marginal necrosis in the
289 *NahG* plants.

290 **Trehalose treatment promotes accumulation of hormones and defense compounds in xylem sap**

291 To better understand the mechanisms of trehalose-mediated wilt resistance, we measured
292 levels of a suite of plant hormones, oxylipins, and defense-related compounds in xylem sap from
293 infected and trehalose-treated Bonny Best tomatoes. We harvested sap from tomatoes displaying a
294 disease index of 1 (DI=1, partially wilted, late-stage infection) and from uninoculated tomatoes at 6, 24,
295 and 48 h after trehalose treatment. Using targeted quantitative mass spectrometry, we measured
296 metabolites from the phenylpropanoid biosynthesis pathway, several oxylipins from the 9-lipoxygenase
297 and 13-lipoxygenase pathways, and other selected defense metabolites (Supp. Table 3).

298 Sap from *R. solanacearum*-infected plants and trehalose-treated plants had different levels of
299 targeted metabolites compared to sap from healthy tomatoes. Sap collected 24 h after trehalose
300 treatment had the highest number of enriched compounds. These were consistent with a shock or
301 wound response even though plants were not wounded (Vallad and Goodman 2004). Surprisingly,
302 trehalose treatment, but not *R. solanacearum* infection, increased SA concentration at 24 h (Fig. 6A).
303 This result was consistent with the possibility that trehalose plays a role in SA signaling and suggested
304 that SA concentration might not have a significant role in xylem sap at late-stage disease. Benzoic acid is

305 a potent antimicrobial compound produced through the phenylpropanoid pathway that can serve as a
306 direct precursor to SA (Chen, Zheng et al. 2009, Widhalm and Dudareva 2015). Benzoic acid levels
307 increased in xylem sap from both trehalose treated and *R. solanacearum* infected plants (Fig. 6B).
308 Interestingly, coumaric acid, another phenylpropanoid defense compound that *R. solanacearum* can
309 degrade, was significantly increased in sap from *R. solanacearum*-infected plants but not in sap from
310 trehalose treated plants (Fig. 6C) (Lowe, Ailloud et al. 2015). Levels of traumatic acid, which results from
311 the oxidation of traumatin produced by wounded plants, were higher in sap from both *R. solanacearum*-
312 infected plants and trehalose-treated plants at 24 h (Fig. 6D) (Zimmerman and Coudron 1979).

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

318 Curiously, several defense-related oxylipins from the reductase and lipoxygenase branches of
319 the LOX pathway were enriched in sap from *R. solanacearum*-infected plants and 24 h trehalose treated
320 plants (Fig. 6H-K). These oxylipins include 9(*S*)-hydroxy-10(*E*),12(*Z*),15(*Z*)-octadecatrienoic acid (9-HOT,
321 Fig. 6H), 9(*S*)-hydroxy-10(*E*),12(*Z*)-octadecadienoic acid (9-HOD, Fig. 6I), 13(S)-hydroxy-9(Z),11(E)-
322 octadecatrienoic acid (13-HOD, Fig. 6J), and 13-oxo-9(*Z*),11(*E*)-octadecadienoic acid (13-KOD, Fig. 6K). All
323 these oxylipins have antimicrobial and signaling activity in defense against pathogens (Prost, Dhondt et
324 al. 2005, Vicente, Cascón et al. 2012, Marcos, Izquierdo et al. 2015). Induction of these oxylipins in this
325 study indicates their potential involvement in resistance to BW. Altogether, trehalose treatment had an
326 unexpectedly large impact on xylem levels of defense metabolites, particularly jasmonates and SA,
327 which have often been considered antagonistic signals.

328 **Discussion**

329 To survive drought, plants must respond to and overcome multiple adverse physiological and
330 molecular events (Shinozaki, Yamaguchi-Shinozaki et al. 2003, Osakabe, Osakabe et al. 2014). Our
331 experiments indicated that *R. solanacearum* affects tomato water physiology; the transcriptomic
332 signatures of infected plants had striking similarities to the gene expression profiles associated with
333 water stress.

334 The roles of trehalose and T6P as nutritional signaling metabolites in plants have been reviewed
335 (Lunn 2007, Paul 2007, Iordachescu and Imai 2008, Paul, Primavesi et al. 2008, Lunn, Delorge et al.
336 2014). The current paradigm is that T6P, and by extension trehalose, are direct indicators of sucrose
337 levels that help plants maintain sucrose-to-starch ratios through SnRK1 signaling (Schluepmann, Pellny
338 et al. 2003, Kolbe, Tiessen et al. 2005, Lunn, Feil et al. 2006, Schluepmann, Berke et al. 2012). However,
339 little is known about the roles of plant trehalose-metabolizing enzymes beyond nutritional signaling.
340 Most organisms, including plants and *R. solanacearum*, synthesize trehalose via a two-step reaction.
341 Trehalose-6-phosphate synthase (TPS) makes trehalose-6-phosphate (T6P) from glucose and UDP
342 glucose. T6P phosphatase (TPP) then dephosphorylates T6P to make trehalose. In *Arabidopsis,* most of
343 these proteins have predicted functions in development or signaling (Vandesteene, Ramon et al. 2010,
344 Vandesteene, Lopez-Galvis et al. 2012). Infected susceptible tomatoes slightly upregulated TPS-7 (0.5 log
345 fold change), suggesting this gene could be responsible for trehalose synthesis during BW. Resistant
346 plants did not upregulate TPS-7. Instead, they downregulated two TPS and three TPP genes, implying
347 that tightly regulated trehalose concentration during disease might contribute to resistance and
348 defense. Experimentally manipulating expression of these TPS and TPP proteins could test this
349 hypothesis.

350 We focused on tomato LEA and aquaporin genes because these proteins accumulate in
351 response to dehydration. However, resistant tomatoes downregulated genes annotated as LEA

352 hydroxyproline-rich glycoprotein (HRGP) proteins (Fig. 1B) (Cao and Li 2015). LEA HRGPs are plant cell
353 wall components, and their downregulation in our data suggests that resistant plants may slow their
354 growth as part of a defense trade-off (Johnson, Cassin et al. 2017). Since tomato aquaporins are
355 encoded by diverse families and channel other solutes in addition to water, it is not easy to generalize
356 about the behavior of aquaporins during BW (Reuscher, Akiyama et al. 2013). However, in response to
357 *R. solanacearum* infection, both susceptible and resistant tomatoes downregulated far more aquaporins
358 than they upregulated, suggesting that plants may try to alter water flow during BW. SlTIP2;2
359 (Solyc03g120470), an aquaporin that improved transpiration and yield when overexpressed in tomato
360 plants (Sade, Vinocur et al. 2009), was downregulated in both resistant and susceptible plants (Fig. 1D).

361 The meta-transcriptome analysis revealed that one ABA hydroxylase gene was strongly
362 upregulated in resistant and susceptible plants, while another was downregulated only in resistant
363 plants (Fig. 1C). In *Arabidopsis* and tomato*,* CYP707A ABA hydroxylases, which catalyze the first step in
364 ABA degradation, are involved in dehydration and rehydration at various developmental stages (Kushiro,
365 Okamoto et al. 2004, Saito, Hirai et al. 2004, Umezawa, Okamoto et al. 2006, Ji, Kai et al. 2014). This
366 strongly suggests both that tomatoes tightly regulate ABA catabolism during BW and that BW causes
367 water stress. Expression of TAS14, a dehydrin inducible by environmental stress and ABA (Godoy, Pardo
368 et al. 1990), was highly upregulated in susceptible and resistant plants, further evidence that BW causes
369 abiotic stress. In *Arabidopsis,* ABA synthesis, signaling, and responsive genes were similarly affected
370 during late-stage BW infection (Hu, Barlet et al. 2008). Additional identification of ABA-responsive
371 networks in tomatoes could identify targets for breeding more hydraulically efficient tomatoes that are
372 also more resistant to BW. It would be interesting to see if the ABA transcription factors identified in our
373 transcriptomic analysis regulate expression of tomato TPP genes; if so, manipulating these genes could
374 contribute to BW resistance.

375 ABA concentration in xylem sap of susceptible tomatoes increased in response to both *R.*
376 *solanacearum* infection and trehalose treatment. Elevated ABA levels are usually associated with
377 drought stress; together with the observation that *R. solanacearum* infection lowers plant stomatal
378 conductance, this indicates that BW significantly disrupts tomato water physiology. However, there
379 might also be a defensive component to ABA concentration and signaling. Deleting several cellulose
380 synthase (*irx*) genes increased *Arabidopsis* resistance to BW in an ABA-dependent manner and ABA
381 synthesis mutants were more susceptible to *R. solanacearum* (Hernández-Blanco, Feng et al. 2007). This
382 suggests that tomatoes that over-produce ABA could have increased resistance to wilt diseases.
383 Treating tomato roots with ABA reduced both stomatal conductance and *R. solanacearum*
384 colonization in stems, suggesting that *R. solanacearum* colonization depends, at least in part, on active
385 hydraulic conductance and a negative pressure gradient in host xylem. *R. solanacearum* is known to use
386 swimming and type IV motility and cell wall degrading enzymes to move towards roots and migrate
387 through the xylem (Tans-Kersten, Huang et al. 2001, Liu, Zhang et al. 2005). However, our results show
388 that *R. solanacearum* also uses plant water transport caused by transpiration to spread within the plant.
389 This suggests that *R. solanacearum* decreases its own spread and pathogenic fitness when it blocks host
390 plant water flow. This idea has interesting implications for physiological differences between susceptible
391 and resistant tomato plants since wilt-resistant varieties thrive even though they are often latently
392 infected. It is possible that ancestors of *R. solanacearum* were once endophytes but lost the ability to
393 check their population growth in the vasculature of susceptible hosts, possibly as a result of evolutionary
394 selection pressures imposed by agriculture, as has been proposed for *Xylella fastidiosa* (Beattie and
395 Lindow 1995).

396 Trehalose likely improves drought tolerance in plants by two mechanisms: by regulating
397 carbohydrate levels during stress conditions, possibly via the SnRK1 kinase; and by altering ABA signaling
398 and stomatal conductance (Garg, Kim et al. 2002, Paul, Gonzalez-Uriarte et al. 2018, Yu, Zhao et al.

399 2019). Members of another kinase family, SnRK2, also integrate with ABA signaling, and trehalose
400 metabolism may also connect to this network via the TOR regulator (Signorelli, Tarkowski et al. 2019,
401 Chen, Li et al. 2020). In our system, exogenous trehalose temporarily improved water conservation in
402 uninfected tomatoes and delayed wilting of drought-stressed plants. This was most likely because
403 trehalose treatment temporarily reduced stomatal conductance and transpiration and increased water
404 use efficiency. Trehalose also increased ABA content in xylem sap 24 h post-treatment. These results
405 confirm and extend findings that trehalose, ABA signaling, and water use interact in complex ways. The
406 *Arabidopsis TPS1* gene responsible for maintaining T6P levels responds to ABA and contributes to
407 regulating stomatal aperture (Avonce, Leyman et al. 2004, Gomez, Gilday et al. 2010). At least two
408 *Arabidopsis* TPP proteins (AtTPPI and AtTPPE) that are controlled by ABA-responsive transcription
409 factors contribute to trehalose production and mitigate drought stress (Lin, Yang et al. 2019, Lin, Wang
410 et al. 2020, Wang, Chen et al. 2020). Another study found applying trehalose to tomato roots improved
411 water conservation, decreased ABA biosynthesis gene expression, increased expression of ABA-
412 responsive genes, kept stomates closed five days after trehalose treatment without water, and
413 increased ABA levels in leaves after 24 h (Yu, Zhao et al. 2019). Our results contrast in some ways with
414 this paper. We found that ABA content temporarily increased in xylem sap 24 h after after trehalose
415 treatment while stomatal conductance was reduced for less than 24 h. These differences may be
416 explained by methodological variation: Yu and co-workers did not include non-drought stressed tomato
417 controls, they measured ABA in leaves rather than xylem, and they measured stomatal aperture rather
418 than conductance. There is a lag between stomatal conductance response in leaves and ABA
419 concentration in the xylem sap in our data, leading us to hypothesize that ABA concentration in leaves
420 might be a more biologically relevant marker for stomatal conductance. It would be interesting to
421 determine if exogenous trehalose or ABA treatment changes trehalose concentration in tomato xylem
422 sap.

423 The plant hormone SA, which mediates resistance to *R. solanacearum* in many systems, is
424 necessary for induction of plant innate immunity and activation of systemic acquired resistance
425 (Deslandes, Olivier et al. 2002, Hirsch, Deslandes et al. 2002, Chen, Lin et al. 2009, An and Mou 2011,
426 Boatwright and Pajerowska-Mukhtar 2013, Jacobs, Milling et al. 2013). SA-responsive tomato defense
427 genes were highly upregulated 6 and 48 h after trehalose treatment. These same genes are upregulated
428 in diseased tomato plants following *R. solanacearum* soil soak infection, suggesting trehalose treatment
429 activates SA-mediated resistance pathways that could affect *R. solanacearum* infection (Milling, Babujee
430 et al. 2011).

431 Trehalose treatment increased wilt resistance of SA-degrading *NahG* tomato plants, but not the
432 parental Moneymaker cultivar. There are several possible explanations for this result. First, trehalose
433 treatment could have increased SA levels so much that overproduction of catechol, the breakdown
434 product of SA, contributed to resistance (Retig and Chet 1974). Second, excess ET and JA in *NahG* plants
435 could promote resistance to *R. solanacearum*. After infection with *P. syringae* DC3000 *NahG* tomatoes
436 produce more ET and JA, both of which can contribute to disease resistance (Spoel, Koornneef et al.
437 2003, López-Gresa, Lisón et al. 2016). ET is involved in *R. solanacearum*-host interactions and ET-
438 responsive defense genes were upregulated at 6 and 48 h post trehalose treatment. However, ET-
439 insensitive *NeverRipe* tomatoes were neither more nor less resistant to *R. solanacearum* than wild type
440 after trehalose treatment. In another study, treatment with a biocontrol strain of the oomycete *Pythium*
441 enhanced resistance to *R. solanacearum* in *NahG* tomatoes, but not in a JA mutant (Hase, Takahashi et
442 al. 2008). Since the ET defense pathway appeared less relevant in our study, it may be useful to
443 determine if JA enhances *R. solanacearum* resistance in tomatoes.

444 Plants use hormones to fine-tune many of their growth, development, and defense functions,
445 and xylem vessels can act as signal transduction highways for long-distance transport of auxin, ABA, JA,
446 and SA (Jackson 1997, Park, Lee et al. 2017). We observed increased SA concentration in xylem sap of

447 trehalose treated plants but not in the xylem sap of symptomatic *R. solanacearum*-infected plants. This
448 was surprising because SA signaling and concentration are integral to plant response to BW (Jacobs,
449 Milling et al. 2013). However, *R. solanacearum* can actively degrade SA, which is toxic to the pathogen
450 (Lowe-Power, Jacobs et al. 2016). It is also possible that SA concentrations might be higher in root
451 xylem than in stem xylem. For instance, an *Arabidopsis* mutant impaired in secondary wall deposition
452 had higher SA concentrations in the root and increased resistance to *R. solanacearum* infection, possibly
453 through vascular immunity (Denancé, Ranocha et al. 2013). Taken together, the increased SA levels in
454 xylem sap, the increased expression of SA-dependent defense genes, and the trehalose-dependent
455 *NahG* resistance phenotype suggest that the protective effect of trehalose requires systemic resistance
456 induced via SA.

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

463 SA- and JA-mediated plant defense responses have often been seen as antagonistic (Glazebrook
464 2005, Derksen, Rampitsch et al. 2013). Our gene expression analysis displayed this antagonism; at six h
465 post trehalose treatment JA-responsive *pin2* was downregulated, while SA-responsive *PR1b* and *gluA*
466 were upregulated. Roots of wilt-resistant Hawaii7996 tomatoes displayed a similar gene expression
467 profile 24h after *R. solanacearum* infection (French, Kim et al. 2018). Despite the antagonism observed
468 in the gene expression data, the concentrations of SA, JA, and the JA precursor 12-OPDA all increased in
469 trehalose-treated plants at 24 h. Recently, it was demonstrated in maize that levels of OPDA and JA are
470 irrelevant for the classic SA/JA-Ile antagonism and that 12-OPDA and SA are co-regulated (Wang,

471 Borrego et al. 2020). Infusing maize xylem sap with 12-OPDA increased resistance to *Colletotrichum*
472 *graminicola* while infusing similar concentrations of JA increased susceptibility. Disease experiments
473 with JA-insensitive tomato mutants could determine if 12-OPDA or JA contribute to resistance and
474 define their relationship to SA signaling.

475 JA signaling also induces leaf senescence, and chlorosis is associated with increased JA
476 production (He, Fukushige et al. 2002). We found that trehalose treatment significantly decreased
477 chlorophyll content 2-10 days post-treatment, which correlates with the increased JA production in
478 trehalose-treated plants. This is consistent with the finding that applying 5 mM trehalose to soybean
479 roots did not affect JA concentration, but it did decrease chlorophyll content at ten days (Asaf, Khan et
480 al. 2017).

481 Trehalose treatment and *R. solanacearum* infection both increased xylem sap levels of several
482 antimicrobial compounds, including phenolics. *R. solanacearum* requires at least two multi-drug efflux
483 pumps and a hydroxycinnamic acid degradation pathway for full virulence and fitness, showing that
484 antimicrobials, including phenolics, protect tomato plants from BW (Brown, Swanson et al. 2007, Lowe,
485 Ailloud et al. 2015). Plants have specialized cells that store and release phenolics into the xylem as part
486 of their defense response, and trehalose treatment and *R. solanacearum* infection both induce this
487 release (Beckman 2000).

488 Levels of the oxylipins 9-HOT, 9-HOD, 13-HOD, and 13-KOD increased in sap from *R.*
489 *solanacearum*-infected plants and trehalose-treated plants 24 h after treatment. These metabolites,
490 which accumulate in wounded maize, bean, and *Arabidopsis*, can be potent inducers of defense gene
491 response (Alméras, Stolz et al. 2003, Walters, Cowley et al. 2006, He, Borrego et al. 2020). Further, these
492 oxylipins have direct antimicrobial activity against fungal and bacterial pathogens (Prost, Dhondt et al.
493 2005). The data generated by this study deserve further analysis since little is known about oxylipin
494 signatures during BW or oxylipin toxicity to *R. solanacearum*. However, 9-LOX-derived oxylipins,

495 including 9-HOT and 9-HOD, increase in tobacco leaves inoculated with *R. solanacearum* GMI100, which
496 is avirulent on tobacco (Montillet, Agnel et al. 2002). This increase in 9-LOX derivatives correlated with
497 the hypersensitive response (HR) in leaves. We speculate that the presence of related oxylipins in our
498 study may indicate vascular HR. It would be interesting to see if defense compound concentration
499 changed in the xylem sap of resistant tomatoes challenged with *R. solanacearum.*

500 Plant systemic resistance typically involves accumulation of SA, JA, and defense oxylipins, and
501 increased expression of SA, JA, and ET-responsive defense genes in response to mechanical wounding or
502 pathogen challenge (Vallad and Goodman 2004, Choudhary, Prakash et al. 2007, Savatin, Gramegna et
503 al. 2014, Wang, Borrego et al. 2020). We found that treating tomato roots with trehalose similarly
504 increased SA, JA, and defense oxylipins, and upregulated SA, JA, and ET defense genes. We propose that
505 tomato plants activated induced systemic resistance because they recognized trehalose as a damage
506 associated molecular pattern (DAMP). DAMPs are waste products produced by pathogen invasion or
507 wounding that alert the plant that it is under attack (Ranf, Eschen-Lippold et al. 2011, De Lorenzo,
508 Ferrari et al. 2018, Hou, Liu et al. 2019). A DAMP can be almost anything that is usually intracellular or
509 results from lignin and cellulose polymer degradation. Our finding adds to the growing evidence that
510 sugars contribute to plant innate immunity (Bolouri Moghaddam and Van den Ende 2012).

511 Could treating roots with compatible solutes like trehalose in the field provide temporary
512 resilience until disease and drought pressure have passed? Other chemical inducers of plant defense
513 responses can reduce BW. Acibenzolar-S-methyl (Actigard) and DL-3-aminobutyric acid (BABA) both
514 lowered wilt disease incidence in greenhouse and field by promoting defense gene expression and
515 defense compound production, respectively (Jakab, Cottier et al. 2001, Pradhanang, Ji et al. 2005,
516 Hassan and Abo-Elyousr 2013). Additionally, growing tomatoes hydroponically with SA and JA increased
517 defense enzyme production and reduced BW incidence (Mandal, Kar et al. 2013). Finally, exogenous L-
518 histidine induced resistance to BW in an ET-dependent manner in *Arabidopsis* and tomato (Seo, Nakaho

519 et al. 2016). Further experiments are needed to determine if treating tomatoes with exogenous
520 trehalose could be a practical BW management strategy.

521 *R. solanacearum* GMI1000 produces and secretes abundant amounts of the TreA trehalase
522 during tomato infection, and ability to degrade trehalose contributes to bacterial fitness and virulence
523 even though trehalose is not an important nutritional source for the pathogen (Jacobs, Babujee et al.
524 2012, Hamilton, Steidl et al. 2021). Interestingly, treating plants with the trehalase inhibitor Validamycin
525 A increased BW resistance (Ishikawa, Fujimori et al. 1996). We previously showed that the trehalose in
526 xylem sap of *R. solanacearum*-infected tomato plants is produced by the plant (MacIntyre, Barth et al.
527 2019). Could *R. solanacearum* secrete trehalase to counter the effects of plant-produced trehalose?

528 Our results suggest several ways *R. solanacearum* could benefit from breaking down plant-
529 produced trehalose. First, the pathogen may degrade xylem sap trehalose in order to reduce host plant
530 defenses by removing this potential DAMP. Second, as discussed above, trehalose reduces tomato
531 transpiration, which helps *R. solanacearum* spread and colonize host xylem. *R. solanacearum* may
532 degrade trehalose to maintain this helpful sap flow by preventing trehalose-mediated stomatal closing.
533 Third, *R. solanacearum* could degrade trehalose to interfere with the trehalose and T6P signaling that
534 plants use to fine tune their sucrose to starch ratios. Trehalose degradation could thus indirectly
535 increase the amount of available sucrose, which is a key carbon source fueling *R. solanacearum* growth
536 in xylem (Jacobs, Babujee et al. 2012, Hamilton, Steidl et al. 2021). The fact that the *R. solanacearum*
537 effector RipTPS is a functional trehalose-6-phosphate synthase is consistent with the hypothesis that this
538 pathogen benefits from manipulating host plant trehalose metabolism (Poueymiro et al., 2014).
539 Additional experiments are needed to test these non-exclusive hypotheses about how trehalase benefits
540 *R. solanacearum*.

541 **Conclusion**
542

543 This study argues that trehalose is present in xylem sap of *R. solanacearum*-infected tomatoes
544 as a response to wilt disease-mediated water stress. Both trehalose levels and the expression of water
545 stress genes change in response to *R. solanacearum* infection. Further, ABA content, an indicator of
546 drought status, increases in xylem sap of diseased plants. We confirmed the link between trehalose and
547 ABA signaling and drought stress in plants by demonstrating that trehalose treated plants temporarily
548 reduce stomatal conductance, contain more ABA in their xylem sap, and are more drought tolerant.

549 When applied to plant roots, trehalose reduced BW incidence and reduced host xylem
550 conductance, which aids bacterial colonization. SA-responsive defense genes were upregulated in
551 trehalose treated plants, SA non-responsive *NahG* plants were more resistant to *R. solanacearum* after
552 trehalose treatment, and SA concentration transiently spiked in xylem sap from treated plants. JA, other
553 oxylipins, and antimicrobial compounds also all increased in xylem sap of trehalose-treated plants.
554 Taken together, these results suggest that plants perceive trehalose as a DAMP and respond with
555 systemic disease resistance. In turn, *R. solanacearum* degrades trehalose as a counter-defense.

556 **Methods**

557 **Total RNA extraction and RNA-seq sample preparation |** RNA extraction and RNA-seq sample
558 preparation for the transcriptomic studies were performed as described (Khokhani, Lowe-Power et al.
559 2017, French, Kim et al. 2018).

560 **RNA-seq data analysis |** Illumina HiSeq2500 paired-end 100-bp; Illumina HiSeq2500 paired-end 150 bp
561 and Illumina HiSeq2000 paired-end 100-bp RNA sequencing were performed on the 18, 6, and 12
562 samples from three independent studies (two previously unpublished [Studies A+B], and [Study C]
563 French, Kim et al. 2018). RNA-sequencing data were analyzed through the same analysis pipeline. Read
564 quality was controlled using FastQC version 0.11.9 (Andrews). Reads were trimmed to remove low-
565 quality sequences and the Illumina sequencing adaptors (TruSeq3-PE-2) using Trimmomatic version 0.39
566 (Bolger, Lohse et al. 2014). After trimming and mapping, a total of 930,004,276, 135,532,392, and
567 103,831,164 paired-end reads were generated for Studies A, B, and C, respectively. Reads for each
568 sample were mapped to the ITAG4.0 *S. lycopersicum* reference genome using STAR version 2.7.4.a
569 (Dobin, Davis et al. 2012). Library type was set to strand-specific (reverse) for Study A and Study B, and
570 set to no-stranded in Study C. Gene expression was measured as the total number of reads for each
571 sample uniquely mapped to the reference using HTSeq version 0.11.1 and samtools version 1.10 (Li,
572 Handsaker et al. 2009, Anders, Pyl et al. 2014). Each sample averaged about 12, 9, and 4 million uniquely
573 aligned reads for Studies A, B, and C, respectively. "EdgeR" package version 3.28.0 was used to filter for
574 low counts such that at least three of the samples in each study had at least three counts per million,
575 resulting in a total of 19123, 18193, and 18917 genes remaining for Studies A, B, and C, respectively
576 (Robinson, McCarthy et al. 2009).

577 Differential gene expression within each study was conducted using the DESeq2 package
578 Bioconductor version 3.11 and a Benjamini-Hochberg false discovery rate multiple testing correction of
579 *P*=.05 (Benjamini and Hochberg 1995, Love, Huber et al. 2014). Meta-transcriptomic analysis was

580 performed using the metaRNASeq package version 1.0, and differential gene expression within the
581 meta-analysis was determined with the DESeq2 results, Fisher, and inverse normal techniques with a
582 *P*=.05, as described (Rau, Marot et al. 2014). Differentially expressed genes were filtered using a log2
583 fold change >|0.585| (corresponds to a fold change of |1.5|).

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

590 Candidate TPS and TPP proteins were identified in the most recent annotations of the tomato
591 (cv. 'Heinz 1706') genome available on NCBI and the Sol Genomics Network (ITAG4.0,
592 https://solgenomics.net/tools/blast/, accessed 03/2020) using BLASTP with standard settings (Sato,
593 Tabata et al. 2012). BLASTP protein sequences were derived from the *E. coli* K-12 *otsA* and *otsB* proteins,
594 as well as *Arabidopsis* trehalose-related proteins (Leyman, Van Dijck et al. 2001, Vandesteene, Ramon et
595 al. 2010, Vandesteene, Lopez-Galvis et al. 2012). Tomato cv. Heinz 1706 contained ten TPS genes, eight
596 TPP genes, and one trehalase gene (Supplemental Table 1). Tomato ABA-related, LEA, and aquaporin
597 were identified by their PANTHER annotation, and many were previously annotated (Ma, Szostkiewicz et
598 al. 2009, Reuscher, Akiyama et al. 2013).

599 The transcriptomic studies were performed and labeled as follows: R1, roots of resistant tomato
600 breeding line Hawaii7966 (HW) sampled 24 h after infection with *R. solanacearum* strain K60; R2, roots
601 of HW sampled 48 h after infection with *R. solanacearum* strain K60; R3, seedling roots of HW sampled
602 24 h after infection with *R. solanacearum* strain GMI1000; S1, roots of wilt-susceptible West Virginia 700

603 tomato plants (WV) sampled 24 h after infection with *R. solanacearum* strain K60; S2, roots of WV
604 sampled 48 h after infection with *R. solanacearum* strain K60; S3, seedling roots of wilt-susceptible cv.
605 Bonny Best (BB) sampled 24 h after infection with *R. solanacearum* strain GMI1000; S4, mid-stems of BB
606 sampled 72 h after inoculation with *R. solanacearum* strain GMI1000.

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

619 For metabolite analysis of sap, 95 µL of xylem sap was mixed with 5 µL of 50 µM of internal
620 standard consisting of; d-ABA ([$^2H_6$](+)-*cis*, *trans*-abscisic acid, Olchem, Olomouc, Czech Republic), d-CA
621 (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
622 jasmonic acid, CDN Isotopes, Pointe Claire, Quebec, Canada), and d-SA (d_6-SA; Sigma-Aldrich, St Louis,
623 MO, USA). Samples were transferred into autosampler vials for LC-MS/MS analysis. Authentic analytical
624 external standards for each metabolite were used to identify select ions and retention times for each
625 metabolite (Supplemental Table 4). The simultaneous detection of several phytohormones used the
626 following methods with modifications (Müller and Munné-Bosch 2011). We used an Ascentis Express C-

627 18 Column (3 cm × 2.1 mm, 2.7 µm) (Sigma-Aldrich, St. Louis, MO, USA) connected to an API 3200 LC-
628 MS/MS (Sciex, Framingham, MA, USA) using electrospray ionization in negative mode with multiple
629 reaction mentoring. The injection volume was 10 µL and had a 500 µL min^{-1} mobile phase consisting of
630 Solution A (0.2% acetic acid in water) and Solution B (0.2% acetic acid in acetonitrile) with a gradient
631 consisting of (time – %B): 0.5 – 10%, 1.0 – 20%, 21.0 – 70%, 24.6 – 100%, 24.8 – 10%, 29 – stop. After
632 integration with Analyst v1.6.3 (Sciex), the concentration of endogenous metabolites was determined by
633 comparing their peak areas to those of isotopically labeled internal standards using calculated response
634 factors.

635 **Stomatal conductance, transpiration, and photosynthesis measurements with LI-COR |**To assess the
636 effect of trehalose treatment on tomato stomatal conductance (g_s), transpiration (E), and
637 photosynthesis (A_{net}), we used a LI-COR portable photosynthesis system (model LI-6400XT, LI-COR
638 Biosciences, Lincoln, NE, USA) equipped with a CO_2-controller and a red-blue LED chamber (model LI-
639 6400-02b). The ambient CO_2 concentration was set to 400 ppm, flow rate to 300 µmol s^{-1}, and the
640 chamber light source set at an intensity of 1200 µmol m^{-2} s^{-1} for all measurements. Fifteen-day-old
641 Bonny Best plants were treated with 50 mL of trehalose solution or water, then watered every day after
642 for the duration of the experiment. LI-COR measurements were taken at 10min, 6 h, 24 h, 48 h, and 72 h
643 post trehalose treatment. The experiment was repeated twice with ten plants per treatment.

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

650 **ABA treatments |** We assessed the effect of ABA treatment on two-week-old tomato stomatal
651 conductance and subsequent bacterial wilt incidence. ABA was prepared at a stock concentration of
652 50mg/mL in DMSO. The water + DMSO control plants were watered daily with 50 mL of water
653 supplemented with the volume of DMSO used to make the 100 µM ABA solution. The ABA-treated
654 groups were watered daily with 50 mL of 50 or 100 µM ABA solution in water. After 48 h of ABA
655 treatment, plants were soil drenched with 50 mL of a $1x10^8$ CFU/mL of *R. solanacearum* GMI1000 with
656 kanamycin resistance (to aid in recovery from the plant) (Lowe, Ailloud et al. 2015). Bacteria were
657 resuspended at the desired O.D. in water + DMSO or the ABA solutions, so plants were inoculated and
658 ABA treated concurrently. Tomatoes were destructively sampled five days post-infection (after 168 h of
659 ABA treatment) to assess bacterial colonization of stems. Stomatal conductance, transpiration, and
660 photosynthesis measurements were taken for the entirety of the experiment with the LI-COR as
661 described 4-6 h after watering/ABA treating in the morning, which was the length of time it took ABA
662 treatment to affect conductance.

663 We assessed ABA toxicity to *R. solanacearum* by supplementing CPG broth with 50-100 µM ABA
664 or the equivalent volume of DMSO, and growing *R. solanacearum* cells in this media for 48 h. Cells were
665 grown in a shaking 96 well plate at 28°C in a BioTek Synergy HT plate reader, which measured Abs_{600}
666 every half hour. The experiment was performed once with eight technical replicates per treatment.

667 **Plant assays with *R. solanacearum* |** Plant growth conditions, colonization, and disease curve assays
668 were performed as described (Khokhani, Tuan et al. 2018, MacIntyre, Barth et al. 2019). All plant assays
669 were grown on 12 h light/dark cycles at 28 °C with ~60% relative humidity unless stated otherwise. For
670 the disease curve assays with trehalose treatments, fifteen-day old Bonny Best tomatoes were treated
671 with either water (control) or 50 mL of 30 mM trehalose solution. Plants were not watered 24 h post-
672 treatment per standard procedure for soil soak assays. At 48 h post-treatment plants were soil drenched
673 with 50 mL of $1x10^8$ CFU/mL of wild type *R. solanacearum* GMI1000 (or Δ*treA* for the later trehalose

674 treatment experiments with this mutant). For the petiole inoculations, 3-week-old tomatoes were
675 treated with trehalose or water as described above; after 48 h a true leaf was removed, and 2000 wild
676 type GMI1000 cells in 10 µL of water were pipetted onto the cut stem. To assess the effectiveness of
677 spraying trehalose to delay BW incidence, fifteen-day-old Bonny Best plants were either treated with 30
678 mM trehalose solution through the soil as described or sprayed with trehalose solution (or water) to
679 run-off. For all assays, plants were inoculated with *R. solanacearum* 48 h post trehalose root or leaf
680 treatment.

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

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

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

694 **Water conservation |** Two-week-old Bonny Best plants were treated with trehalose or water. Water
695 was subsequently withheld and plants were assessed daily to see how long it took them to wilt. Wilting

696 was rated using the same scale as disease curve assays. The experiment included four biological
697 replicates, each with ten plants per treatment.

698 **QRT-PCR measurement of tomato trehalose and defense gene expression |** JA, SA, and ET defense
699 gene primers were previously characterized in tomato *R. solanacearum* response (Supplemental Table 2)
700 (Milling, Babujee et al. 2011). The *rd22* and *dhn_tas* ABA-responsive primers were previously described
701 (Yamaguchi-Shinozaki and Shinozaki 1993, Kissoudis, Seifi et al. 2016). Actin and *dnaJ* primers served as
702 our constitutively expressed normalizing genes.

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

717 **Relative water content |** To assess the water status and osmotic adjustment of tomato plants treated
718 with trehalose, we measured relative water content (RWC) in leaves. This directly measures cellular

719 hydration. Three-week-old Bonny Best tomato plants were treated with 50 mL of water or 30 mM
720 trehalose solution. 48 h post-treatment, leaves were excised at the petiole and weighed for fresh
721 weight. Leaves were then rehydrated by floating on distilled water in closed petri dishes for 4 h, at
722 which time they were blotted, and their turgid weight was assessed. Leaves were then thoroughly dried
723 in a food dehydrator oven for 24 h, and dry weight was evaluated. RWC was calculated as follows: %
724 RWC= [(Leaf fresh weight – dry leaf weight)/(turgid leaf weight-leaf dry weight)] x 100 (Barrs and
725 Weatherley 1962). The data represent two biological replicates with five technical reps (leaf from an
726 individual plant) per treatment.

727 **Root length assays |** Root length assays to quantify trehalose treatment effect on root length were
728 performed as described with modification (Tran, MacIntyre et al. 2016). Tomato seeds were sterilized in
729 10% bleach for 10 min, then 95% ethanol for 10 min, then washed five times with sterile water and
730 germinated in the dark on 1% water agar plates at room temperature. Four-day-old tomato seedlings
731 were transferred to germination pouches (Mega International, Newport, MN) then watered with either
732 10 mL of water or 30 mM trehalose solution and incubated in a 28°C growth chamber. Following the
733 initial trehalose treatment, pouches were kept moist as needed with sterile water and imaged ten days
734 post-treatment. This experiment was performed three times with ten tomato
735 seedlings/pouch/treatment. New root growth was calculated as % new root growth= (new growth/total
736 root length) x 100.

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

741 **Bacterial leaf spot of tomato inoculations |** *Xanthomonas gardneri* culture conditions, infection, and
742 colonization analysis were as described (Potnis, Soto-Arias et al. 2014). *X. gardneri* strains were cultured
743 on nutrient agar plates (Millipore-Sigma) supplemented with nalidixic acid (50 µg/mL) at 28°C. Inoculum
744 was grown for 48 h in a shaking incubator at 28°C in nutrient broth supplemented with nalidixic acid.
745 The inoculum was adjusted to OD_{600}=0.3 and diluted 1:100 in 400 mL sterile water with 75 µL Silwet
746 (Fisher Scientific). Four-week-old Bonny Best tomato plants that were treated with water or 50 mL of 30
747 mM trehalose solution 72 h previously were dip-inoculated for 30 s in the inoculum. Plants were
748 incubated in high humidity conditions (85-90%) in plastic containers in the growth chamber for 48 h.
749 After 48 h, plants were moved to low humidity conditions during the day (taken out of containers, 45-
750 60%) and high humidity conditions at night on a 12 h cycle. Bacterial spot symptoms appeared 5-6 days
751 post-inoculation. Leaf tissue from individual plants was sampled at 3, 6, and 9 days post-inoculation.
752 Two 2 cm^2 portions of leaf were sampled from each plant, ground in a Powerlyzer (Qiagen) in water, and
753 serially diluted and enumerated on nutrient agar + nalidixic acid. The data represent three biological
754 replicates with 12 technical reps/treatment/timepoint.

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

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

766

767

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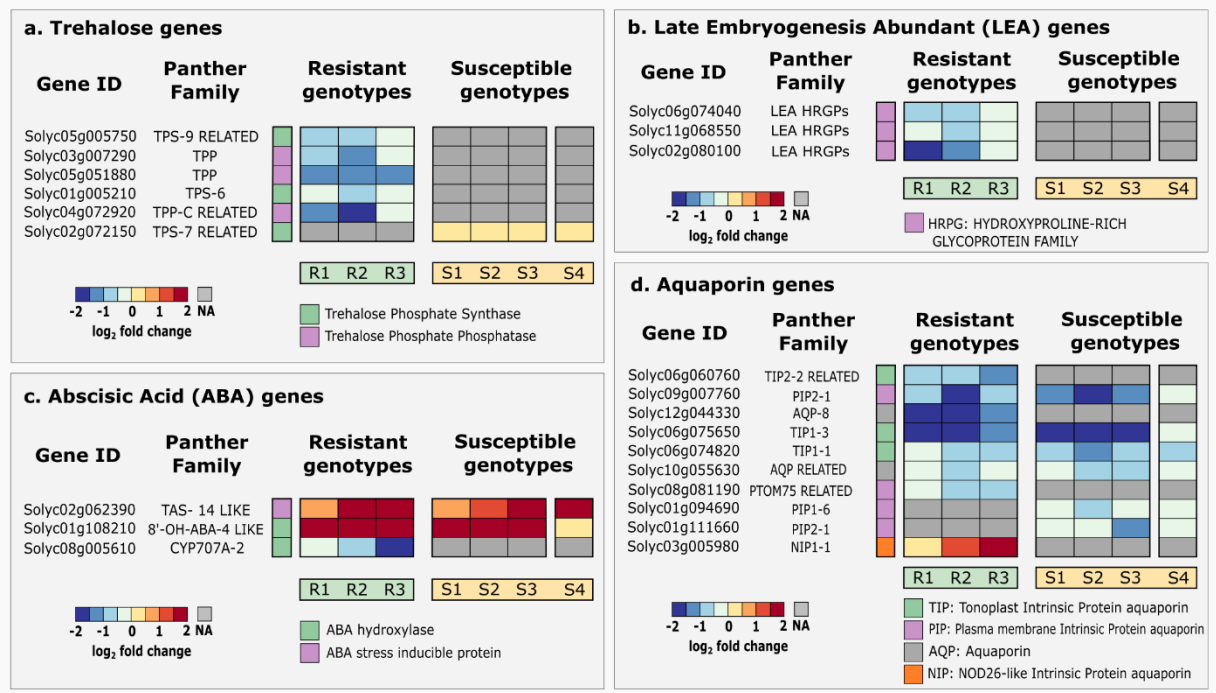
770 *gardneri* cultures and technical advice. April MacIntyre and Caitilyn Allen designed the experiments and
771 wrote the text with contributions from Valerian Meline, Anjali Iyer-Pascuzzi, and Zachary Gorman. April
772 MacIntyre carried out most of the experiments and created most of the figures. Valerian Meline
773 performed the meta-transcriptomic analysis and created Figure 1. Zachary Gorman performed the xylem
774 sap metabolite analysis. Steven Augustine advised and assisted with all LI-COR experiments and created
775 the trehalose stomatal conductance, photosynthesis, transpiration, and water efficiency graphs. Carolyn
776 Dye assisted with the trehalose physiology, ABA treatment, and *Xanthomonas* experiments. Corri
777 Hamilton advised on xylem sap collection. Anjali Iyer-Pascuzzi, Michael Kolomiets, and Kate McCulloh
778 provided feedback and edited the manuscript.

779

780 **Figures**



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782

783 **Figure 1. *R. solanacearum* infection changes expression of tomato genes involved in trehalose**
784 **metabolism and water stress physiology.** Comparative analysis of transcriptomic responses to *R.*
785 *solanacearum* from seven experiments measuring commonly differentially expressed genes across all
786 data sets in wilt-resistant and susceptible tomato plants. Heatmaps show relative expression (as $\log_2$
787 fold change) compared to mock-inoculated control plants of: **A)** Genes encoding trehalose metabolism;
788 **B)** Late embryogenesis/dehydrin (LEA) genes; **C)** Genes encoding ABA synthesis and signaling; and **D)**
789 Aquaporin genes. Gray boxes (NA) in heatmaps indicate that a gene's expression was not different from
790 that of mock-inoculated control plants. Transcriptomic datasets are: R1, roots of wilt-resistant tomato
791 breeding line Hawaii7966 (HW) sampled 24 h after infection with *R. solanacearum* strain K60; R2, roots
792 of HW sampled 48 h after infection with *R. solanacearum* strain K60; R3, seedling roots of HW sampled
793 24 h after infection with *R. solanacearum* strain GMI1000; S1, roots of wilt-susceptible West Virginia 700
794 tomato plants (WV) sampled 24 h after infection with *R. solanacearum* strain K60; S2, roots of WV
795 sampled 48 h after infection with *R. solanacearum* strain K60; S3, seedling roots of wilt-susceptible cv.
796 Bonny Best (BB) sampled 24 h after infection with *R. solanacearum* strain GMI1000; S4, mid-stems of BB
797 sampled 72 h after inoculation with *R. solanacearum* strain GMI1000. Tomato gene names and locus
798 tags are from NCBI and the Sol Genomics Network, accessed in 03/2020. Transcriptomic data were
799 normalized across experiments to allow comparisons as described in Methods.

800

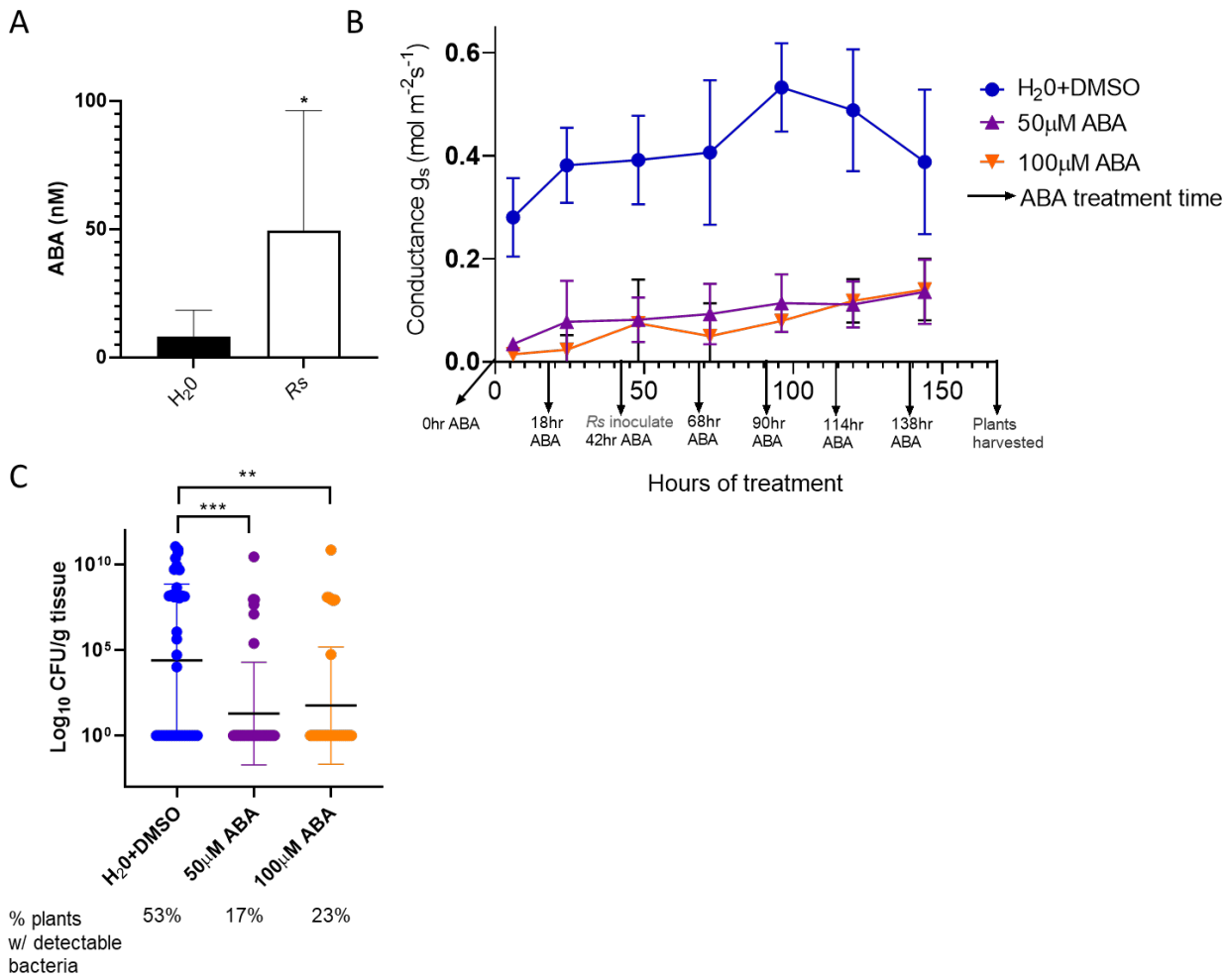


Figure 2. ABA concentration increased in *R. solanacearum*-infected plants, and ABA treatment reduced stomatal conductance, which correlated with reduced *R. solanacearum* stem colonization. A) ABA concentration in xylem sap harvested from healthy and *R. solanacearum*-infected tomato plants showing early wilt symptoms (1-25% of leaf area wilted) (T-test, **P*=.03). Bars show means of xylem sap ABA levels, measured by GC-MS, from four to 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, with three plants analyzed per treatment per time point. **C)** *R. solanacearum* population sizes were 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 (Kruskall Wallis, 50 uM ABA, ****P*<.001; 100 uM ABA, ***P*<.01). The proportion of 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. The horizontal bars represent the geometric mean of the data and vertical bars indicate standard deviation. Plants with undetectable *R. solanacearum* populations were given a value of 1 in the statistical analysis. Data represent three biological replicate experiments, each with 12 plants per treatment.

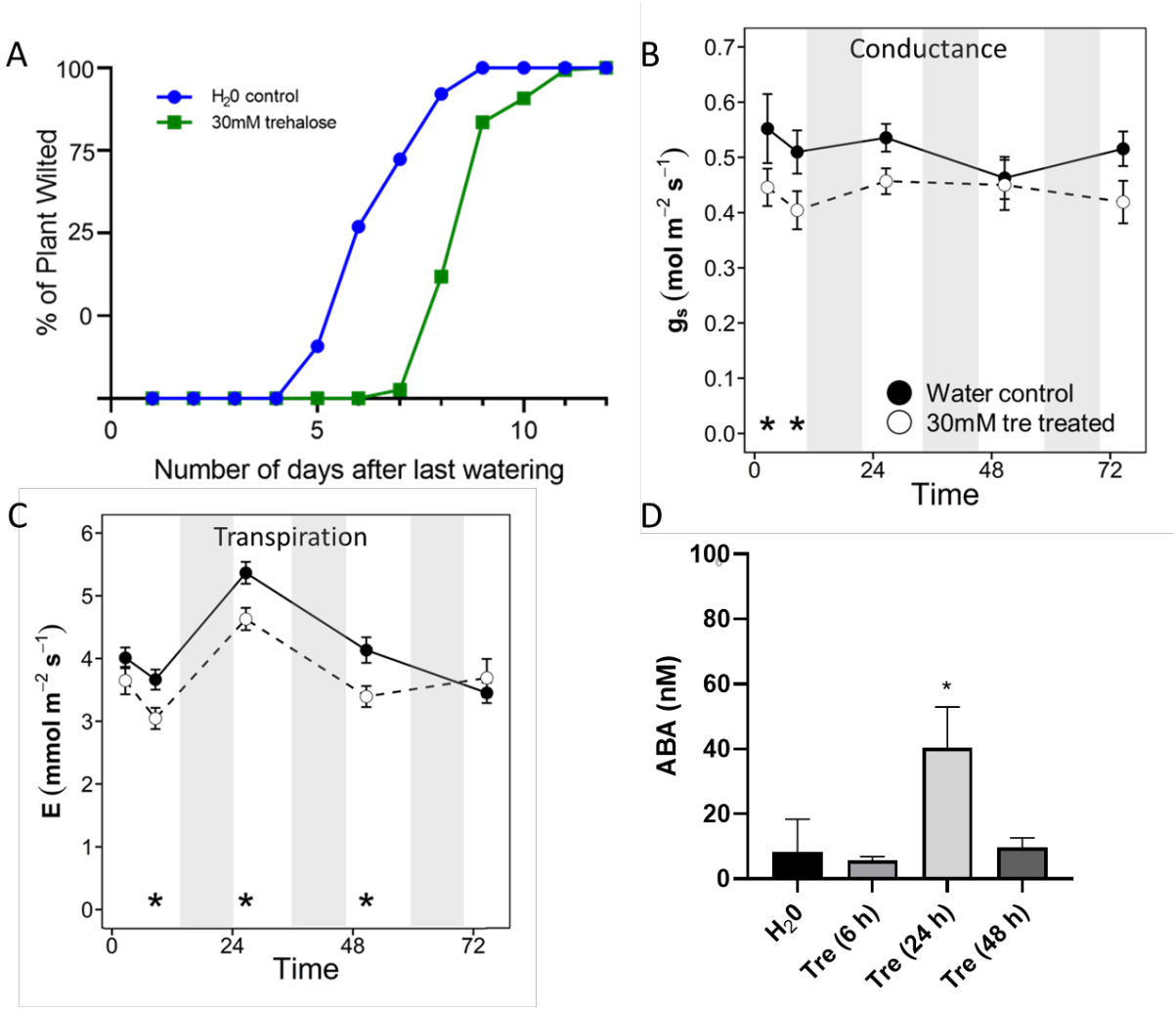
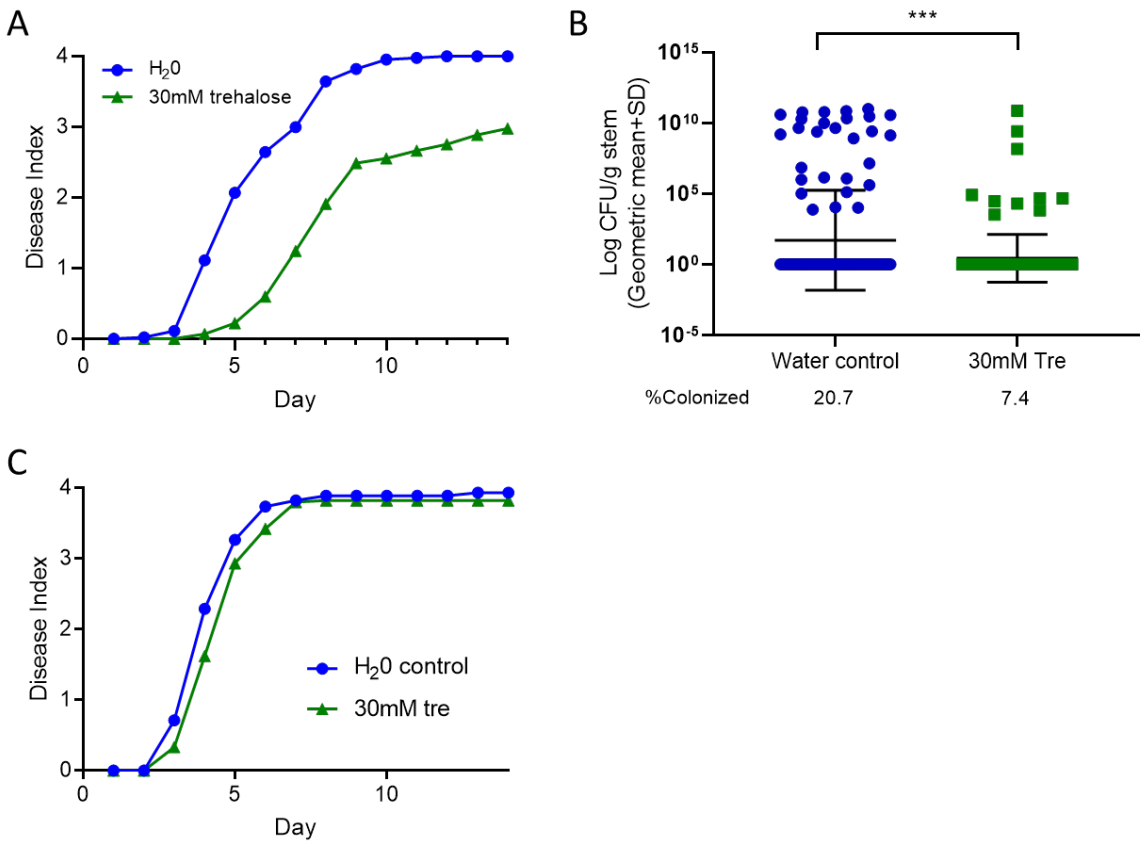


Figure 3. Trehalose treatment delayed tomato wilting due to drought, likely by transiently decreasing stomatal conductance and transpiration and increasing ABA content in xylem sap. A) Curves showing how long it took plants to wilt following a single treatment with either water (blue) or 30 mM trehalose (green) and then no water for two weeks (*P*=.01, T-test of areas under the curve). Data shown represent four biological replicates with ten plants/treatment/replicate. **B)** Stomatal conductance and **C)** transpiration were measured with LI-COR (*P*>0.05, repeated measures ANOVA). Data shown represent 20 plants/treatment. Measurements were taken 10 min after treatment with trehalose or water, then each day after that in the morning two hours after growth chamber lights turned on. The shaded bars on graphs represent night periods. All data were calculated from the same LICOR time points. **D)** ABA concentration in trehalose treated plants (One-way ANOVA Fisher's LSD multiple comparisons to water-treated control: 6 h tre *P*=.85, 24 h tre *P*=.016, 48 h tre *P*=.95). Data shown represent measurements of xylem sap samples from four to six plants.



834

835 **Figure 4. Treating plants with trehalose at the root delayed BW symptoms and reduced *R.***
836 ***solanacearum* stem colonization, but only following soil inoculation. A)** Wilt disease progress of
837 tomato plants treated with trehalose, then after 48 h soil soak inoculated with *R. solanacearum*
838 GMI1000. Trehalose treatment delayed appearance of wilt symptoms (*P*=.0082, T-test comparing areas
839 under the curves). Data represent three replicate experiments, each with 15 plants/treatment. Disease
840 symptoms were rated on a 0-4 disease index scale where 0=no wilt, 1=1-25% of the leaves wilted,
841 4=plant is 76-100% wilted or dead. **B)** Bacterial population sizes were smaller in stems of trehalose-
842 treated plants at 5-7 days after inoculation (Mann-Whitney, *P*=.0009). Data represent three biological
843 replicates with 15 plants/day sampled/treatment; each dot represents the *R. solanacearum* population
844 size in one plant (including plants without detectable *R. solanacearum*). Black bars represent the
845 geometric mean and standard deviation of the data. The percentage of plants that contained any
846 detectable *R. solanacearum* cells is shown below the X-axis; the limit of detection is ~10^2 cells. Relative
847 to controls, fewer trehalose-treated plants contained detectable *R. solanacearum* populations (chi-
848 square, *P*=.0016). C**)** Disease progress of plants treated with trehalose (green) or water (blue), then 48 h
849 later inoculated by introducing *R. solanacearum* directly into the xylem through a cut leaf petiole.
850 Trehalose treatment did not affect wilt disease development in petiole-inoculated plants (*P*=.2067, T-
851 test of areas under curves). Data represent three replicate experiments, each containing 15
852 plants/treatment.
853

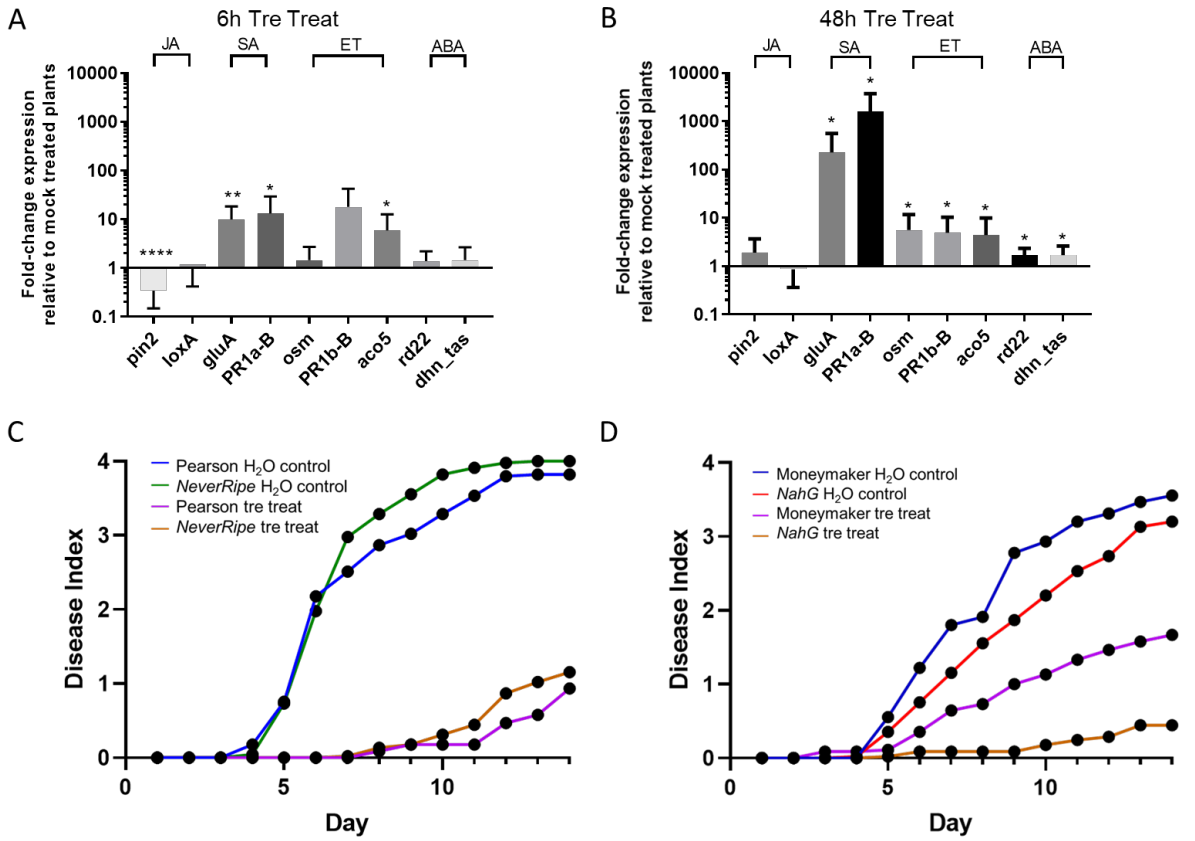


Figure 5. Exogenous trehalose treatment affects the expression of hormone-responsive tomato defense genes, and trehalose-mediated disease resistance is partially regulated by salicylic acid signaling. Effects of trehalose treatment on expression of diverse defense genes in Bonny Best tomato stems, determined 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 water-treated plants. Data represent three biological replicates per time point, each containing five plants per treatment. **C)** Wilt disease progress following *R. solanacearum* inoculation of water- or trehalose-treated *NeverRipe* ET-insensitive tomatoes and their 'Pearson' parent cultivar (One-way ANOVA of AUC; 'Pearson' H_20 vs. *NeverRipe* H_20, *P*=.91; 'Pearson' tre vs. *NeverRipe* tre, *P*=.98). **D)** 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).

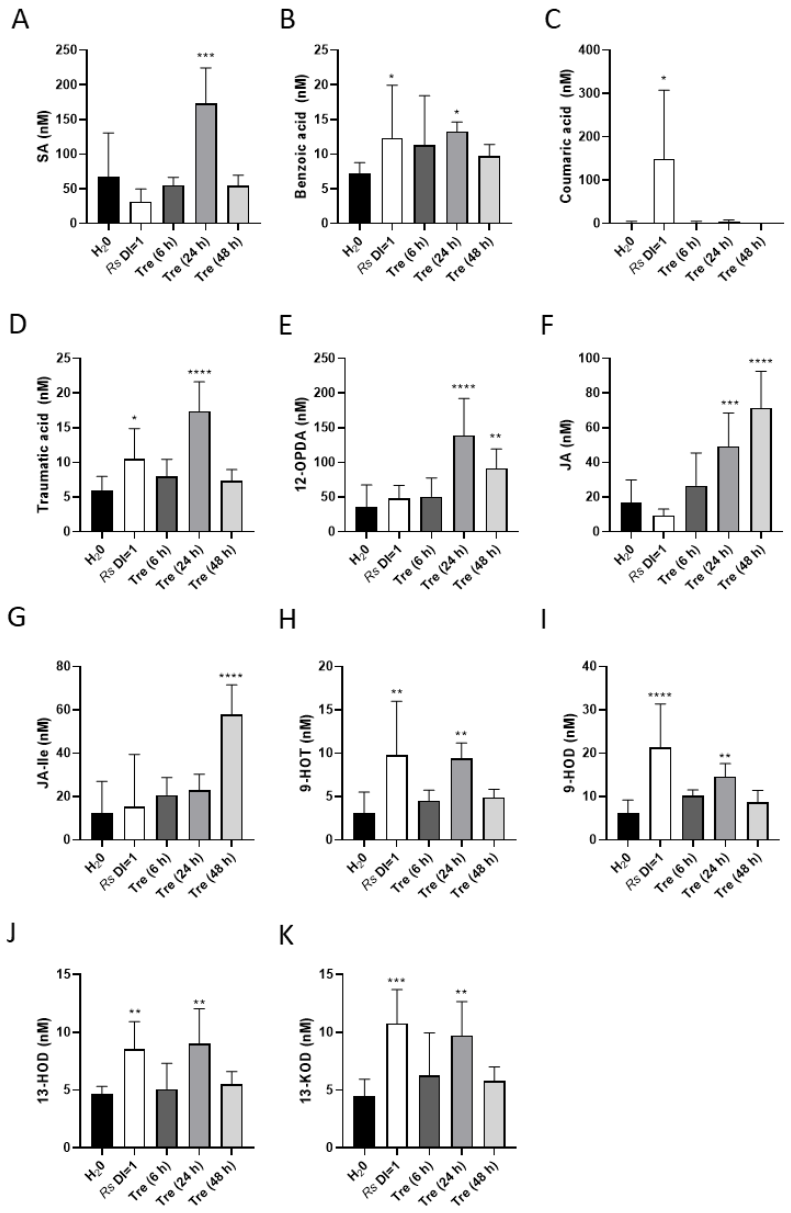


Figure 6. Trehalose treatment increased metabolites related to defense and wounding in tomato xylem sap. LC-MS/MS was used to measure concentrations of hormones, oxylipins, and other metabolites in xylem sap collected from cv. Bonny Best tomato plants that were either mock-inoculated (H_2O), inoculated with *R. solanacearum* GMI1000 (*R. solanacearum* DI=1), or had been treated at the roots with 30mM trehalose (Tre treat (6h), etc.) Differences from water-treated control plants were determined by one-way ANOVA Fisher's LSD multiple comparison test, *, *P*=.01-.05; **, *P*=.001-.01; ***, *P*=.0001-.001; ****, *P*<.0001). Data shown represent the means of samples from 4-6 individual plants. Bars indicate the mean with standard deviation. **A)** SA=salicylic acid; **B)** BA=benzoic acid; **C)** COUMA=coumaric acid; **D)** TA= Traumatic acid; **E)** 12-OPDA= 12-oxo-8(*Z*),13(*Z*)-phytodienoic acid; **F)** JA=jasmonic acid; **G)** JA-Ile=jasmonic acid isoleucine; **H)** 9-HOT= 9(*S*)-hydroxy-10(*E*),12(*Z*),15(*Z*)-octadecatrienoic acid; **I)** 9-HOD= 9(*S*)-hydroxy-10(*E*),12(*Z*)-octadecadienoic acid; **J)** 13-HOD= 9(*S*)-hydroxy-10(*E*),12(*Z*)-octadecadienoic acid; and **K)** 13-KOD= 13-oxo-9(*Z*),11(*E*)-octadecadienoic acid.

883 **Supplemental Tables and Figures**

884 MacIntyre et al *Trehalose induces tomato defenses and increases drought tolerance and bacterial wilt*
885 *disease resistance*

886

887 **Supplemental Table 1. Predicted TPS/TPP and trehalase genes in tomato cv. 'Heinz1706'**

Protein Name[a]	Locus tag (Solgenomics)[b]	Protein Accession (NCBI)[c]	Renamed[d]	Protein Length	Notes
trehalose-phosphate synthase [UDP-forming] 1-like isoform X2	Solyc02g071590.3.1	XP_010316884.1	SlTPSX2	943	
trehalose-phosphate synthase [UDP-forming] 6	Solyc01g005210.4.1	XP_004228746.1	SlTPS6	857	
trehalose-phosphate synthase [UDP-forming] 9	Solyc04g025940.3.1	XP_004237260.1	SlTPS9a	857	
trehalose-phosphate synthase [UDP-forming] 9	Solyc05g005750.3.1	XP_004238680.1	SlTPS9b	851	
trehalose-phosphate synthase 5	Solyc08g076650.3.1	XP_004245918.1	SlTPS5	862	
trehalose-phosphate synthase 7	Solyc07g055300.3.1	XP_004243268.1	SlTPS7a	858	
trehalose-phosphate synthase 7	Solyc02g072150.3.1	XP_004233035.1	SlTPS7b	851	
trehalose-phosphate synthase 7	Solyc10g007950.4.1	XP_004248198.1	SlTPS7c	857	
trehalose-phosphate synthase isoform X1	Solyc07g006500.3.1	XP_010323144.1	SlTPS11	852	Homologous to *Arabdopsis SlTPS1* (Singh and Shah 2012)
trehalose-phosphate synthase 1	Solyc07g062140.3.1	NP_001234879.1	SlTPS1	926	
trehalose-phosphate phosphatase H isoform X1	Solyc05g051880.4.1	XP_019069376.1	SlTPP_HX1	348	May have two additional splice variants

Protein Name[a]	Locus tag (Solgenomics)[b]	Protein Accession (NCBI)[c]	Renamed[d]	Protein Length	Notes
trehalose-phosphate phosphatase 2	Solyc03g007290.4.1	XP_004234173.1	SlTPP_2	348	
trehalose-phosphate phosphatase J	Solyc04g072920.4.1	XP_004237894.1	SlTPP_Ja	365	
trehalose-phosphate phosphatase J	Solyc04g054930.3.1	XP_004237406.1	SlTPP_Jb	388	
trehalose-phosphate phosphatase F	Solyc08g079060.4.1	XP_004245739.1	SlTPP_Fa	386	
trehalose-phosphate phosphatase F	Solyc06g060600.3.1	XP_004242008.1	SlTPP_Fb	339	
trehalose-phosphate phosphatase D	Solyc04g082550.2.1	XP_025886308.1	SlTPP_D	296	
trehalose-phosphate phosphatase A	Solyc03g083960.3.1	XP_025885725.1	SlTPP_A	384	
trehalase	Solyc08g082860.4.1	XP_025888255	SltreA	532	

[a] Tomato TPS/TPP and trehalase proteins were identified via a BLASTP search through NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) and the SolGenomics network (https://solgenomics.net/tools/blast/)(accessed: 3/11/2020) as described in the Methods section using the most up to date tomato reference genome (SL4.0 and ITAG4.0)
[b] Locus tags assigned by the SolGenomics network (https://solgenomics.net/)
[c] Protein accession tags through NCBI (https://www.ncbi.nlm.nih.gov/protein/)
[d] Proteins were renamed/abbreviated by the authors for clarity

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893 **Supplemental Table 2. Strains and primers used in this study**

Strain	***Notes***	***Reference***
Wild type GMI1000	*Ralstonia solanacearum* phylotype one sequevar 18, isolated from tomato in French Guyana	(Boucher, Barberis et al. 1985)
GMI1000 Δ*treA*	GMI1000 trehalase mutant cannot degrade trehalose; Spectinomycin/streptomycin-resistant	(MacIntyre, Barth et al. 2019)
Primer Name	***Sequence***	***Reference***
loxA F	TGGTAGACCACCAACACGAA	(Milling, Babujee et al. 2011)
loxA R	GACCAAAACGCTCGTCTCTC	"
osmF	TGTACCACGTTTGGAGGACA	"
osmR	ACCAGGGCAAGTAAATGTGC	"
PR1b B F	TTGGTGACTGCGGGATGA	"
PR1b B R	GGCGGCGGCTAGGTT T	"
PR1a B F	GAGGGCAGCCGTGCAA	"
PR1a B R	CACATTTTTCCACCAACACATTG	"
GluA F	TCA GCA GGG TTG CAA AAT CA	"
GluA R	CTCTAGGTGGGTAGGTGTTGGTTAA	"
Pin2 F	TGATGCCAAGGCTTGTACTAGAGA	"
Pin2 R	AGCGGACTTCCTTCTGAACGT	"
ACO5 F	AGATGGGCATTGGGTGAACA	"
ACO5 R	TTCAGCCATCACTCGGTGTC	"
DnaJ F	ATGAAGCGCCAGATACCATC	"
DnaJ R	TCAAGGCTCAATGTGTGCTC	"
Actin F	TCAGCAACTGGGATGATATG	"
Actin R	TTAGGGTTGAGAGGTGCTTC	"
rd22_F	ACGTGGCGTTATTTTTCCTG	(Yamaguchi-Shinozaki and Shinozaki 1993)
rd22_R	ATCTCCGGCATCTTCTCTGA	"
dhn_tas_F	CACCATGAGGGGCAACAGCA	(Kissoudis, Seifi et al. 2016)
dhn_tas_R	TCACCTTCATGTTGTCCAGGCATC	"

894

895 **Supplemental Table 3. Concentration of hormones, oxylipins, and phenylpropanoids in tomato xylem**
896 **sap following various treatments**

Compound (nM)[a]	Healthy Avg[b]	WT avg[c]	WT FC[d]	WT p-val[e]	6 hr tre avg[f]	6 hr tre FC	6 hr tre p-val	24 hr tre avg[g]	24 hr tre FC	24 hr tre p-val	48 hr tre avg[h]	48 hr tre FC	48 hr tre p-val
12,13-diHOM	0.83	1.30	2	0.04	1.26	2	0.27	2.69	3	0.00	1.50	2	0.02
12,13-Ep-9KOM	5.38	6.86	1	0.24	7.93	1	0.02	4.82	1	0.64	7.62	1	0.04
12OH-JA	1.43	1.70	1	0.63	5.21	4	0.30	1.38	1	0.84	1.17	1	0.06
12OH-JA-Ile	12.37	15.23	1	0.81	20.67	2	0.29	22.97	2	0.14	57.67	5	0.00
12-OPDA	35.86	47.89	1	0.44	50.75	1	0.43	138.72	4	0.00	91.84	3	0.01
13-HOD	4.66	8.52	2	0.00	5.09	1	0.66	9.02	2	0.01	5.49	1	0.14
13-KOD	4.50	10.78	2	0.01	6.27	1	0.31	9.72	2	0.00	5.81	1	0.12
13,12-KODA	0.27	0.75	3	0.34	0.11	0	0.37	4.89	18	0.00	0.22	1	0.80
13,12-KOMA	1.78	3.18	2	0.23	1.23	1	0.47	9.67	5	0.00	2.23	1	0.58
2-HOD	0.24	0.35	1	0.44	0.36	1	0.18	0.30	1	0.55	0.29	1	0.66
9,10,11-THOD	88.94	191.22	2	0.02	134.71	2	0.09	214.49	2	0.00	153.30	2	0.02
9,10,11-THOM	237.57	811.16	3	0.00	430.01	2	0.02	572.57	2	0.00	379.05	2	0.00
9,10,13-THOD	3.47	4.47	1	0.28	9.91	3	0.00	5.78	2	0.00	6.06	2	0.02
9,10,13-THOM	62.08	100.56	2	0.03	147.39	2	0.00	128.47	2	0.00	96.87	2	0.01
9,10-diHOM	0.95	1.25	1	0.43	1.48	2	0.52	3.64	4	0.00	1.02	1	0.86
9,12,13-THOD	39.77	50.62	1	0.18	43.68	1	0.56	84.76	2	0.00	60.09	2	0.02
9,12,13-THOM	127.69	231.37	2	0.01	153.78	1	0.36	305.89	2	0.00	179.49	1	0.04
9-HOD	6.15	21.29	3	0.01	10.27	2	0.02	14.60	2	0.00	8.64	1	0.17
9-HOT	3.04	9.78	3	0.03	5.02	2	0.16	9.75	3	0.00	4.87	2	0.12
9-KOD	4.35	8.90	2	0.15	6.60	2	0.34	19.32	4	0.00	12.16	3	0.00
9-KOT	1.06	3.68	3	0.17	5.51	5	0.05	7.61	7	0.00	3.90	4	0.05
9OH-10KOM	8.81	5.36	1	0.07	10.81	1	0.15	9.05	1	0.88	8.97	1	0.92
9OH-12KOD	0.20	0.13	1	0.51	0.27	1	0.53	0.69	4	0.00	0.34	2	0.02
9OH-12KOM	1.88	2.48	1	0.03	2.96	2	0.24	3.97	2	0.00	2.11	1	0.36
9-oxo-NA	13.94	17.47	1	0.37	16.53	1	0.56	16.06	1	0.51	17.25	1	0.32
ABA	8.20	49.59	6	0.03	5.71	1	0.60	40.37	5	0.00	9.72	1	0.73

Compound (nM)[a]	Healthy Avg[b]	WT avg[c]	WT FC[d]	WT p-val[e]	6 hr tre avg[f]	6 hr tre FC	6 hr tre p-val	24 hr tre avg[g]	24 hr tre FC	24 hr tre p-val	48 hr tre avg[h]	48 hr tre FC	48 hr tre p-val
AZA	14.08	19.39	1	0.11	19.21	1	0.00	20.44	1	0.00	18.03	1	0.01
BA	7.18	12.37	2	0.13	11.34	2	0.19	13.29	2	0.00	9.74	1	0.02
CA	7.49	5.19	1	0.52	5.30	1	0.56	22.69	3	0.00	14.19	2	0.16
COUMA	2.04	149.10	73	0.05	2.42	1	0.83	5.22	3	0.11	0.00	0	0.12
dnOPDA	0.12	0.21	2	0.25	0.26	2	0.02	0.53	4	0.00	0.44	4	0.00
IAA	3.26	36.76	11	0.03	2.84	1	0.59	4.01	1	0.29	2.20	1	0.09
JA	16.71	9.42	1	0.22	26.64	2	0.33	49.41	3	0.01	71.36	4	0.00
JA-Ile	26.59	3.67	0	0.09	35.48	1	0.62	60.08	2	0.04	103.68	4	0.00
OPC-4	0.31	0.53	2	0.09	0.52	2	0.13	1.09	4	0.00	1.05	3	0.00
p12,13-DiHOD*	0.16	0.91	6	0.00	0.85	5	0.05	0.82	5	0.00	0.18	1	0.69
p9,10-DiHOD*	0.63	1.75	3	0.02	1.48	2	0.04	1.73	3	0.01	0.91	1	0.42
p9OH-TAN*	3.62	3.35	1	0.62	3.36	1	0.71	8.86	2	0.00	4.07	1	0.46
pOTD*	0.47	0.42	1	0.72	0.52	1	0.71	1.19	3	0.00	0.36	1	0.33
SA	67.53	31.73	0	0.21	55.50	1	0.69	173.44	3	0.01	55.09	1	0.65
TA	6.03	10.56	2	0.04	7.94	1	0.19	17.41	3	0.00	7.35	1	0.23
TAN	0.54	0.77	1	0.32	0.53	1	0.91	0.74	1	0.01	0.60	1	0.51

[a] Compound color key: Blue fill= 13-LOX pathway derived compound; Red fill= 9-LOX pathway derived compound; Purple fill= phenylpropanoid pathway derived compound; Gray fill= other; metabolites were measured with LC/MS/MS
[b] Xylem sap harvested from mock-inoculated/treated healthy BB plants
[c] Sap harvested from DI=1 *Rs* infected plants
[d] Fold-change values ≥ 2 are shaded in light red with dark red text.
[e] P-value derived from t-test of treatment compared to healthy; $P<0.5$ was shaded yellow with darker yellow text.
[f-g] Xylem sap harvested from uninoculated, trehalose treated plants 6, 24, and 48 h post-treatment

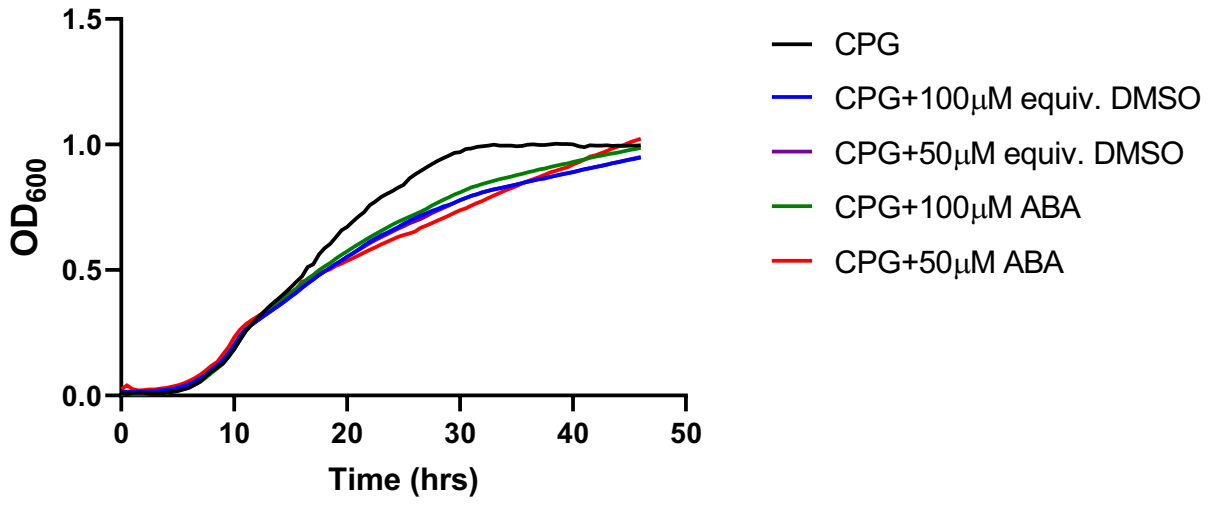
897

898 **Supplemental Table 4. Metabolite key for hormone analysis**

Q1[a]	Q3[b]	RT[c]	Metabolite	Formal Name
313.3	183.3	10.16	12,13-DiHOM	threo-12,13-dihydroxy-9(*Z*)-octadecenoic acid
309.4	208.9	12.46	(*E*)-KODA	12,13-epoxy-9-oxo-10(*E*)-octadecenoic acid
225	59	2.44	12OH-JA	12-hydroxy-jasmonic acid
338.3	130.1	3.19	12OH-JA-Ile	12-hydroxy-jasmonic acid isoluecine
291.3	165	11.13	12-OPDA	12-oxo-10(*Z*),15(*Z*)-phytodienoic acid
295.3	195.2	13.46	13-HOD	13(*S*)-hydroxy-9(*Z*),11(*E*)-octadecatrienoic acid
293.2	113.2	14.16	13-KOD	13-oxo-9(*Z*),11(*E*)-octadecadienoic acid
309.4	180.8	10.32	13,12-KODA	13-hydroxy-12-oxo-9(*Z*),15(*Z*)-octadecadienoic acid
311.3	194.9	11.42	13,12-KOMA	13-hydroxy-12-oxo-9(*Z*)-octadecenoic acid
293.2	191.1	15.15	2-HOD	2-hydroxy-9(*Z*),15(*Z*)-octadecadienoic acid
327.1	137	5.57	9,10,11-THOD	9(*S*),10(*S*),11(*R*)-trihydroxy-12(*Z*),15(*Z*)-octadecadienoic acid
329.5	201	6.69	9,10,11-THOM	9(*S*),10(*S*),11(*R*)-trihydroxy-12(*Z*)-octadecenoic acid
327.1	137	4.94	9,10,13-THOD	9(*S*),10(*S*),13(*S*)-trihydroxy-11(*E*),15(*Z*)-octadecadienoic acid
329.5	139	5.8	9,10,13-THOM	9(*S*),10(*S*),13(*S*)-trihydroxy-11(*E*)-octadecenoic acid
313.2	201.2	10.46	9,10-DiHOM	threo-9,10-dihydroxy-12(*Z*)-octadecenoic acid
326.9	229.1	5	9,12,13-THOD	9(*S*),12(*S*),13(*S*)-trihydroxy-10(*E*),15(*Z*)-octadecadienoic acid
329.5	210.9	5.74	9,12,13-THOM	9(*S*),12(*S*),13(*S*)-trihydroxy-10(*E*)-octadecenoic acid
295.3	171.1	13.53	9-HOD	9(*S*)-hydroxy-10(*E*),12(*Z*)-octadecadienoic acid
293.2	171.2	12.04	9-HOT	9(*S*)-hydroxy-10(*E*),12(*Z*),15(*Z*)-octadecatrienoic acid
293.2	195.1	12.25	9-KOD	9(*S*)-oxo-10(*E*),12(*Z*)-octadecatrienoic acid
291.3	184.7	13.04	9-KOT	9(*S*)-oxo-10(*E*),12(*Z*),15(*Z*)-octadecatrienoic acid
311.3	293.2	11.76	9,10-KOMA	9-hydroxy-10-oxo-12(*Z*)-octadecadienoic acid
309.1	291.3	9.02	9,12-KODA	9-hydroxy-12-oxo-10(*E*),15(*Z*)-octadecadienoic acid
311.3	293.1	10.29	9,12-KOMA	9-hydroxy-12-oxo-10(*E*)-octadecenoic acid
263.4	153.1	3.33	ABA	Abscisic acid
186.9	124.9	2.83	AZA	Azelaic Acid
121.1	76.9	2.54	BA	Benzoic acid
147	102.9	3.6	CA	Cinnamic acid
162.9	118.9	2.05	COUMA	Coumaric acid
263.1	165	8.29	dn-12-OPDA	dinor-12-oxo-8(*Z*),13(*Z*)-phytodienoic acid
174	129.9	2.77	IAA	Indole-3-acetic acid
209.1	59	4.24	JA	(+)-7-iso-jasmonic acid
322.2	130	6.82	JA-Ile	(+)-7-Jasmonoyl-L-isoleucine
237	165.1	6.38	OPC-4	8-[3-oxo-2-cis-[(*Z*)-2-pentenylcyclopentyl]butanoic acid
311.2	183.2	10.3	12,13-DiHOD*	threo-12,13-dihydroxy-9(*Z*),15(*Z*)-octadecenoic acid
311.2	201.2	10.35	9,10-DiHOD*	threo-9,10-dihydroxy-12(*Z*),15(*Z*)-octadecenoic acid
226.9	208.7	3.2	9OH-TAN*	9-hydroxy-12-oxo-10(*E*)-dodecenoic acid
223	195	7.3	OTD*	13-oxo-9(*Z*),11(*E*)-tridecadienoic acid
137	92.9	2.45	SA	Salicylic acid
226.9	182.7	5.36	TA	Traumatic acid
210.9	182.7	6.56	TAN	Traumatin

[A-B] Select m/z ions for compound identification (see Methods section)
[C] Compound retention time

899 **Supplemental Figures**



900

901 **Supplemental Figure 1. ABA and DMSO are not toxic to *Rs*.** Growth of *Rs* cultured in rich CPG broth

902 supplemented with ABA in DMSO, or with DMSO alone since ABA stock solutions were dissolved in

903 DMSO (100 µM treatments contained 0.1 µL of DMSO or ABA stock solution, and 50 µM treatments

904 contained 0.05 µL DMSO or ABA stock solution) (ANOVA AUC, Fisher's LSD multiple comparisons to CPG

905 control, 100 µM DMSO, *P*=.55; 50 µM DMSO, *P*=.53; 100 µM ABA, *P*=.67; 50 µM ABA, *P*=.45). Growth

906 was measured spectrophotometrically using a Bio-Tek plate reader; the data represent eight technical

907 reps/treatment.

908

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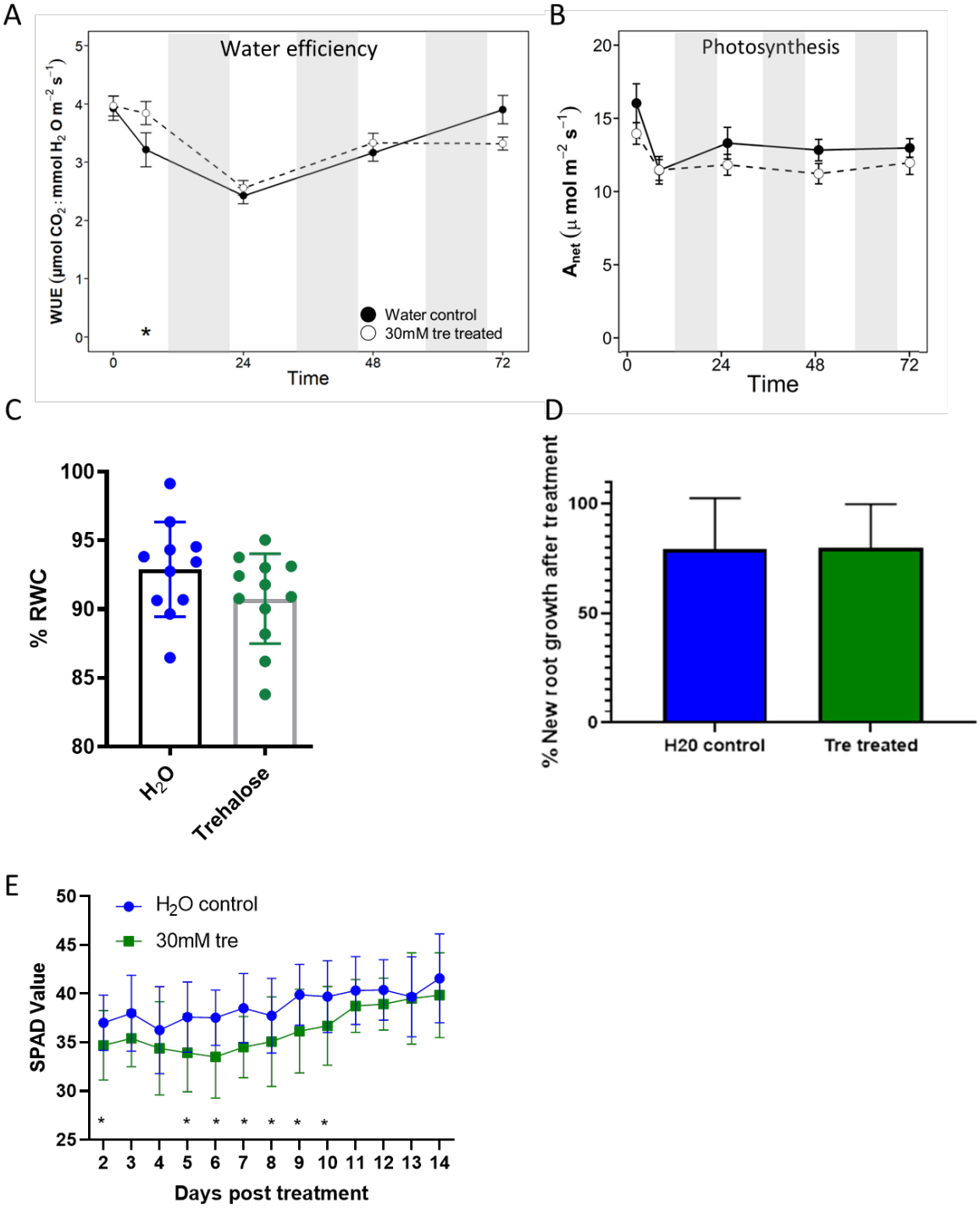


910

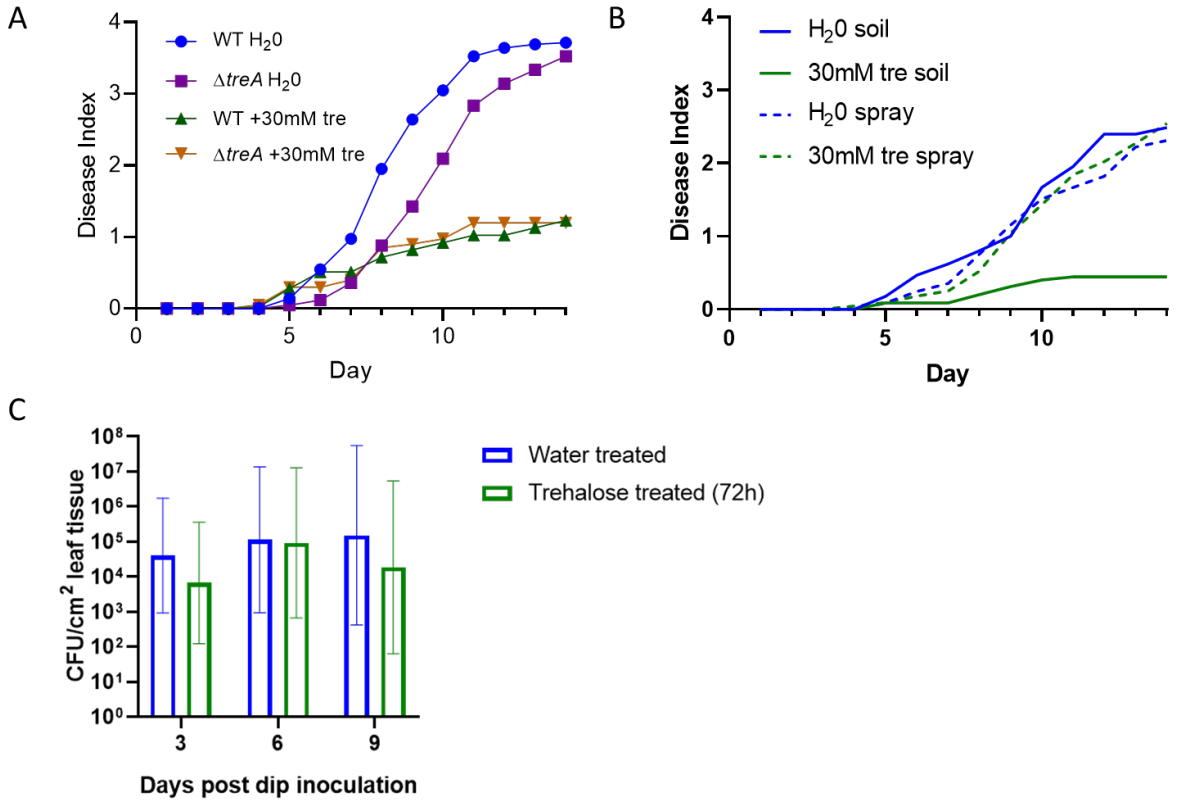
911 **Supplemental Figure 2. Trehalose treatment temporarily improves water efficiency without affecting**

912 **photosynthesis; treatment does not affect root growth and relative water content of leaves but**

913 **lowers chlorophyll concentration. A)** Water efficiency, measured with a LI-COR as photosynthetic

914 rate/transpiration, in trehalose treated plants compared to water only controls (t-test, *P*<0.5). **B)**
915 Photosynthesis, measured as A_{net}, in trehalose treated plants (repeated measures ANOVA, *P*>0.05). The
916 water efficiency and photosynthetic data represent 20 plants/treatment. LI-COR measurements were
917 taken ~10min after treatment and then in the morning every time point after. Shaded bars on the
918 graphs represent night periods. All data were calculated from the same LICOR time points. **C)** Relative
919 water content of leaves was calculated from the fresh weight, hydrated weight, and dry weight of leaves
920 from water control or trehalose treated BB tomatoes (t-test, *P*=.14). The data represent two biological
921 replicates with five technical replicates (leaves)/treatment). **D)** 'Bonny Best' tomato seedlings were
922 placed in root growth pouches, watered with 10mL of either water or trehalose for one day, then
923 watered as needed for ten days. Root length was measured after ten days (t-test, *P*=.31). The data
924 represent three biological replicates with ten roots/treatment. %New root growth was calculated as
925 %new root growth= (new growth/total root length)*100. **E)** SPAD meter measurements of chlorophyll
926 content in leaves following trehalose treatment (2-way ANOVA Fishers LSD multiple comparison, * =
927 *P*<0.5 or lower).

928



929

930 **Supplemental Figure 3 Trehalose treatment does not enrich the soil environment, and its protection is**

931 **limited to roots. A)** Disease progress curve of plants treated with trehalose or water, then infected with

932 either wild-type *Rs* or a Δ*treA* mutant unable to catabolize trehalose (One-way ANOVA of areas under

933 the curve, WT H_20 vs. WT tre, *P*=.0008, Δ*treA* H_20 vs. Δ*treA* tre, *P*=.017). The data represent three

934 bioreps with 13-15 plants per treatment. **B)** Disease development in plants sprayed once with 30 mM

935 trehalose or water, and then soil-soak inoculated with *Rs* 48 h later (ANOVA of AUC, Fisher's LSD

936 multiple comparisons to H_20 soil, H_20 spray, *P*=.67; tre spray, *P*=.63; tre soil, *P*=.63). The data represent

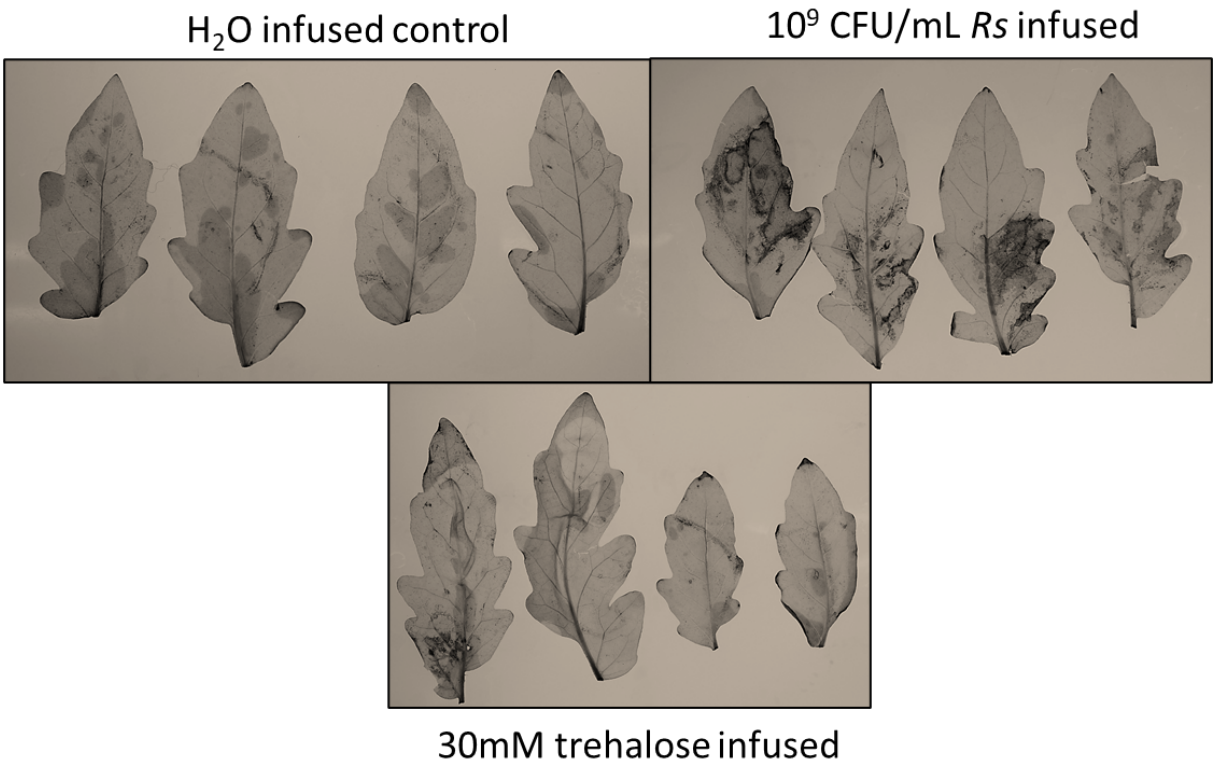
937 three biological replicates with fifteen plants per treatment. **C)** Colonization of tomato leaves by the

938 bacterial leaf spot pathogen *Xanthomonas gardneri*; plants were dip-inoculated with *X. gardneri* 72 h

939 after root treatment with water or 30 mM trehalose (Mann-Whitney, day 3, *P*=.06; day 6, *P*=.61; day 9,

940 *P*=08). The data represent three biological replicates with twelve plants/treatment/timepoint. The graph

941 displays the geometric means; bars indicate the geometric standard deviation of the data.



942

943 **Supplemental Figure 4 Infusing leaves with trehalose did not trigger ROS production in 'Bonny Best'**

944 **tomato leaves.** DAB staining of 'Bonny best' tomato leaves infused with water, 10^9 CFU/mL *Rs*, or 30

945 mM trehalose solution to assess the effect of trehalose treatment on ROS production. The data

946 represent three biological replicates, with four plants per biological replicate per treatment. Photos are

947 representative samples and sharpened 25% for contrast.

948

949

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