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1 **Unravelling physiological signatures of tomato bacterial wilt and xylem**
2 **metabolites exploited by *Ralstonia solanacearum***

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17 Keywords: metabolism, plant pathogen, transpiration, growth, glutamine, putrescine, NMR,
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19 Running title: Xylem metabolites exploited during bacterial wilt

20 **Originality-Significance Statement.**

21 The plant pathogen *Ralstonia solanacearum* colonizes plant xylem vessels, reaching 10^{10} colonies per
22 gram of plant. To proliferate so dramatically and thwart host responses, the nutritive resource supply
23 is central to successful infection, but is still poorly understood. Through a combination of automated
24 phenotyping, quantitative metabolomics and genetic approaches, we identify on the one hand sensitive
25 markers of the infectious process and on the other hand nutritional substrates that allow the rapid
26 growth of the pathogen in vascular tissues. In particular, we show that during bacterial colonization,
27 xylem is depleted in two amino acids abundantly present: glutamine and asparagine. In addition, we
28 show that sugars have little role to sustain growth of the pathogen and progression of the disease, using
29 a mutant unable to catabolize glucose, sucrose and fructose. These findings challenge the classical
30 view that the pathogen's infection process depends on abundant sugars in plants to colonize xylem.
31 Furthermore, the monitoring of plant physiological markers during infection reveals that the phase of
32 amino acid assimilation and simultaneous bacterial proliferation corresponds to a tipping point of the
33 infection, associated with a strong alteration of plant physiology including growth arrest and
34 transpiration arrest.

35

36 **Summary**

37 The plant pathogen *Ralstonia solanacearum* uses plant resources to intensely proliferate in xylem
38 vessels and provoke plant wilting. We combined automatic phenotyping and tissue/xylem quantitative
39 metabolomics of infected tomato plants to decipher the dynamics of bacterial wilt. Daily acquisition
40 of physiological parameters such as transpiration and growth were performed. Measurements allowed
41 us to identify a tipping point in bacterial wilt dynamics. At this tipping point, the reached bacterial
42 density brutally disrupts plant physiology and rapidly induces its death. We compared the metabolic
43 and physiological signatures of the infection to drought stress, and found that similar changes occur.
44 Quantitative dynamics of xylem content enabled us to identify glutamine (and asparagine) as primary
45 resources *R. solanacearum* consumed during its colonization phase. An abundant production of
46 putrescine was also observed during the infection process and was strongly correlated with *in planta*
47 bacterial growth. Dynamic profiling of xylem metabolites confirmed that glutamine is the favored
48 substrate of *R. solanacearum*. On the other hand, a triple mutant strain unable to metabolize glucose,
49 sucrose and fructose appears to be only weakly reduced for *in planta* growth and pathogenicity.

50

51 **Introduction**

52 The *Ralstonia solanacearum* species complex (RSSC) includes a diverse group of pathogenic strains,
53 causative agents of bacterial wilt on many plants (Álvarez et al., 2010; Hayward, 1991). These strains
54 were classified in four main evolutionary lineages also called phylotypes (Fegan & Prior, 2005) which
55 were recently assigned to three distinct species (Prior et al., 2016; Safni et al., 2014). The bacterial wilt
56 disease caused by RSSC strains on tomato results from a multi-stage infection process in which, after
57 the root infection stage and colonization of the root cortex tissues, bacteria invade the xylem vessels.
58 This allows their spread to the aerial part of the plant, concomitantly with a strong multiplication
59 (Caldwell et al., 2017; Vasse et al., 1995; Xue et al., 2020). High population density in the xylem
60 vessels, along with the production of exopolysaccharide (EPS), reduces the sap flow leading to the
61 appearance of wilting symptoms (Hikichi et al., 2017). Resistance of the plant to limit bacterial
62 colonization proceeds at different steps including root invasion (Morel, Guinard, et al., 2018;
63 Poueymiro et al., 2009), vertical movement upwards to the stem, and vessel to vessel diffusion in the
64 xylem (Caldwell et al., 2017; Planas-Marquès et al., 2020; Xue et al., 2020). On the other hand, *R.*
65 *solanacearum* spp. relies on effectors delivered by the type-three secretion system to circumvent plant
66 immunity (Landry et al., 2020) as well as many other virulence factors which contribute to pathogenic
67 fitness at the different infection steps (Genin & Denny, 2012; Hikichi et al., 2017).

68 Once in xylem vessels, *R. solanacearum* spp. reaches massive bacterial density up to 10^{10} colony
69 forming units (CFU) per g of fresh stem (Lowe-Power, Khokhani, et al., 2018; Perrier et al., 2019).
70 Therefore, bacterial infection success is highly dependent on the rapid assimilation of nutrients to
71 sustain both production of biomass (proliferation) and factors subverting host immunity (virulence)
72 (Lowe-Power, Khokhani, et al., 2018). There is evidence for intertwined regulation between virulence
73 genes and metabolic genes (Khokhani et al., 2017; Peyraud et al., 2016, 2018), but how metabolic
74 resources are acquired *in planta* remains poorly understood. A recent study, however, showed that sap

75 from infected plants is enriched in several nutrients that improve *R. solanacearum* growth in sap
76 (Lowe-Power, Khokhani, et al., 2018), notably sucrose (Hamilton et al., 2021).

77 The consumption of host resources associated with intense bacterial proliferation, as well as the effects
78 of virulence factors, should induce strong alterations of the plant physiology. In the case of bacterial
79 wilt, only qualitative observations of wilting are usually made, and little is known about these
80 physiological alterations in terms of dynamic and quantitative data. The progression of the disease is
81 generally evaluated by a disease index (i.e a visual scoring of the wilting symptoms) or by counting of
82 the bacterial population extracted from infected plants. Although the latter method appears more
83 sensitive for monitoring the course of the disease, it sometimes happens that the bacterial load is not
84 correlated with disease symptom progression (Angot et al., 2006; Lebeau et al., 2011).

85 In this study, we aimed at quantifying the dynamics of the bacterial wilt disease on tomato plants in
86 greenhouse conditions, using *Ralstonia pseudosolanacearum* GMI1000 (Salanoubat et al., 2002), a
87 model strain representative of the RSSC. We used automated plant phenotyping and metabolomics of
88 both xylem sap and plant organs to determine the evolution of various physiological parameters at
89 different stages of xylem vessels colonization. This quantitative kinetic study unraveled a critical point
90 in the *R. solanacearum* colonization process beyond which the host-pathogen interaction switches to
91 the side of the disease, with strong physiological effects on the plant and a rapid progression of
92 symptoms. In a 48h window after infection, the pathogen drains the major substrates allowing for its
93 strong proliferation. Quantitative metabolomics of infected xylem confirmed that putrescine
94 concentration is a relevant marker of the infection process and strongly suggested that glutamine and
95 asparagine are the major carbon sources sustaining bacterial proliferation *in planta*. On the other hand,
96 the generation of a triple mutant strain defective for the assimilation of glucose, sucrose and fructose
97 revealed that these sugars play little role in sustaining pathogen's growth *in planta*, as evidenced by
98 the phenotype of this strain on tomato plants.

99

100 **Results**

101 **High-throughput phenotyping of tomato plants during bacterial wilt**

102 To evaluate the physiological and metabolic parameters associated with the infection of *R.*
103 *solanacearum* strain GMI1000 of its tomato host, we monitored the progression of the bacterial wilt
104 symptoms over 9 days in a controlled conditions greenhouse using an automated high-throughput plant
105 phenotyping facility (<http://tpmp.inrae.fr>). To improve the synchronization of plant infection, the
106 inoculation was carried out on root-damaged plants by soil drenching with a suspension of 5.10^6
107 CFU/ml. Control plants (hereafter referred to as ‘healthy plants’) were inoculated with water only.
108 Two independent experiments comprising respectively 90 and 81 plants were performed. Each
109 experiment was analyzed separately (automatic phenotyping, metabolomics) and a similar response
110 profile was observed for both. The data presented in the following sections correspond to an
111 experiment (three replicates, 81 plants with 63 infected / 18 healthy); the data of the second experiment
112 are presented in Fig. S4, Fig. S6, Table S1. A larger number of infected plants was monitored given
113 the greater variability expected in the case of the interaction with the pathogen. Automated
114 phenotyping included weighing for the estimation of transpiration and imaging. Analysis of acquired
115 images (Fig. S1) allowed to estimate the position (vertical) of the plant center of gravity (Fig. S2 and
116 Fig. S3) and the chlorophyll content (Liang et al., 2017). Plants were also manually sampled at different
117 days post inoculation (1, 3, 4, 5, 7, 8) to determine organ fresh/dry weight, as well as organ and xylem
118 biochemical profiling and bacterial population (CFU). The appearance of bacterial wilt symptoms,
119 from 0 (no symptoms) to 4 (complete wilting) (Arlat & Boucher, 1991) was also followed.

120

121 ***R. solanacearum* alters the plant physiological balance rapidly after infection**

122

123 *Figure 1*

124 Physiological dynamics of the disease progression clearly revealed a tipping point in plant physiology
125 and infection kinetic. In our experimental conditions, this point is at 4 dpi and corresponds to a
126 colonization stage of 10^8 bacterial colonies per g of FW (Figure 1). Before this tipping point, plant
127 general physiology remains similar between healthy and infected individuals: growth/transpiration
128 rates, chlorophyll content/center of gravity vertical position estimation (Figure 1, Figure 2). At 4 dpi,
129 transpiration begins to decrease significantly for infected plants, indicating that bacterial colonization
130 is starting to affect plant physiology in a detectable way, although symptoms are barely visible (50%
131 of plants at disease index 0). Early impact on transpiration was expected as xylem occlusion limits the
132 transmission of water to aerial parts. This very early stage of symptom development shows that before
133 disease index score 2 the plant has already been strongly colonized by bacteria. Position of the plant
134 center of gravity, which is a proxy of how upwards the plant is growing, is also significantly affected
135 (Figure 2) at 4 dpi. Some physiology parameters such as plant growth and chlorophyll content were
136 impacted later and correlated with disease symptom appearance (disease index of 1 and above). From
137 5 dpi only, plant growth began to slow down for infected plants and chlorophyll content started to
138 decrease, further showing that a major transition occurred around 4 dpi. We will refer below to this
139 major transition as the “tipping point”.

140 After this tipping point, infected plants almost stopped growing: the growth rate was 0.044 d^{-1} for
141 infected plant versus 0.2126 d^{-1} for healthy plants between 4 and 8 dpi. Transpiration also strongly
142 decreased, from nearly 100 mL to less than 10 mL per day, while it reached around 200 mL per day
143 for larger healthy plants. Furthermore, the fastest bacterial proliferation was observed around the
144 tipping point (bacterial growth rate of 0.09 h^{-1} between 3 and 5 dpi), before slowing down (bacterial
145 growth rate of 0.02 h^{-1} between 5 and 8 dpi). It indicated that the first colonization stage in xylem
146 vessels is a fast proliferation of *R. solanacearum* with limited visible impacts (symptoms) but which
147 induces a major shift in plant physiology. After this shift, bacterial proliferation slows down as the

148 high-density population of the pathogen begins to become a burden to plant development. This is
149 probably due to lack of nutrients because of plant stopped growth.

150 *Figure 2*

151 **Tomato organ-specific changes induced by the bacterial wilt disease**

152 Fresh and dry weights of healthy and infected plant organs were used to determine the impact of *R.*
153 *solanacearum* proliferation on plant water status (Figure 3A). Organ water content remains constant
154 for healthy plants. As expected from our transpiration results (Figure 1), the percentage of water in
155 infected plants decreased during bacterial infection for leaves and stems. Organ dehydration was
156 observed close to the previously defined tipping point (a detachment is observed at 4 dpi, although it
157 is significant only at 5 dpi), almost simultaneously to the onset of transpiration deficiency (as observed
158 in Figure 1), and close to the appearance of wilting symptoms (disease index). For roots, water content
159 was in a similar range for infected and healthy plants, confirming that major bacterial proliferation in
160 xylem vessels does not affect root hydration (Lowe-Power, Khokhani, et al., 2018). At later infection
161 stage (7 and 8 dpi), corresponding to a disease index score of 3 (75% of the plant wilted) with plant
162 transpiration close to 0 mL/day, leaves and stems remained however well-hydrated, only decreasing
163 from 87% to 80% water and 95% to 92%, respectively.

164 Plant dehydration is suspected to affect central metabolism as high-water supply is essential for
165 photosynthesis as well as for plant development/growth since water is necessary to constitute the turgor
166 pressor. We thus performed quantitative metabolomics of plant tissues at 3, 5 and 7 dpi (Figure 3B,
167 Fig. S5), to analyze different steps of the infection process. We did not find significant differences at
168 3 and 5 dpi. At 7 dpi, a drastic and significant impact on leaf and stem starch was observed (Figure
169 3B). In leaves, the proportion of starch was high in healthy plants (8.58 ± 6.06 %) while it was depleted
170 in infected plants (0.08 ± 0.12 %). Amino acids also decreased significantly but less drastically. In
171 accordance with the chlorophyll drop as deduced from image analysis (Figure 2), the quantification of
172 chlorophyll content in leaves also seems to decrease in infected tissues but not significantly.

173 *Figure 3*

174 **The bacterial wilt disease modifies metabolite availability in tomato xylem sap**

175 We investigated further how *R. solanacearum* is able to proliferate intensely in xylem vessels by
176 determining the carbon sources supporting bacterial proliferation in xylem sap. We extracted xylem
177 sap of infected and healthy plant at different dpi for quantitative analysis NMR (see Materials and
178 Methods). To assess which xylem metabolites are affected by infection, we performed a PCA. This
179 multivariate analysis revealed that two principal components (PCs) explained 65% of the variability
180 (see Fig. S7), on which we focused our analysis. Each individual point plotted in Figure 4A
181 corresponds to a xylem sap sample (15 metabolites concentrations) transformed into the two
182 uncorrelated PCs and associated with the corresponding bacterial population. We found that the first
183 PC (explaining 30% of the variability) is not correlated with infection stage and reveals an important
184 variability between plants independent of the infection. However, the second PC (explaining 25% of
185 the variability) is strongly associated with the bacterial population level. Interestingly, uninfected
186 xylem samples have very similar metabolic profiles as all the points are clustered whereas infected
187 samples display a scattered profile. This suggests that impact of bacterial infection on xylem sap
188 composition is heterogeneous between plants and that there is therefore a certain level of variation at
189 this stage.

190 We plotted the contribution of the variables (metabolite concentrations) to the two PCs in Figure 4B.
191 Variables differing most from zero on the horizontal axis (e.g ethanol, lysine, phenylalanine and
192 valine) have an important contribution to the first PC, which implies that for these compounds there is
193 an important inter-plant variability not correlated to infection. Conversely, variables differing most
194 from zero on the vertical axis contribute to the second PC and are therefore associated with the bacterial
195 population level. High levels of asparagine and glutamine (bottom of the plot) are associated to healthy
196 plants and plants with low bacterial population, while high levels of putrescine and acetate are

197 associated with high bacterial population (see complete list in Table 1). We found that for healthy
198 plants, concentrations are constant between 1 and 8 dpi (no statistical differences observed between
199 sampling days, and no apparent temporal dynamics) so we determined a global mean for each plant
200 (Table 1, Table S1). Glutamine appeared as the major component of xylem sap (75 percent of molar
201 organic carbon) whereas the other compounds were found in much lower concentrations (sixteen times
202 less at least).

203 *Figure 4*

204 *Table 1*

205

206 We found that the progression of the infection was associated with glutamine and asparagine decrease
207 and provoked the appearance of putrescine (non-detectable in healthy plants) and an increase of acetate
208 content. We also estimated that aspartate, phenylalanine, lysine, glutamic acid and leucine were not
209 affected by *R. solanacearum* infection as the absolute loading weights are close to zero (inferior to
210 0.25). It was difficult to conclude firmly for the other variables as they were in an intermediate (and
211 so less discriminating) area, between 0.25 and 0.75.

212

213 **Dynamic profiling of tomato xylem fluid metabolites during *R. solanacearum* infection**

214 *Figure 5*

215 PCA showed that some tomato xylem metabolites concentration vary during infection by *R.*
216 *solanacearum*. In order to get a dynamic view of these changes, we monitored metabolite
217 concentrations over several days in healthy and infected plants. The kinetic data for the 7 compounds
218 with statistical differences for one time point or more are represented in Figure 5 (and in Fig. S8 for
219 alanine). We noticed that statistical differences were mostly observed at 3, 4 or 5 dpi, certainly due to

220 a higher number of plants analyzed and sometimes high variability. For additional compounds tested,
221 no statistical difference was observed (Fig. S8).

222 Putrescine was detected when plant infection resulted in high bacterial proliferation (between 3 and 5
223 dpi, see Figure 1) and its concentration stabilized in the last days of infection (7 and 8 dpi) when
224 bacterial multiplication started to slow down. Putrescine therefore appears to be a sensitive and precise
225 biomarker of the infection: its dynamics is strongly correlated with the onset and evolution of the
226 bacterial wilt disease. Three other metabolites increase importantly and significantly in infected plants:
227 3-hydroxybutyric acid increases at 4 and 5 dpi, while it seems to decrease afterward, acetate increases
228 from 4 to 7 dpi, and alanine (Fig. S8) seems to follow the same pattern than 3-hydroxybutyric acid.

229 Glutamine depletion is striking as the initial concentration (almost 3 mM) decreases to around 1 mM
230 at the end of the sampling. Simultaneously, asparagine, which is observed at a smaller amount in
231 healthy plants (around 1.5 mM) is also quickly depleted: our data indicate a drastic drop between 4
232 and 5 dpi. After this drop, asparagine concentration is around 0 (or at concentrations under the limit of
233 detection). These parameters prove an intense metabolic activity over a short time window (48 hours)
234 in xylem vessels, exactly around the tipping point of the infection previously reported (4 dpi).

235 **Putrescine production is a reliable marker of *R. solanacearum* infection**

236 *Figure 6*

237 The progression of bacterial wilt disease is usually monitored in two ways: either by disease index
238 scoring or by enumerating bacteria extracted from infected tissues. We plotted the relationship between
239 these two phenotypical markers and some of the physiological parameters we monitored during the
240 infection assay: plant transpiration, putrescine production and glutamine concentration in xylem
241 (Figure 6). The calculation of the regression coefficients (R^2) shows that these three markers are
242 globally well correlated with the bacterial density (in \log_{10} CFU/g of fresh weight) and the disease
243 index, although we observe for the latter a greater dispersion of the points in the early stages of

244 infection. As expected, bacterial population best correlated with wilt symptom development ($R^2 = 0.81$
245 with disease index). Plant transpiration is an early marker of infection impact on plant physiology (Fig.
246 1) and accordingly a clear negative relationship was also observed with the bacterial population rate,
247 confirming that bacterial proliferation has a strong negative impact on plant transpiration rate. It turned
248 out that the bacterial population rate in xylem is also well correlated to the metabolic profile of infected
249 xylem: positive correlation between putrescine concentration (in decimal logarithm) is high ($R^2=0.66$),
250 indicating that appearance and accumulation of putrescine is directly associated with *R. solanacearum*
251 infection. Negative correlation with glutamine concentration is also significant but weaker ($R^2 = 0.54$).

252

253 **Genetic evidence that sugars are not major carbon sources supporting growth of *R.***
254 ***solanacearum* in xylem**

255 *Figure 7*

256 Xylem metabolomics revealed a significant decrease of glutamine over time as well as a negative
257 correlation between bacterial density and glutamine content, whereas sugar content was found to not
258 vary. To validate the hypothesis that *R. solanacearum* growth is mostly supported by glutamine and
259 not sugars, we generated a triple mutant strain unable to uptake (and so metabolize) three major sugars
260 found in plants: sucrose, glucose and fructose, glucose and sucrose being detected in our xylem content
261 analysis. The strain GMI1000 glucose transport operon (RSp1632-RSp1635) was identified after
262 screening a mutant library to identify genes essential for growth on glucose (Peyraud et al., 2018), but
263 these genes were incorrectly annotated as a xylose transport system. Cumulative deletions in each
264 sugar transport system were generated to create the mutant strain GRS941. We used *in vitro* growth
265 assays on microplates to validate that strain GRS941 growth on sucrose (Figure 7A), glucose (Figure
266 7B) and fructose (Fig. S9) was severely impacted. We then performed automated phenotyping of strain
267 GRS941 on tomato plants under conditions similar to those used for the wild-type strain. We observed

268 that growth *in planta* of strain GRS941 was similar to the wild-type and reached high population levels
269 (Figure 7C). The transpiration rate was significantly lower for plants infected by GRS941 compared
270 to the wild-type at 6 to 8 dpi, but the difference was small with a similar overall profile and an identical
271 tipping point at 4 dpi (Figure 7D). Small but statistically significant variations of plant dry weight,
272 disease index and imaging profiles were also observed (Figure 7, Fig. S9), but the mutant strain was
273 still able to provoke full plant wilting symptoms. We therefore concluded that glucose, fructose and
274 sucrose are not critical carbon sources sustaining the pathogen growth during the xylem infection stage,
275 but rather complementary nutritive sources..

276 **Discussion**

277 In this study, we followed the dynamics of infection of the pathogen at the time of xylem colonization
278 and its impact on plant physiology. Unlike several studies that have focused on the *R. solanacearum*-
279 plant interaction using miniaturized in vitro pathosystems, we have conducted this work on 4-week
280 old tomato plants grown in greenhouse conditions to get closer to *in natura* interactions. In order to
281 synchronize the infection process, we carried out infections with scarified roots, enabling easier entry
282 of bacteria into vascular tissues. This root wounding procedure made it possible to follow the infection
283 kinetics with relatively homogeneous values of the measured parameters (see Figure 1, 2, 3, 5),
284 contrary to what is observed with soil soaking inoculations where greater variability is observed
285 (Morel, Peeters, et al., 2018). We used our experimental system to follow not only symptoms but also
286 general growth and water status of the plant, as well as measure metabolites without a priori. We were
287 then able for the first time to correlate them in a time series for a finer understanding of the overall
288 process.

289

290 **Physiological markers define a tipping point during of bacterial wilt infection.**

291 The use of automated phenotyping has enabled us to collect many measurement points, thus allowing
292 for a sensitive estimation of major parameters linked to the infection of tomato by *Ralstonia*
293 *pseudosolanacearum*. In particular, our study reveals that markers such as the plant transpiration rate
294 in plant tissue are excellent proxies to follow the progression of bacterial infection. Moreover, the
295 comparison of the measurements in these standardized conditions show that the quantification of the
296 bacterial load in planta, despite being destructive, is much more reliable than the rating of the
297 symptoms by disease index which is still commonly used to follow the evolution of an infection (see
298 Figure 6). The absence of visible symptoms is observed with plants already highly infected (up to 10^9
299 CFU per g of FW) and disease index scores of 0, 1 and 2 include very diverse *in planta* bacterial loads.

300 It is only when the disease index reaches 3 that the profiles become more homogeneous, but they
301 correspond to an already very advanced stage of bacterial propagation and alteration of plant
302 physiology. This emphasizes that disease index is a late marker of the infection process and some
303 physiological markers such as the plant transpiration rate or vertical position of the center of gravity
304 (proxy of how upwards the plant is growing) have a much better correlation with bacterial colonization.

305 The fine tracking of parameters (weight, transpiration, center of gravity, chlorophyll content) of the
306 infection highlighted a specific step that we consider a tipping point beyond which the disease
307 progresses to plant death. This tipping point corresponds to a stage where macroscopic disease
308 symptoms are barely visible (disease index=0.5) and to the first detection of bacteria after extraction
309 from plant tissues for counting (CFU per g of FW). This reveals that the plant can afford a load of
310 bacteria in its vascular system before seeing its immunity overwhelmed by the pathogen, which is
311 consistent with the observation of bacterial proliferation reaching 10^6 CFU per g of fresh weight in the
312 resistant tomato plant cultivar Hawaii 7996 (Nakaho et al., 2004). We assume that the dynamics around
313 a tipping point described here is a conserved trait of bacterial wilt, however its temporal positioning
314 could vary depending on the plant species and plant age (which could influence immune response or
315 root infection).

316 The plant water loss observed during *R. solanacearum* infection (Figure 3) is in a similar range to that
317 observed during drought on tomato (Sánchez-Rodríguez et al., 2010), around 10% of water content,
318 showing that bacterial wilt imposes significant drought stress on the plant. Photosynthesis requires a
319 big amount of water, so water deficit should quickly impair photosynthesis and, therefore, have an
320 impact on the ability to store carbon such as starch during the daytime phase of the day. In agreement
321 with this, we observed in our metabolomic analysis a clear drop in starch storage in infected aerial
322 parts at 7 dpi (Figure 3). The estimation of chlorophyll content by imaging revealed a progressive
323 decrease after infection, which remained limited at 7 dpi (Figure 3). The incidence of bacterial wilt is
324 probably less apparent on the chlorophyll content than on the accumulation of starch since chlorophyll,

325 even though described as decreasing under stress, is subjected to a turnover slower than starch
326 (Scialdone et al., 2013).

327

328 **Quantitative insights in *R. solanacearum* preferred carbon sources in xylem.**

329 In a second part of this work, we studied the kinetic variations of metabolites of xylem sap associated
330 with the bacterial wilt disease. The fast proliferation of *Ralstonia* spp in xylem must be sustained by
331 nutrients that the pathogen uptakes in this environment. We therefore studied specifically the
332 metabolites that were less abundant in the sap of infected plants compared to that of healthy plants,
333 presumably used by bacteria for efficient growth. The PCA multivariate analysis of metabolomic data
334 revealed that glutamine and asparagine are significantly depleted in infected plants. This is particularly
335 striking for glutamine for which the initial concentration (2.66 ± 1.34 mM at 1 dpi) decreases to lower
336 than 1 mM at the last days of sampling. Glutamine is described as the major organic component of
337 xylem sap in plants of different botanical families (Andersen & Brodbeck, 1989; Montes Borrego,
338 Miguel; Jiménez-Díaz et al., 2017; Zuluaga et al., 2013). Our data are in line with these reports:
339 glutamine in tomato xylem sap was measured 3.29 ± 1.25 mM for non-infected plants, which
340 represents 75% of the molar organic carbon in this compartment. We showed that glutamine
341 concentration was negatively correlated with bacterial growth, supporting the view that glutamine is
342 most probably the main carbon source sustaining fast proliferation in vascular tissues. As glutamine is
343 by far the most abundant amino acid in xylem, we hypothesize that *R. solanacearum* has adapted to
344 preferentially metabolize this compound. The five different transport systems for this compound
345 present in the genome of GMI1000 highlight the high importance of this metabolite.

346 Simultaneously to glutamine, the asparagine pool (around 0.178 ± 0.074 mM in sap of healthy plants)
347 is quickly depleted after 4 dpi, falling under the detection limit (between 1 μ M and 10 μ M). According
348 to our data, sugars do not appear to play a major role as sources of energy supply for the bacteria in

349 xylem vessels. Indeed, sugars are present in scarce proportions compared to amino acids such as
350 glutamine or asparagine. Glucose can be detected but in concentration at the limit of reliable detection,
351 and the sucrose content also appears rather low. Thus, the sugars concentration does not seem sufficient
352 to explain the strong bacterial growth. Bacterial infection should lead to the enrichment of the sap with
353 sugars such as sucrose and glucose, as reported by (Lowe-Power, Hendrich, et al., 2018). However,
354 based on the kinetic and quantitative values we hypothesized that these metabolites might have a role
355 in signaling rather than being primary carbon sources to sustain rapid growth, unless sucrose/glucose
356 dynamics is too fast to be captured by a day by day kinetics. This was confirmed by the behavior of a
357 triple mutant strain that is unable to grow *in vitro* when glucose, sucrose and fructose are provided as
358 sole carbon sources. This strain however is still able to colonize the plant, reaching high density in the
359 stem, and causes physiological alteration in plants (suppression of growth and transpiration) and
360 symptoms (disease index) delayed but similar to the wild-type strain. We thus concluded that sugars
361 do not play a major role in the pathogen's growth at this stage.

362 Our metabolomics study also revealed that several potential nutrients (such as aspartate, phenylalanine
363 and leucine) remain in xylem sap even at later infection stages in concentrations similar to that of
364 healthy plants. This suggests that these metabolites are not consumed by *R. solanacearum*, probably
365 due to the lack or non-expression of the appropriate transporter genes. For various other lower
366 abundant nutrients, the high variability observed in infected plants prevents making clear conclusions.
367 In an earlier study, Zuluaga et al. (2013) analyzed the xylem sap content of tomato and it should be
368 noted that some carbon sources reported in their study were not detected in ours. This mainly concerns
369 amino acids such as GABA (gamma-aminobutyric acid), histidine and serine. The presence of fructose
370 was also detected while our data reveal the presence of sucrose but not fructose. Higher quantities of
371 glucose, fructose and sucrose were found in the tomato apoplast (Zuluaga et al., 2013). However, as
372 the GRS491 triple mutant strain was still able to cause effective bacterial wilt, this suggests that

373 metabolization of these sugars is also not essential for the growth of *R. solanacearum* in the apoplastic
374 environment.

375 A recent study revealed glutamic acid as a major amino acid promoting expression of some *R.*
376 *solanacearum* pathogenic traits (Shen et al. 2020) . Inversely to glutamine, glutamic acid is present in
377 trace amounts in our xylem sap analysis (Table 1) and its concentration remains constant during
378 infection. The NMR analysis of xylem sap unambiguously discriminated between the two amino acids
379 (Fig. S10). Therefore, we favor a role of glutamic acid in signaling to induce production of virulence
380 factors rather than as a major carbon source metabolized in xylem vessels.

381

382 **Metabolic signatures of bacterial wilt.**

383 Beside the depletion of some metabolites as mentioned above, we also observed the increase of other
384 compounds in xylem following *R. solanacearum* infection. The emergence of such compounds could
385 be from bacterial origin (excreted product such as putrescine (Lowe-Power, Hendrich, et al., 2018;
386 Peyraud et al., 2016)), or part of a plant metabolic response to infection (Zeiss et al., 2019). In some
387 cases, these compounds could come simultaneously from the bacteria and from the plant. In the case
388 of putrescine, the role of the molecule may be dual since bacterial production has been shown to
389 contribute to the wilt symptoms (Lowe-Power, Hendrich, et al., 2018) and also be produced by tomato
390 cells hijacked by a *Ralstonia spp* TAL effector (Wu et al., 2019).

391 We found that upon infection xylem sap accumulated 3-hydroxybutyric acid, acetate, ethanol and
392 potentially proline (discriminated by PCA, Figure 4 although no significant difference in the kinetics
393 on Fig. S8). Accumulation of proline and ethanol has been associated with drought stress (Kelsey et
394 al., 2014; Sánchez-Rodríguez et al., 2010) and one can assume that the bacterial wilt disease induces
395 a sudden and brutal water deficit for the infected plant (Zeiss et al., 2019). Ethanol production could
396 also be a physiological response to xylem occlusion by *R. solanacearum*: the formation of bacterial

397 biofilm would limit the diffusion of oxygen in the xylem vessels, thus inducing a switch to anaerobic
398 respiration in plant cells and therefore the production of ethanol as a fermentation product. Concerning
399 proline, its release into the xylem by plant cells may be due to the need to compensate for the depletion
400 of glutamine / asparagine consumed by the bacteria. Acetate was also found to appear in infected
401 xylem; similarly to ethanol, acetate could be a plant fermentation product due to a limitation of oxygen
402 diffusion, but it could also result from an overflow of the bacterial metabolism (El-Mansi & Holms,
403 1989). As previously reported by Lowe-Power et al. (2018), we observed an increased synthesis of 3-
404 hydroxybutyric acid. 3-hydroxybutyric acid is a precursor of PHB (polyhydroxybutyrate), a storage
405 polymer identified in *R. solanacearum* and phylogenetically close β -proteobacteria. Kinetics showed
406 an increase followed by a decrease phase of 3-hydroxybutyric acid concentration (Figure 4C), in
407 agreement with the hypothesis that this metabolite could be excreted by *Ralstonia* and then consumed
408 before returning to the soil (Lowe-Power et al., 2018). However, it cannot be excluded that bacteria
409 induce production of 3-hydroxybutyric acid by the plant, as the biosynthetic pathway is present in
410 tomato plant (Yuan et al., 2016).

411 In a complementary approach, Lowe-Power and collaborators (2018) have recently performed GC-
412 MS metabolomics of tomato xylem sap infected by *R. solanacearum*. This allowed a non-targeted
413 approach but not an absolute quantification of the metabolites as we were able to achieve it by the
414 NMR approach. Based on the relative fold-change of metabolites in the xylem sap of healthy versus
415 infected plants, several carbon sources such as galactose, 3-hydroxybutyrate, gluconate and glucose
416 were identified as important carbon sources for *R. solanacearum* in the xylem (Lowe-Power, Hendrich,
417 et al., 2018). In our study, except for 3-hydroxybutyric acid and glucose (at a concentration below 0.1
418 mM), the other compounds were not detected, indicating that these compounds are in concentration
419 below 1 – 10 μ M, which is the sensibility limit of NMR. Significant change folds can be observed
420 more easily for low-concentration metabolites than for abundant ones. This thus suggests that the
421 metabolites, even if they had high fold change, are probably not primary carbon sources supporting

422 bacterial growth. Given the quantities of carbon source available in tomato xylem sap and their
423 observed consumption by *R. solanacearum* (Figure 5), the most likely hypothesis is that co-
424 consumption of glutamine and asparagine is the main means used by the pathogen to support its high
425 proliferation in xylem. The redundancy observed in the genome at the level of glutamine transporter
426 genes (five unlinked loci identified) reinforces the view that this amino acid is probably crucial to the
427 *R. solanacearum* fitness inside the plant vascular system.

428 **Experimental procedures**

429 **Plant cultures and automatic phenotyping**

430 Two independent experiments were conducted for this study. In both cases, tomato seeds (*Solanum*
431 *lycopersicum* M82) were grown in soil (SB2, Proveen, The Netherlands) supplemented with
432 Osmocote® coated fertilizer at a rate of 4g/L. Seed were germinated in a growth chamber (26°C, 67%
433 HR, 12h- LED light per day). Around a hundred of plantlets were transplanted in individual plastic
434 pots (8x8cm) 8 days after sowing. 16 days after sowing, 90 young plants were chosen and repotted in
435 3L pots until the end of the experiment. Foam cover discs were placed on each pot to limit the
436 evaporation.

437 On the first experiment, 90 plants were loaded on the Phenoserre robot facility of the Toulouse Plant
438 Microbe Phenotyping infrastructure, of which 45 plants were inoculated with *R. pseudosolanacearum*
439 GMI1000 strain (Salanoubat et al., 2002) and another 45 mock-inoculated with sterile water (referred
440 to as “Healthy plants”). 12h-light per day at 28°C and 50% humidity were programmed. On the second
441 experiment, 81 plants were loaded on the Phenoserre robot, of which 63 infected and 18 healthy plants.

442 All the plants were watered with 100 ml three times on the loading day and weighted, in order to define
443 a well-watered target weight. The subsequent watering was conducted automatically once a day to the
444 target weight. Imaging was programmed four times a day (starts at 2.00 a.m., 9.00 a.m., 4.30 p.m, and
445 7.30 p.m, for a total duration of around one hour and a half per imaging job), the conveyor belt bringing
446 the individual plants to a side-RGB camera. The plants were then automatically weighted after each
447 imaging session. The daily transpiration was determined as the weight differences between two
448 consecutive days, at the time of watering. Temperature, hygrometry and light intensity were recorded
449 during the whole experiment.

450 **Plant image analysis**

451 Fig. S1 presents the step-by-step segmentation performed using a set of python algorithms allowing
452 for the testing of the segmentation pipeline. Every plant is imaged using an RGB camera with a blue
453 background, under six angles (starting at 0° and then by 60° steps). Three different “Region of Interest”
454 or ROIs are defined to allow easier analysis, in white containing all the plant information, with a “safe
455 zone” in green and a “danger zone” in red. As the images are acquired in the exact same conditions,
456 the ROIs are static. Masks are extracted corresponding to the bulk of the plant (“mask top”), as well
457 as two additional masks to gather the plant information close to the pot and conveyor (“mask middle”
458 and “mask bottom”). All three masks are merged in order to build a coarse mask, this latter ask is
459 then cleaned by removed additional noise to generate a clean mask and allowing for the final features
460 extraction.

461 Fig S2, displays the “centroid y” (or Cy) morphological traits, which is the y coordinate of the plant
462 area center of gravity. The origin of the y axe is positioned at the top of the image. A larger Cy value
463 indicates a center of gravity closer to the pot (further from the top of the image) and is a trait that is
464 very well associated with the bacterial wilt symptoms, as any “lowering” of the leaves (wilting) has an
465 impact of the Cy trait. Fig S3 displays some Cy values of plants with different disease index (DI).

466 **Plant inoculation**

467 For both experiments, three replicates of the strain GMI1000 were grown in BG complete medium
468 (Plener et al., 2010) at 28°C. Bacterial cells were washed, resuspended in sterile water and each plant
469 was inoculated with one of the three replicates by pouring 50 ml of a suspension at $5 \cdot 10^6$ CFU/ml at
470 the stem base 4 days after the loading on the robot. Just before inoculation, roots were scarified with a
471 scalpel blade in order to synchronize the infection rate.

472 **Collection and preparation of plant samples**

473 For the first experiment, plants were removed each day from the conveyor belt for samplings during 9
474 consecutive days. On the inoculation day and at 7 and 8 dpi, 3 healthy and 3 infected plants were

475 removed. From 2 to 6 dpi, 6 healthy and 6 infected plants were removed each day. For the second
476 experiment, plants were removed from the conveyor belts for samplings at 1, 3, 4, 5, 7 and 8 dpi. Each
477 sampling day, 3 healthy plants and respectively, 3, 6, 14, 16, 14, 10 infected plant were removed.

478 In both experiments, the following procedures were then applied at the same period of the day (between
479 10 am and 2 pm) to avoid the effect of daily variations in xylem sap/organ chemistry. For the plant
480 that were removed from the experimental setup, stems were cut just above the cotyledon node, rinsed
481 with approximately 1 ml of water and the upcoming xylem sap was collected by repeated pipetting
482 and collection in Eppendorf tubes placed on ice. For the infected plants, the tubes were centrifuged
483 and the supernatants were transferred in new tubes while the pellets were discarded. The tubes with
484 xylem sap were placed at -80°C for further quantitative NMR analyses.

485 The different organs of each plant were collected separately (stems, leaves, roots and flowers whenever
486 they occurred) for fresh and dry weights measurements. Approximately 300 mg fresh weight for each
487 collected organ was frozen in liquid nitrogen and stored at -20°C for further biochemical analyses.

488 1 cm of stem was sampled above the cotyledons, cut up in small pieces and placed in a 2 ml centrifuge
489 tube containing glass beads (2 mm diameter) and ground at 30 Hz with a mixer mill (MM 400, Retsch,
490 Germany) until total grinding (1 to 3 minutes). Extracted bacteria were resuspended in 1 ml of sterile
491 water, serial-diluted and plated on complete BG medium supplemented with triphenyl tetrazolium
492 chloride, for counting as previously described (Guidot et al., 2014).

493

494 **Quantitative NMR analyses**

495 The xylem saps were analyzed by 1D ¹H NMR on MetaToul analytics platform (UMR5504, UMR792,
496 CNRS, INRAE, INSA 135 Avenue de Ranguel 31077 Toulouse Cedex 04, France), using the Bruker
497 Avance 800 MHz equipped with an ATMA 5mm cryoprobe. Each xylem sap sample was centrifuged
498 to remove the residues (5min, 13520 RCF, Hettich Mikro 200 centrifuge), then placed in 3 mm NMR

499 tubes. TSP-d4 standard (Sodium 3-(trimethylsilyl)(1-¹³C,2H₄)propanoate), at a final concentration of
500 100 μM, was used as a reference. pH 6.0 phosphate buffer was used to standardize the chemical shifts
501 among samples. Acquisition conditions were as follows: 30° pulse angle, 20.0287 ppm spectral width,
502 64 scans per acquisition for a total scan time of approximately 8 minutes per sample, and zgpr30 water
503 pre-saturation sequence. The samples were kept at a temperature of 280 K (6.85 °C) all along the
504 analysis. Resonances of metabolites were manually integrated and the concentrations were calculated
505 based on the number of equivalent protons for each integrated signal and on the TSP final
506 concentration. For one equivalent proton, half of the glucose signal was overlapped by the residual
507 water, as shown on Fig. S10 (around 5.2 ppm). Therefore, its contribution was extrapolated based on
508 the analysis of a glucose standard.

509

510 **Biochemical analyses of metabolites**

511 Quantifications of metabolites were performed at the HitMe platform (INRAE - IBVM - 71 avenue E.
512 Bourlaux - CS 20032 - 33882 Villenave d'Ornon Cedex, France). The plants samples, previously
513 frozen in liquid nitrogen, were ground to a powder using liquid nitrogen to avoid thawing. A quantity
514 of 20 +/- 10 mg of each were weighted in previously frozen Micronic tubes. Free amino acids, glucose,
515 fructose, malate, proteins, starch, sucrose and chlorophylls in leaves, stems and roots were quantified
516 as described in (Biais et al., 2014). Briefly, ethanolic extracts from every samples were obtained using
517 three consecutive incubation of the frozen ground powder aliquots. Ethanol 80% v/v with
518 HEPES/KOH 10 mM pH6 buffer was used for the two first incubations, and ethanol 50% v/v with
519 HEPES/KOH 10 mM pH6 buffer was used for the third. Supernatants were pooled and used for the
520 quantification of chlorophylls, glucose, fructose, sucrose, malate and free amino acids. Pellets were
521 used for the determination of protein and starch contents. The extracts and pellets were stored at -20°C
522 between each quantification. For each sample, chlorophylls were quantified by measuring optical
523 densities at 645 and 665 nm on a mix of 50 μl of extract supplemented with 150 μl of analytics grade

524 ethanol. Amino acids were quantified using the fluorescamine method. Excitation wavelength was
525 405nm and emission was measured at 485 nm. The proteins were quantified using Bradford reagent.
526 Starch was quantified in glucose equivalent after full pellet digestion in an oven at 37°C for 18 hours.
527 For the other analytes cited above, the NADH/NADPH appearance was measured, and the analytes
528 were quantified using a 1:1 stoichiometric coefficient.

529 **Generation and phenotyping of *R. solanacearum* triple mutant strain defective for glucose,**
530 **sucrose and fructose assimilation**

531 Strain GRS941 was engineered by cumulating deletions in the glucose transport operon (RSp1632-
532 RSp1635), the sucrose assimilation operon (RSp1280-RSp1286) and the fructose transport operon
533 (RSc2861-RSc2863). Complete procedures for the triple mutant generation are available on Text S1.
534 For *in vitro* growth assay, preculture on BG complete medium (Plener et al., 2010), cell
535 centrifugation/washing and resuspension were performed. Strains were then inoculated at an OD (600
536 nm) of 0.01 on minimal medium (Plener et al., 2010) supplemented with respectively glucose, sucrose,
537 fructose, glutamine as sole carbon source (at 50 mM of carbon). Growth was assessed using microplate
538 spectrophotometer (FLUOstar Omega, BMG Labtech, Germany) during 96h at 28°C and under
539 shaking at 700 rpm. For in planta phenotyping, the same procedure than described for the GMI1000
540 strain was followed on two independent experiments, with respectively 31 and 41 tomato plants
541 infected by the mutant strain and similar number of plants infected by the wild-type strain..

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545

546 **Supporting Information**

547 Fig. S1. Step-by-step segmentation pipeline.

548 Fig. S2. The Centroid-y trait.

549 Fig. S3. Examples of Cy values on healthy and diseased plants.

550 Fig. S4. Biological replicate of the physiological measurements.

551 Fig. S5. Impact of *R. solanacearum* infection on plant tissues metabolic content.

552 Fig. S6. PCA on the biological replicate data: experimental points (A) and variables (B).

553 Fig. S7. Explained variance of the principal components.

554 Fig. S8. Concentrations profiles for metabolites with non-statistically significant infection effect.

555 Fig. S9. *In vitro* and *in planta* phenotyping of *R. solanacearum* triple mutant strain defective for
556 glucose, sucrose and fructose assimilation.

557 Fig. S10. NMR spectrum example and spectrum differences between glutamine and glutamic acid (or
558 glutamate).

559 Table S1. Biological replicate of the composition of non-infected xylem.

560 Table S2. Loading weights on the second component of the PCA.

561 File S1. Script for the segmentation pipeline.

562 Text S1. Generation of *R. solanacearum* triple mutant strain defective for glucose, sucrose and
563 fructose assimilation.

564

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579 **Author contributions**

580 Conceptualization: SG, CB. Methodology: LG, AE, NP, SG, CB. Formal analysis: LG, AE, FMM,
581 CB. Investigation: LG, AE, CC, CB. Resources: NP. Writing – Original Draft: LG, AE. Writing –
582 Review & Editing: LG, AE, NP, SG, CB. Visualization: LG, AE. Project administration: CB. Funding
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584 **Data availability statement**

585 All data supporting the findings of this study are available within the paper and within its
586 supplementary materials published online.

587 **Conflict of interests.** The authors declare no competing interests.

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744

745 **Figure and table legends**

746 **Table 1. Xylem metabolite content and the impact of *R. solanacearum* infection**

747 1: Effect of infection was deduced from the PCA presented in Figure 4. Metabolite concentration
748 decrease was assigned for compounds with loading weights on the second component inferior to -0.3
749 whereas increase in concentration was assigned for those superior to 0.3. Values ranging between -
750 0.15 and -0.3 correspond to compounds with putative decrease in concentration, those between 0.15
751 and 0.3 to putative increase. Values ranging from -0.15 and 0.15 defined compounds with no
752 significant variation in concentration (ie not impacted by infection). Putrescine was considered as
753 ‘appearing’ as it was absent in healthy plants and had a loading weight on the second component
754 superior to 0.3. (see Table S2)

755 2: Usage as carbon source (+: growth, -: no growth) was assessed from published Biolog Phenotype
756 Microarrays data (Peyraud et al., 2016).

757 **Figure 1**

758 Impact of *R. solanacearum* colonization on plant physiology (dry weight/transpiration) and monitoring
759 of disease kinetics (disease index/bacterial density). The data presented are from an experiment on 81
760 plants, with at least 3 plants per conditions at each sampling point. Bars indicate standard deviation.
761 Healthy and infected plant data (transpiration and dry weight) were compared by Wilcoxon-Mann-
762 Whitney test (*: p-value<0.05, **: p-value<0.01, ****: p-value <0.0001).

763 **Figure 2**

764 Physiological parameters determined by automatic imaging. Chlorophyll content was estimated
765 through RGB values of plant images as described by Liang et al (*Liang et al., 2017*). Center of
766 gravity position (vertical) was estimated through plant imaging with 0 pixel representing the top of
767 the plant. The data presented are from an experiment on 81 plants with at least 3 plants per
768 conditions at each sampling point. Bars indicate standard deviation. Healthy and infected plant data

769 were compared by Wilcoxon-Mann-Whitney test (*: p-value<0.05, **: p-value<0.01, ****: p-value
770 <0.0001).

771 **Figure 3**

772 Impact of *R. solanacearum* infection on plant tissues. A. Tissue hydration. Fresh weight and dry weight were
773 used to determine the proportion of water in healthy and infected tissues. Bars indicate standard deviation. B.
774 Metabolic profile of tissues expressed in percentage of dry weight for starch and amino acids. The mean and
775 standard deviation displayed were obtained after combining the acquired data of the two biological replicates
776 (total of 12 samples for leaf, 10 for stem, 12 for root at each time point). In A. and B., healthy and infected
777 plant data were compared by Wilcoxon-Mann-Whitney test (*: p-value<0.05, **: p-value<0.01).

778 **Figure 4**

779 Principal Component Analysis on metabolite concentrations from samples at different dpi.
780 Representation of individual points (A) and the variables (B). Zero bacterial population corresponds
781 to healthy plant samples. Data points at the top of the plot correspond to uninfected or low bacterial
782 population samples while data points at the bottom correspond to high bacterial population samples.
783 Principal component 1: 35% of variability. Principal component 2: 27% of variability. ETOH: ethanol,
784 PUTR: putrescine, FUM: fumarate, SUC: sucrose, GLC: glucose.

785 **Figure 5**

786 Kinetics of metabolic contents in xylem sap of infected or healthy plants based on a total of 57 xylem
787 sap samples obtained at different dpi. Healthy and infected plant data were compared by Wilcoxon-
788 Mann-Whitney test (*: p-value<0.05, **: p-value<0.01).

789 **Figure 6**

790 Correlations between markers of the infection. For CFU per g of fresh weight, only data for which the
791 counting was above 10^7 CFU per of fresh weight (corresponding to the “tipping point”) were included.

792 **Figure 7**

793 In vitro and in planta behavior of the *R. solanacearum* mutant strain GRS941 (carrying deletion in the
794 sucrose, glucose and fructose transporter genes), compared to the wild-type strain GMI1000. Growth
795 on minimal medium supplemented with sucrose (A) and glucose (B) as sole carbon source monitored
796 in microplates with three independent biological replicates. Means and standard deviations are
797 represented by the lines and colored areas, respectively. Bacterial density in planta (C) was determined
798 on at least 9 plants per condition and per day, and plant transpiration (D) on at least 7 plants per
799 condition and per day for infected WT/GRS941 (except 3 at 8 dpi) and at least 3 plants per day for
800 healthy plants. Means are presented and bars indicate standard-deviation.

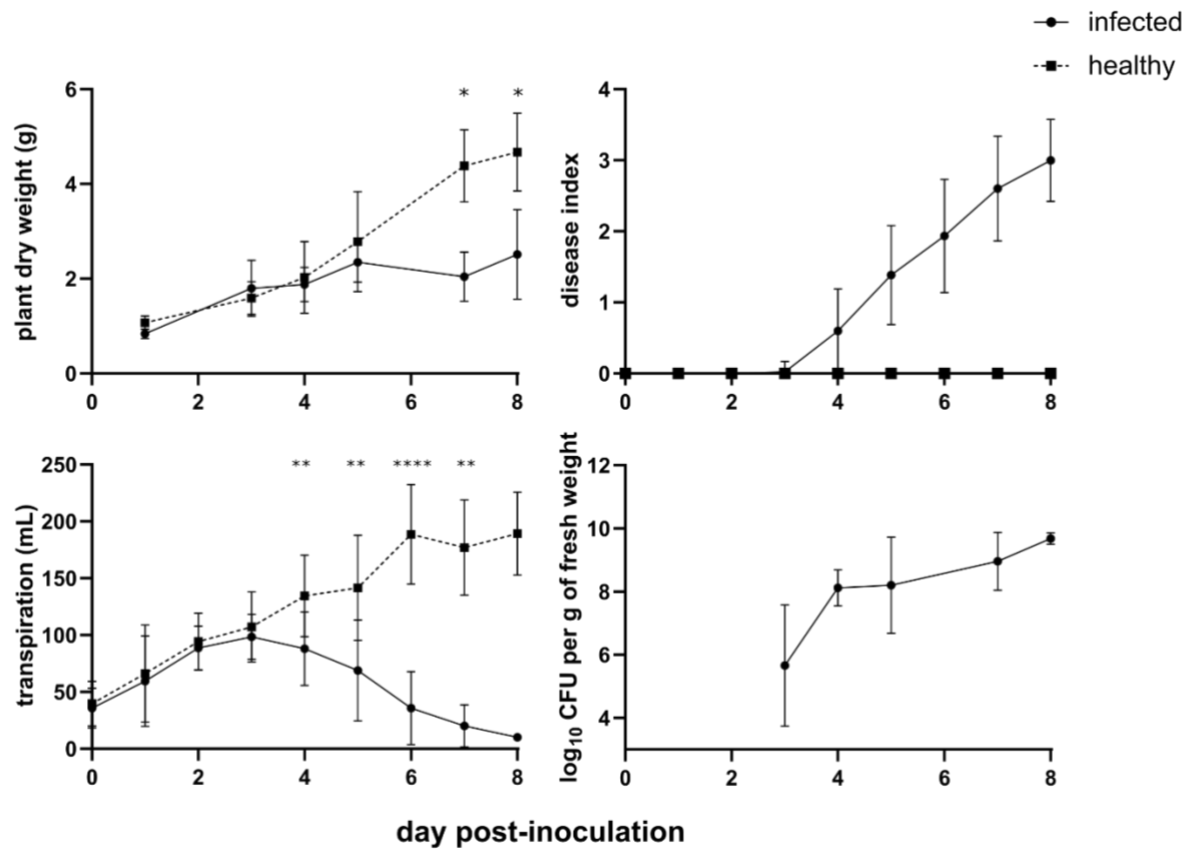


Figure 1

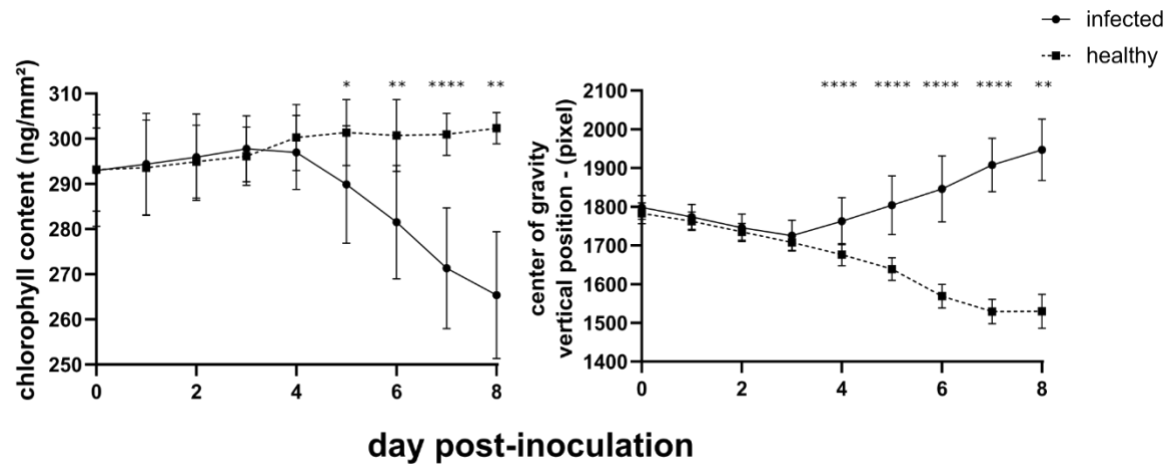


Figure 2

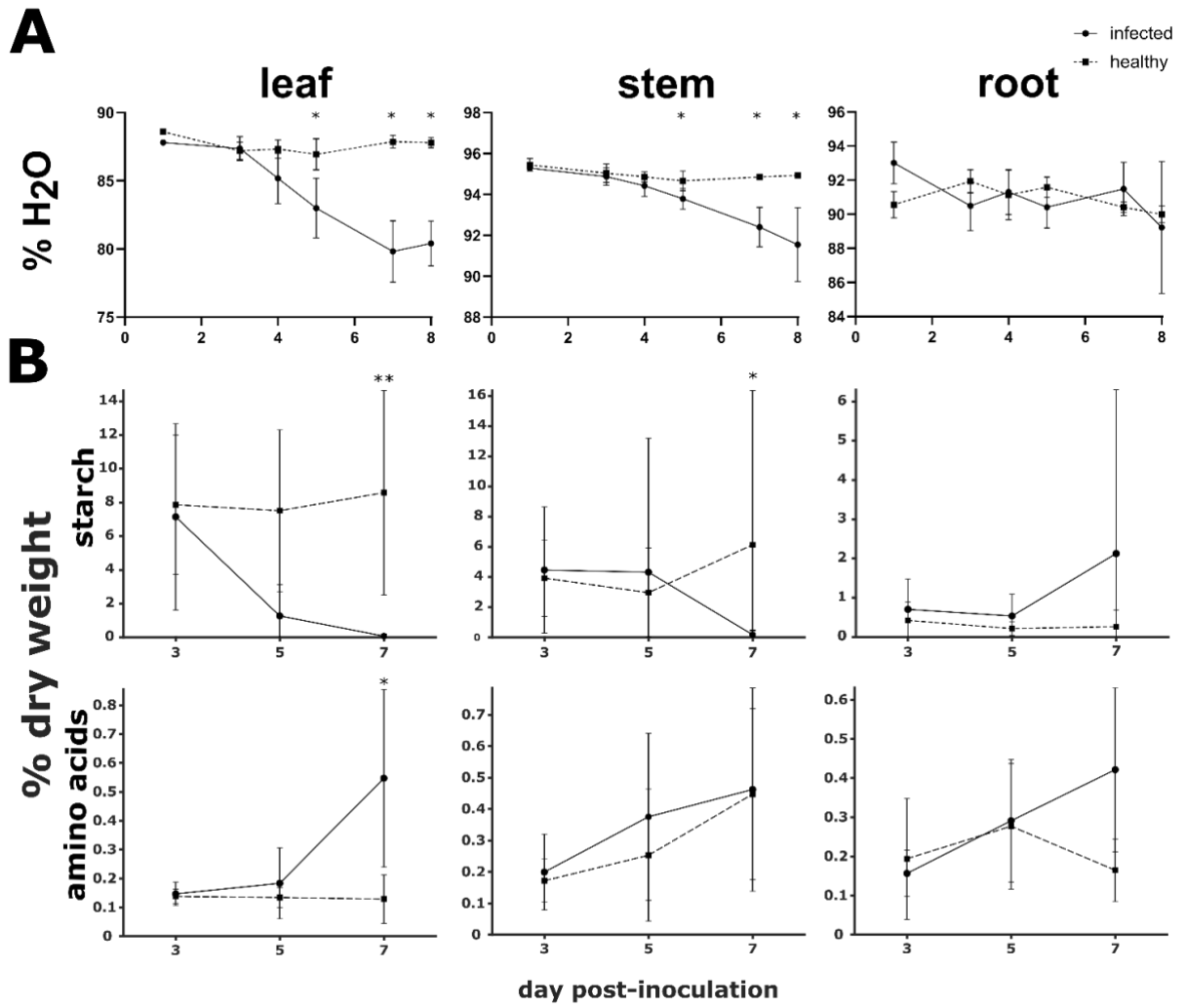


Figure 3

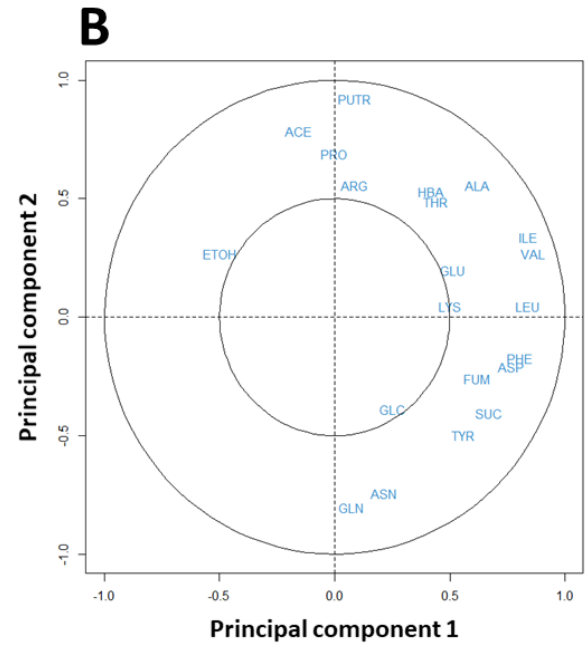
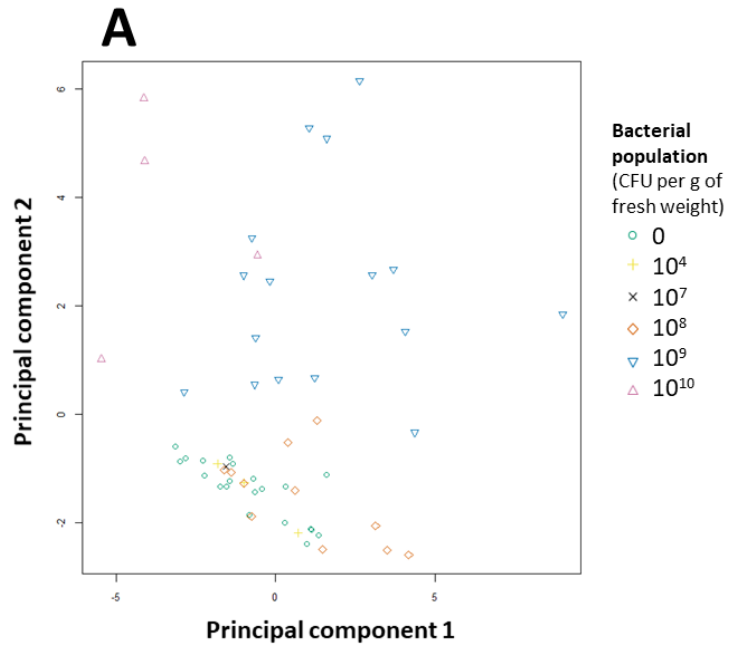


Figure 4

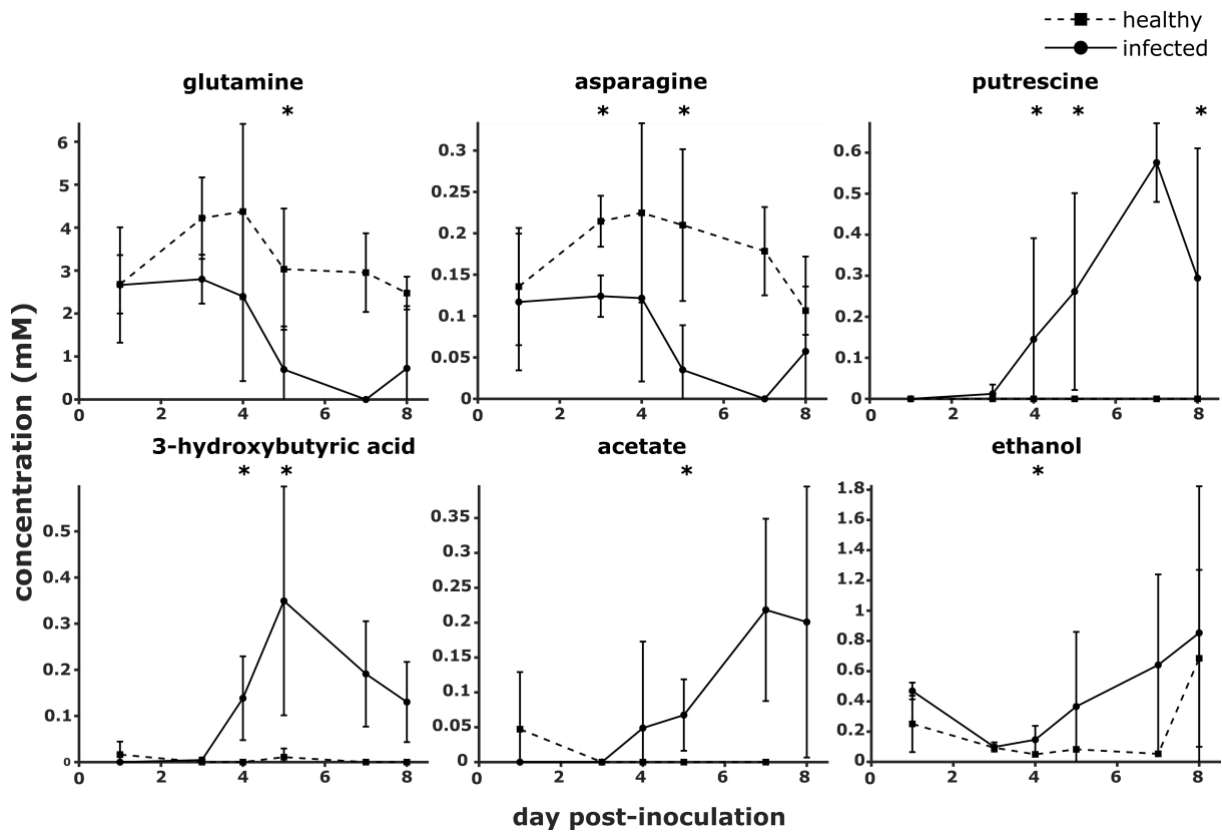


Figure 5

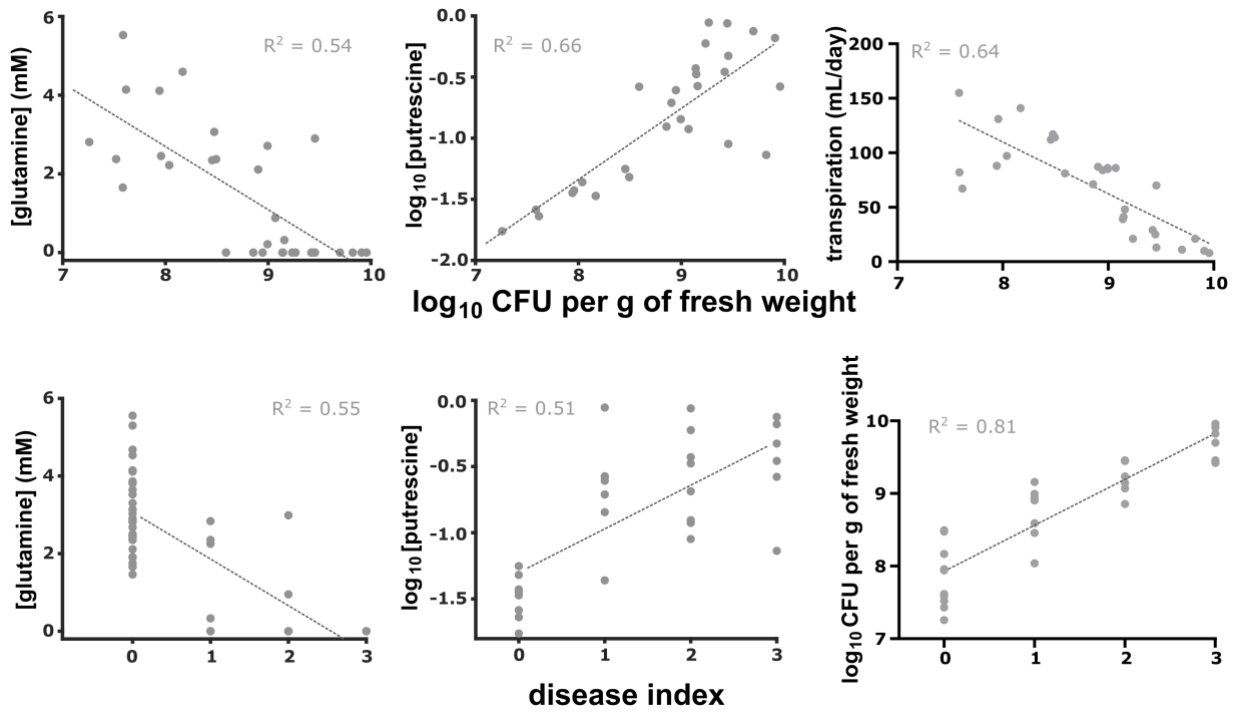


Figure 6

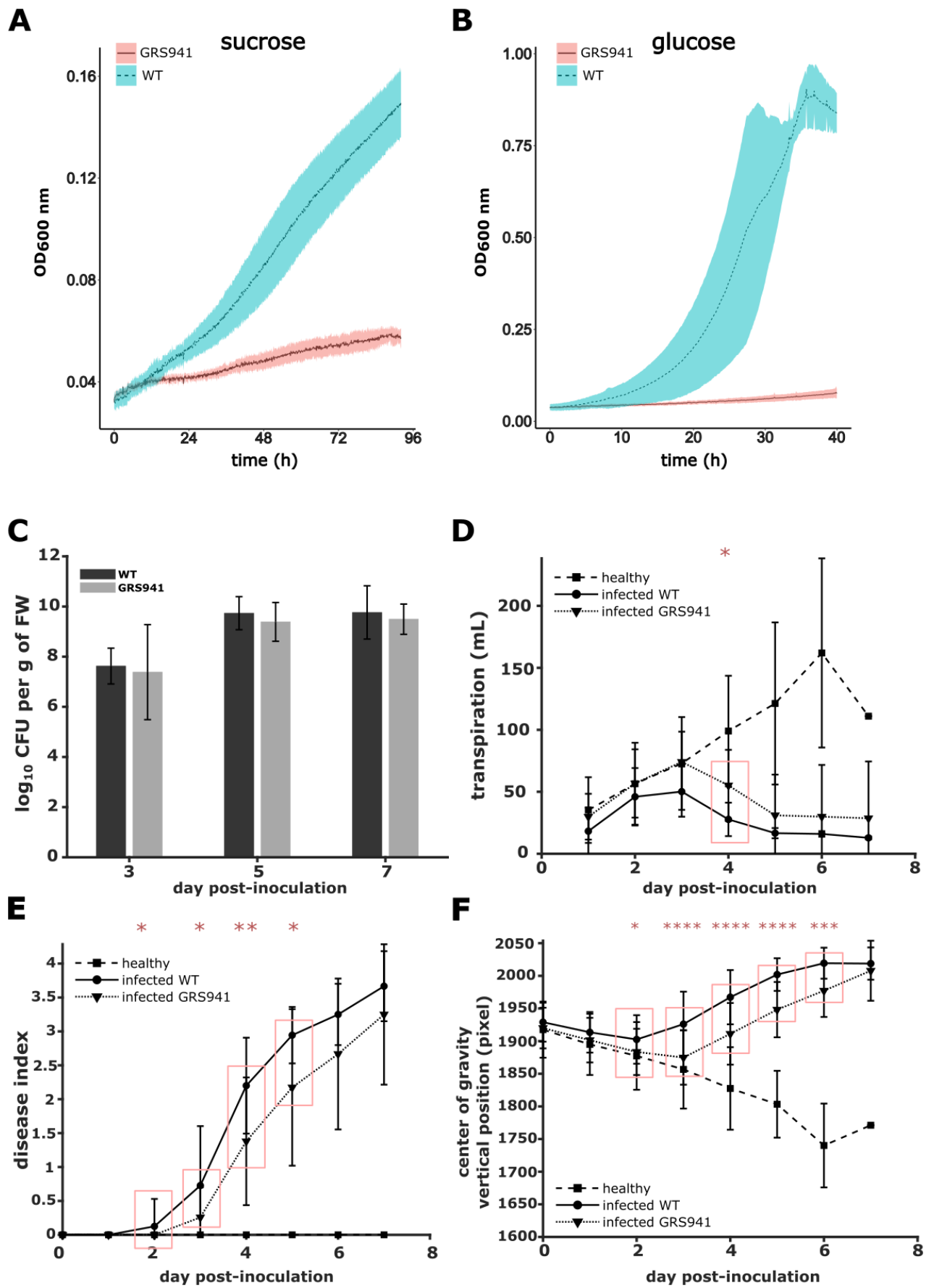


Figure 7

Table 1. Xylem metabolite content and the impact of *R. solanacearum* infection

Metabolite	Concentration in healthy plant (mM)	Effect of the infection¹	Growth of <i>R. solanacearum</i> as sole carbon source²
glutamine	3.29 ± 1.25	decrease	+
asparagine	0.178 ± 0.074	decrease	+
tyrosine	0.022 ± 0.010	putative decrease	NA
glucose	0.010 ± 0.026	putative decrease	+
sucrose	0.028 ± 0.018	putative decrease	+
fumarate	0.004 ± 0.006	putative decrease	+
aspartate	0.015 ± 0.014	no effect	+
phenylalanine	0.035 ± 0.022	no effect	-
leucine	0.088 ± 0.029	no effect	-
lysine	0.123 ± 0.062	no effect	-
glutamic acid	0.020 ± 0.041	no effect	+
threonine	0.062 ± 0.019	putative increase	+
valine	0.083 ± 0.025	putative increase	+
proline	0.066 ± 0.033	putative increase	-
alanine	0.012 ± 0.007	putative increase	+
isoleucine	0.048 ± 0.015	putative increase	-
ethanol	0.203 ± 0.314	putative increase	NA
arginine	0.094 ± 0.051	putative increase	-
3-hydroxybutyric acid	0.005 ± 0.040	putative increase	+
acetate	0.008 ± 0.033	increase	+
putrescine	not detected	appearance	-

Supplementary Figures and Tables

Figure S1. Step-by-step segmentation pipeline.

Are represented the major steps of the segmentation process. The JSON script of this specific pipeline is available as supplementary file 1 and can be uploaded, run and modified using the IPSO-Phen software package available at Github https://github.com/tpmp-inra/ipso_phen.

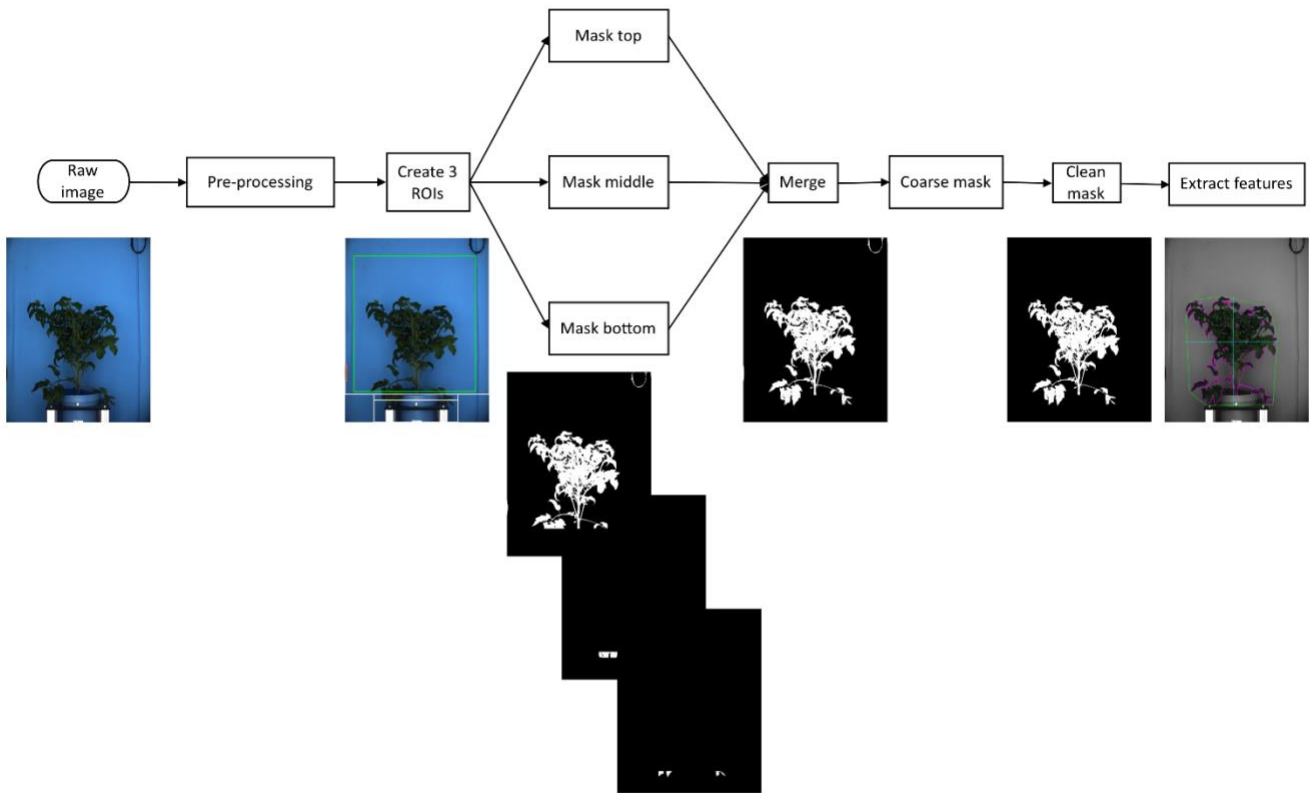


Figure S2. The centroid-y trait (Cy).

The Centroid-y trait "Cy" is the y coordinate of the center of gravity of the plant area (Cy is one of the traits extracted as a final step of the image analysis process (see Supplementary figure X0). The origin of the Y axis is set at the top of the image. Plant growth, as an upwards expansion, lowers the Cy value. Bacterial wilt, with leaves dropping and later decaying, increases the Cy value.



Figure S3. Examples of Cy values on healthy and diseased plants.

Two distinctive plant images corresponding to disease index 0, or healthy, and diseased (DI=2, 3 or 4) are showed with their respective Cy values (in pixel).

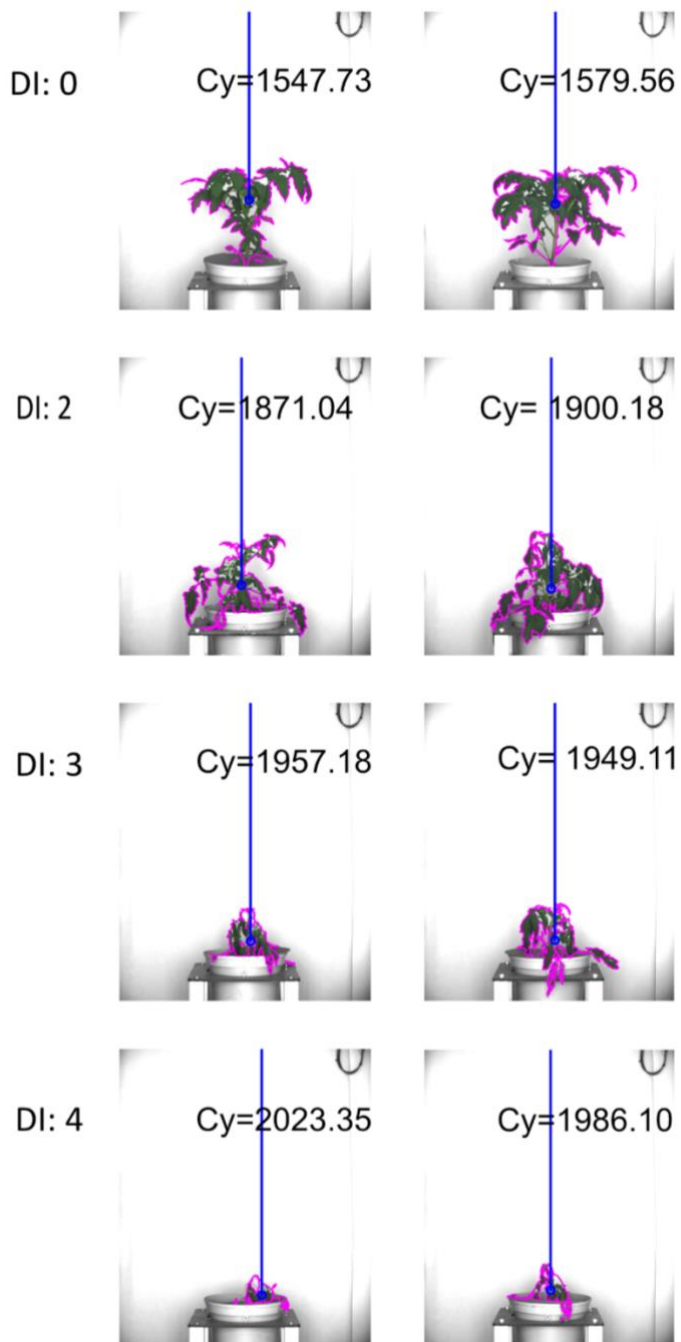


Figure S4. Biological replicate of the physiological measurements.

Impact of *R. solanacearum* colonization on plant physiology (dry weight/transpiration) and monitoring of disease kinetics (disease index), imaging parameters (chlorophyll/center of gravity position) and water content. Chlorophyll content was estimated through RGB values (automatic phenotyping) of plant images as described by Liang et al (Liang et al., 2017). Center of gravity position (vertical) was estimated through plant imaging (automatic phenotyping) with 0 pixel representing the top of the plant. The data presented are from an experiment on 90 plants with at least 3 plants per conditions at each sampling point. Fresh weight and dry weight were used to determine the proportion of water in healthy and infected tissues. Bars indicate standard deviation. Healthy and infected plant data were compared by Wilcoxon-Mann-Whitney test (*: p-value<0.05, **: p-value<0.01, ***: p-value<0.001, ****: p-value <0.0001).

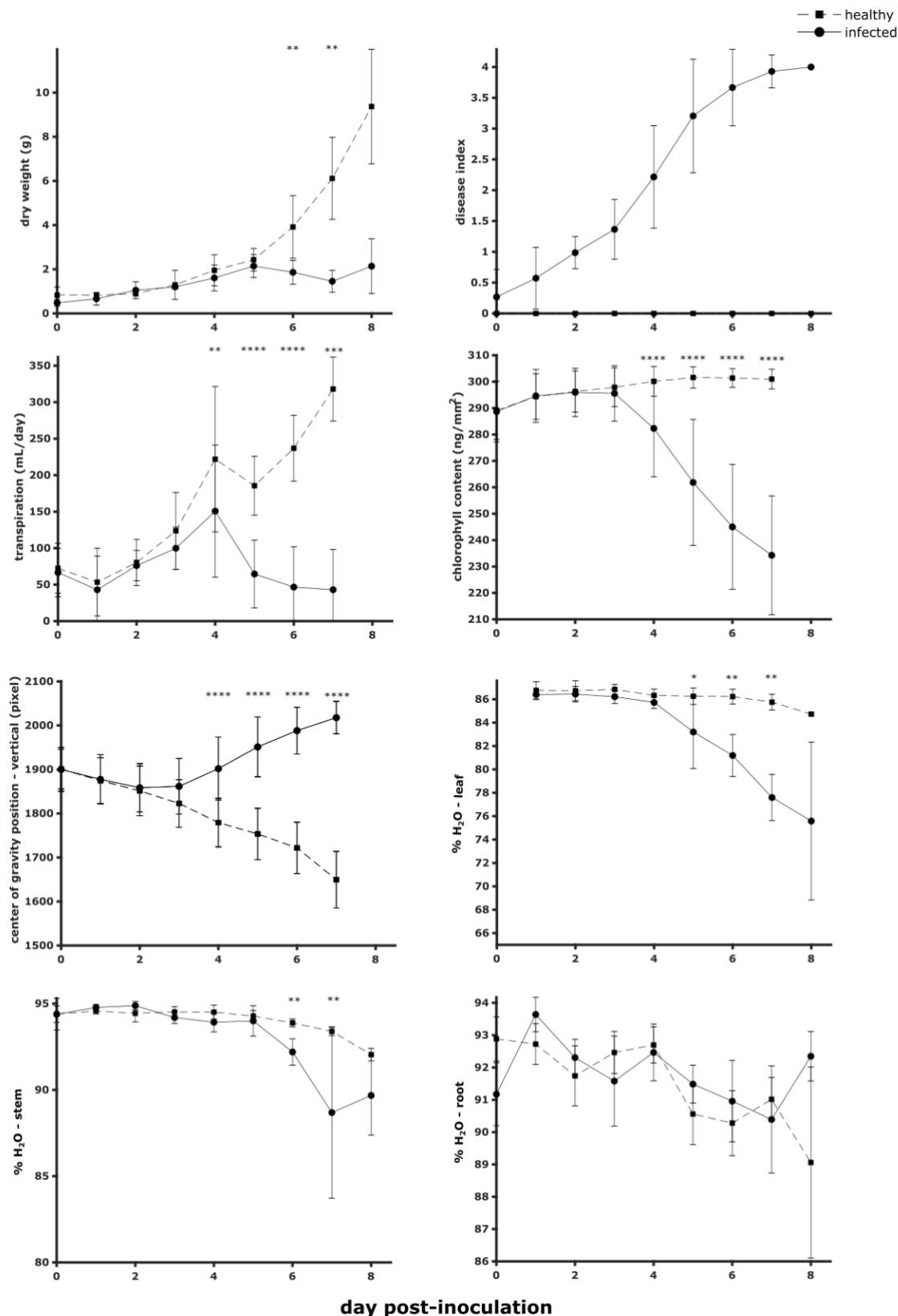


Figure S5. Impact of *R. solanacearum* infection on plant tissues metabolic content.

The mean and standard deviation displayed were obtained after combining the acquired data of the two biological replicates (total of 12 samples for leaf, 10 or 12 for stem, 12 for root at each time point). Healthy and infected plant data were compared by Wilcoxon-Mann-Whitney test (no significant change on the metabolites presented on this figure).

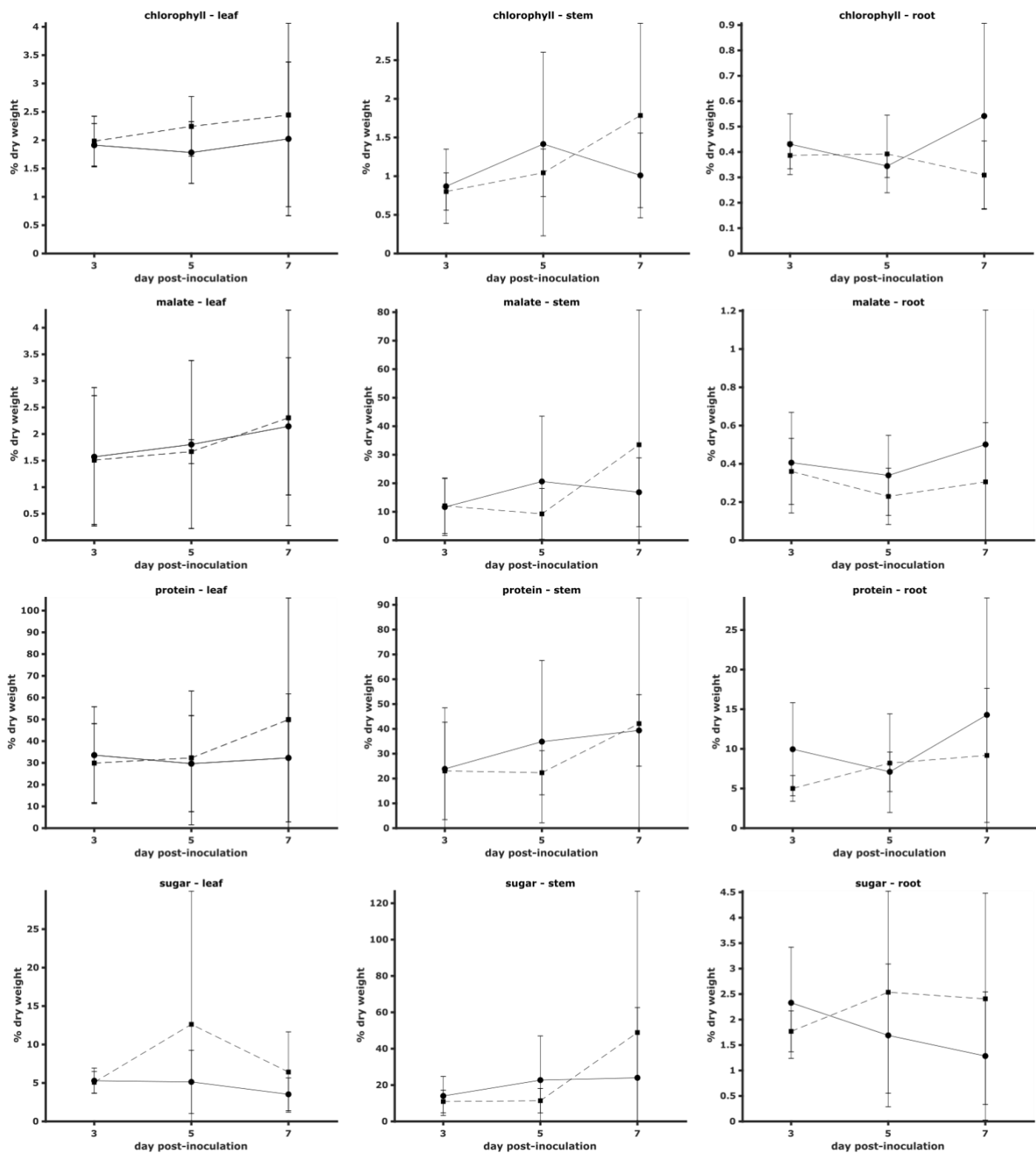


Figure S6. PCA on the biological replicate data: experimental points (A) and variables (B).

As CFU per gram of fresh weight data were not available, infection stage (ni: non-infected, ir_bf_3dpi: infected, sampling before or at 3dpi, ir_af_3dpi: infected, sampling after 3dpi) was mapped on the analyzed data. Similarly to the PCA presented in the Results section, glutamine are the compounds the most correlated with no infection or limited infection stage as it is oriented at the top left (B) which is associated with no or early infection. Oppositely, putrescine and acetate are at bottom right (B) which is associated with late infection. Other compounds associated with late infection include acetate, also noticed in the Results figure as associated with the infection. Other compounds appear to be associated with the infection so as fumarate but this association was not confirmed by the replicate presented in the Results figure.

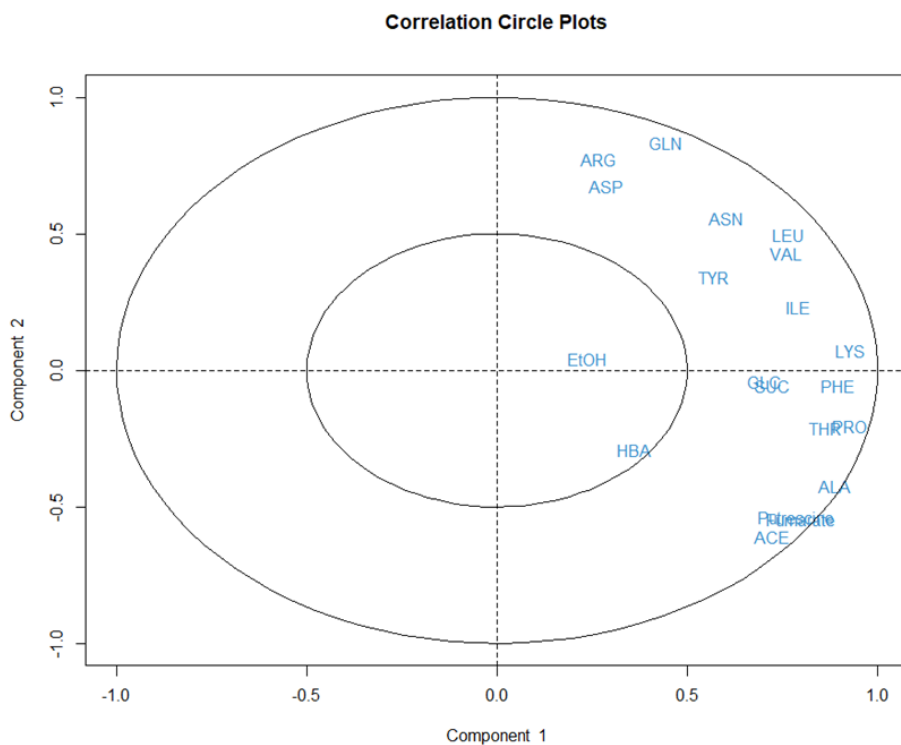
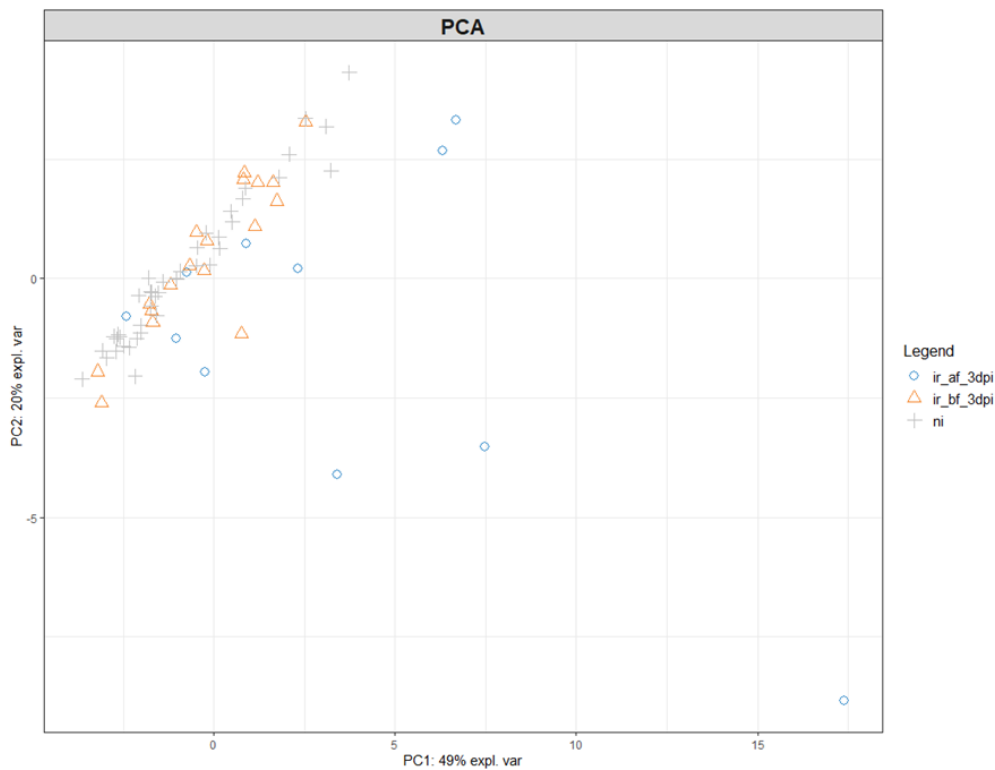


Figure S7. Explained variance of the principal components.

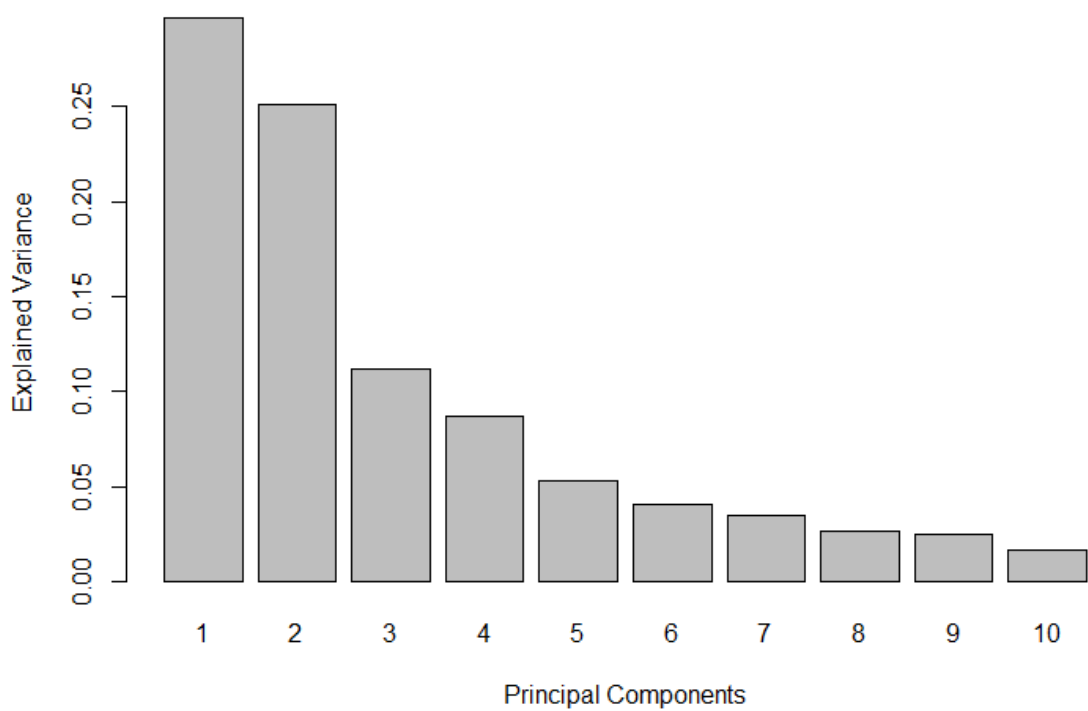
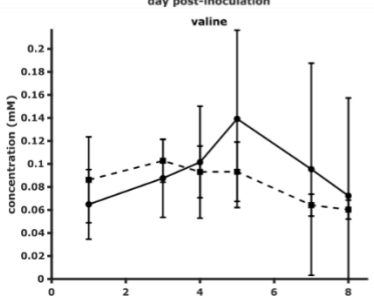
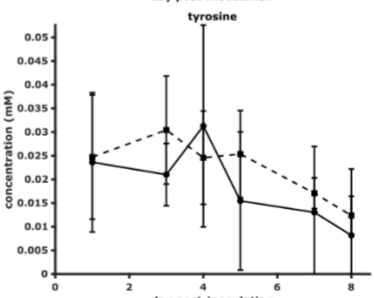
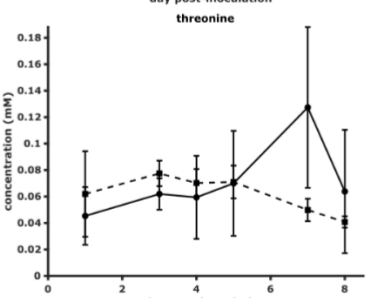
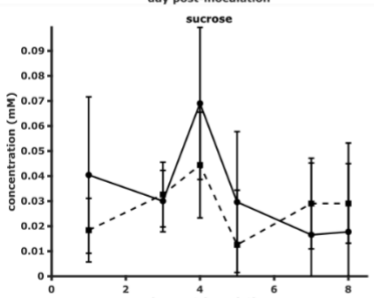
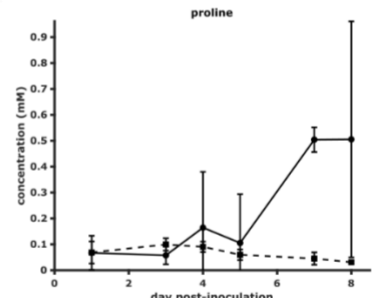
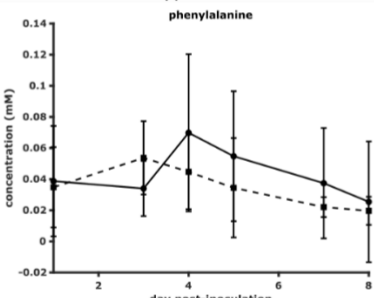
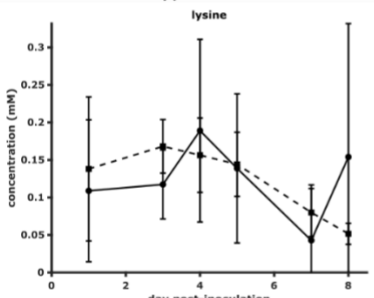
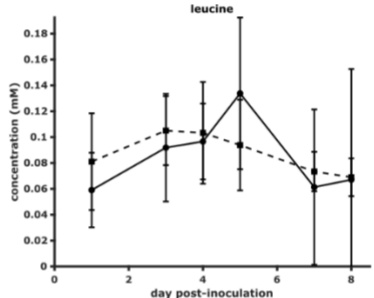
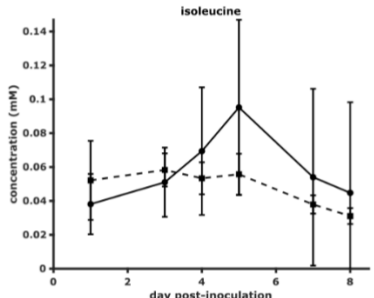
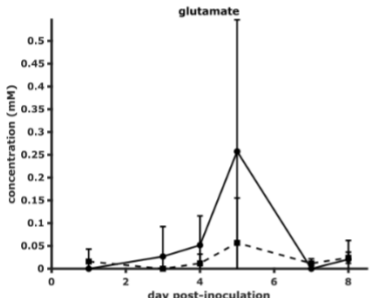
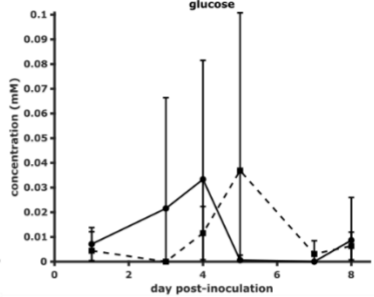
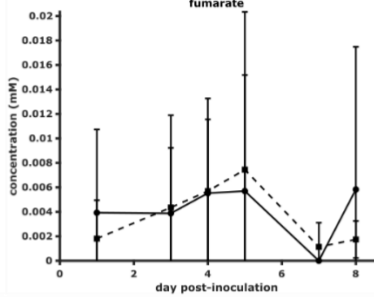
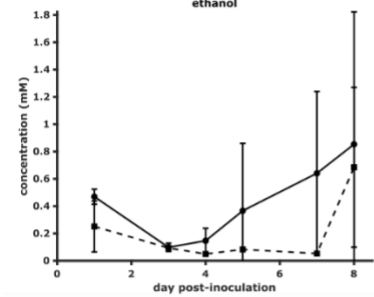
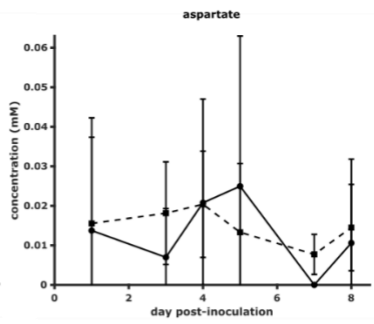
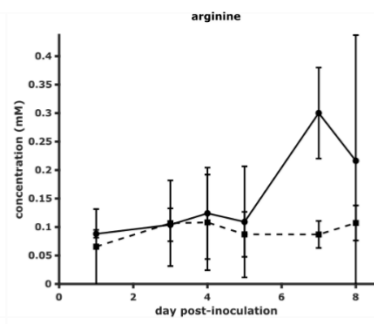
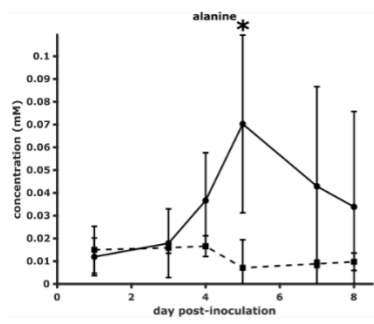


Figure S8. Concentration profiles for alanine and other metabolites with non-statistically significant infection effect.



— healthy
— infected

Fig S9. In vitro and in planta phenotyping of *R. solanacearum* triple mutant strain defective for glucose, sucrose and fructose assimilation.

Growth on minimal medium supplemented with glutamine (A) and sucrose (B) as sole carbon source monitored in microplates with three independent biological replicates. Means and standard deviations are represented by the lines and colored areas, respectively. Plant dry weight (C, F) and bacterial population (H) were determined on at least 9 plants per condition and per day for infected WT/GRS941 (except 3 at 8 dpi for F) and at least 3 plants per condition and per day for healthy plants. Disease index, transpiration and chlorophyll content (D, E, G) were determined on at least 7 plants per condition and per day for infected WT/GRS941 (except 3 at 8 dpi for E, G) and at least 3 plants per day for healthy plants. E, F, G, H represent an independent experimental repeat, and C, D are complementary figures of the experiment presented in the results section of the article. Means are presented and bars indicate standard-deviation. Infection by wild-type (WT) and GRS941 strains data were compared by Wilcoxon-Mann-Whitney test (*: p-value<0.05, **: p-value<0.01).

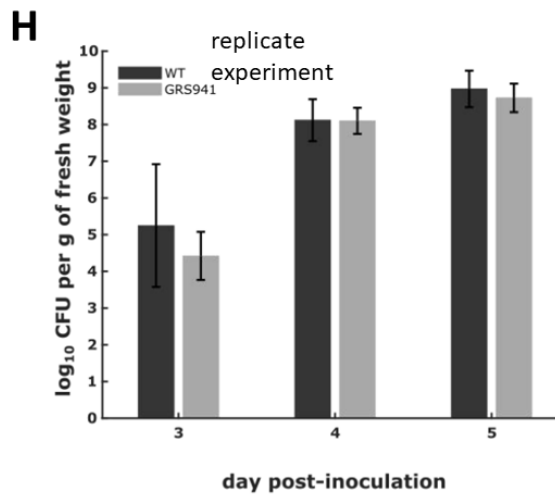
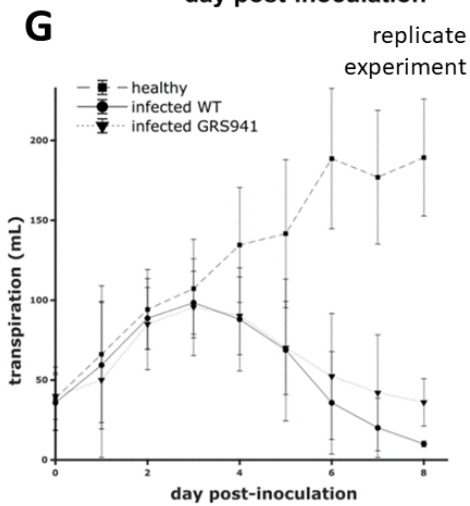
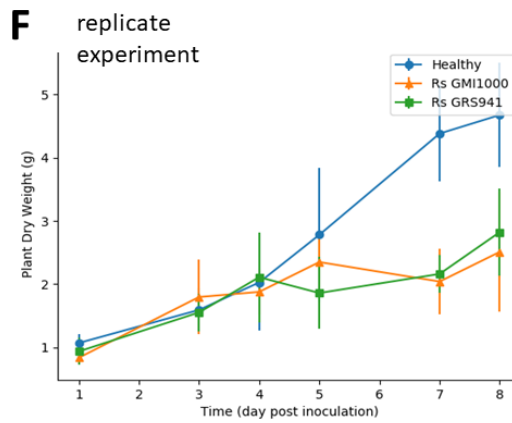
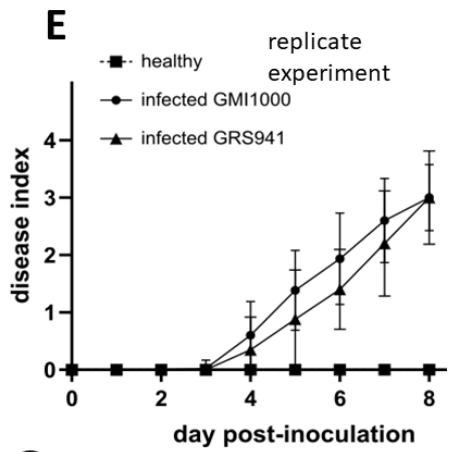
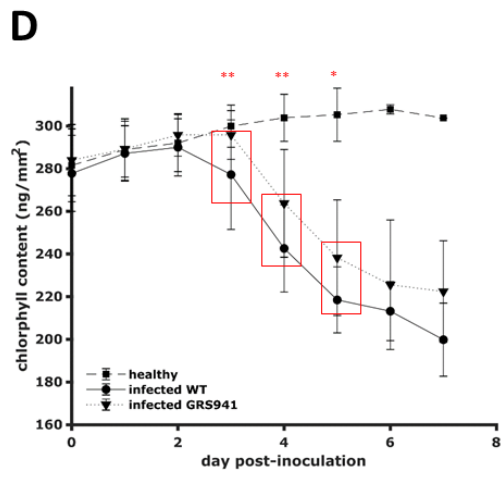
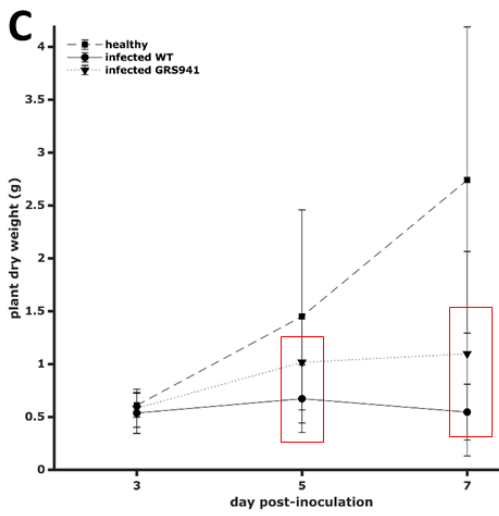
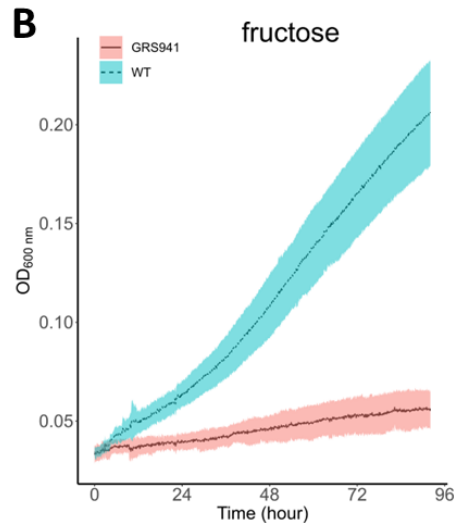
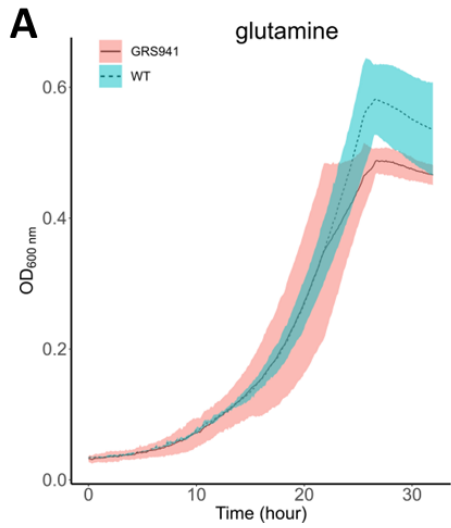
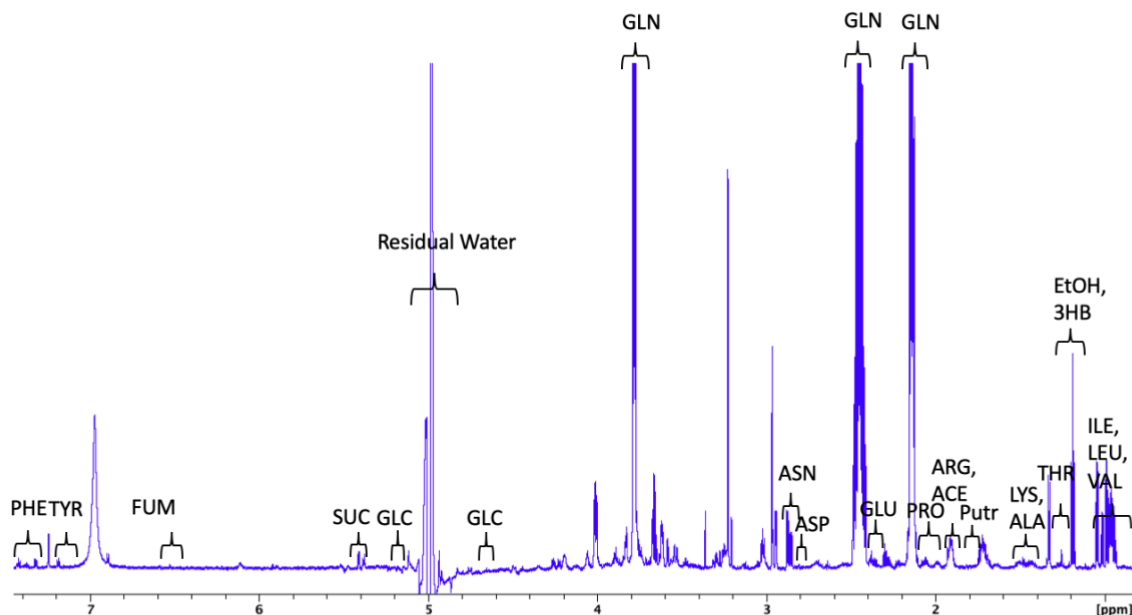


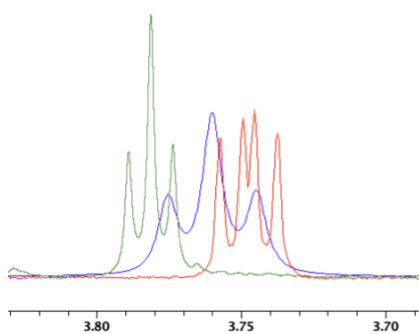
Fig S10. NMR spectrum example.

A. PHE: phenylalanine, TYR: tyrosine, SUC: sucrose, GLC: glucose, ASP: aspartate, ASN: asparagine, GLN: glutamine, GLU: Glutamate, PRO: proline, ARG: arginine, ACE: Acetate, Putr: Putrescine, LYS: lysine, ALA: Alanine, THR: threonine, EtOH: ethanol, 3HB: 3-hydroxybutyric acid, ILE: isoleucine, LEU: leucine, VAL: valine. B and C highlight the difference of spectrum aspects between glutamine and glutamate (or its conjugated acid glutamic acid), and a xylem sample with very few glutamate but high glutamine content: on B, the spectrum profile between 3.7 and 3.8 ppm is different (aspect and number of peaks) and on C, glutamine and glutamate profiles are very different: location and number of group of peaks.

A



B



C

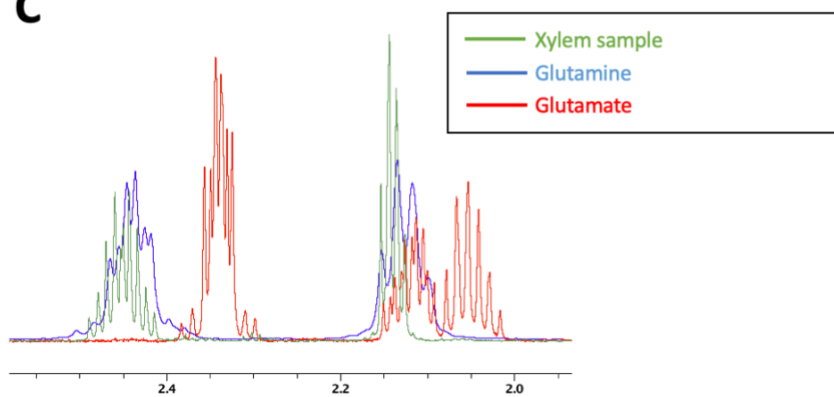


Table S1. Biological replicate of the composition of non-infected xylem.

Metabolite	Concentration in healthy plant (mM)
glutamine	3.259 ± 1.633
asparagine	0.143 ± 0.078
tyrosine	0.025 ± 0.007
glucose	0.031 ± 0.058
sucrose	0.070 ± 0.052
fumarate	0.002 ± 0.003
aspartate	0.017 ± 0.014
phenylalanine	0.029 ± 0.011
leucine	0.081 ± 0.029
valine	0.076 ± 0.026
proline	0.118 ± 0.058
lysine	0.163 ± 0.052
isoleucine	0.052 ± 0.017
arginine	0.132 ± 0.051
ethanol	0.771 ± 0.910
putrescine	not detected
threonine	0.058 ± 0.019
glutamate	not detected
acetate	not detected
3-hydroxybutyric acid	not detected
alanine	0.021 ± 0.007

Table S2. Loading weights on the second component of the PCA. (update)

Metabolite	Loading weight
PUTR	0.401
GLN	-0.350
ACE	0.339
ASN	-0.325
PRO	0.300
ARG	0.242
ALA	0.242
HBA	0.2309
TYR	-0.217
THR	0.212
SUC	-0.177
GLC	-0.169
ILE	0.146
ETOH	0.116
VAL	0.116
FUM	-0.113
ASP	-0.091

GLU	0.086
PHE	-0.075
LEU	0.020

Generation of *R. solanacearum* triple mutant strain defective for glucose, sucrose and fructose assimilation

Strain GRS941 was engineered by cumulating deletions in the glucose transport operon (RSp1632-RSp1635), the sucrose assimilation operon (RSp1280-RSp1286) and the fructose transport operon (RSc2861-RSc2863).

An unmarked internal deletion in of the glucose transport operon (Δ RSp1632-RSp1635) gene was created using the *sacB*-mediated counter selection system (Schäfer et al., 1994). Briefly, upstream (RSp1632) and downstream (RSp1635) regions were PCR amplified using the primer pairs 1632A/1632B and 1635C/1635D, respectively, and cloned into the *EcoRI-HindIII*-digested pK18mobsacB to generate pK18- Δ RSp1632/1635 (listed below). The circularized plasmid was recombined in strain GMI1000 through natural transformation (Perrier et al., 2018).

Kanamycin-resistant and sucrose-sensitive recombinant clones were first selected and in a second step, after overnight culture in BG medium (Plener et al., 2010), kanamycin-sensitive and sucrose-resistant clones were screened by PCR using the primer pair 1632A/1635D to identify a Δ RSp1632-RSp1635 recombinant. The resulting mutant was named GRS903.

A deletion of the RSp1280-RSp1286 region was created by double recombination using a selectable antibiotic interposon cassette from pHP45 (Prentki & Krisch, 1984): upstream (RSp1286) and downstream (RSp1280) regions were PCR amplified using the primer pairs 1280R1 /1280Sc and ScrR-Xba/ScrR-R1, respectively, cloned in the pGEMT vector (Promega), and then the Ω interposon was inserted in the unique *EcoRI* site. The resulting plasmid pSG804 was recombined in strain GRS903 through natural transformation as described above, and spectinomycin-resistant clones were selected and PCR-validated, yielding strain GRS936.

A deletion of the *fruBKA* operon (RSc2861-RSc2863) was created by double recombination using the selectable integrative plasmid pCM184 (Marx & Lindstrom, 2002): upstream (RSc2861) and downstream (RSc2863) regions were PCR amplified using the primer pairs fru-bgl-2/fru-nde-2 and fru-Sac2/fru-Sc1, respectively, and cloned in pCM184 as *BglIII-NdeI* and *SacII-SacI* inserts, respectively. This recombinant plasmid, pSG753, was recombined in strain GRS936 through natural transformation as described above, then gentamycin-resistant clones were selected and PCR-validated, yielding strain GRS941.

List of oligonucleotides used in this study

1632A	GAATTCAGGAGACAGTCCATGGAGCTC
1632B	TCTAGATCAGCGCGCCGGTGGTG
1635C	TCTAGATCTCCGTCACGGGCATG
1635D	AAGCTTGACGATCATCTGCCACG
1280R1	GCCTGCTCACGCGCCCGGAGG
1280Sc	GAGCTCGCCCAACAAGTTCGAG
ScrR-Xba	TCTAGATCAATTCGTACACCAC
ScrR-R1	GAATTCAACCTCGGCGCTATTGC
fru-bgl-2	AGATCTGCTGGTGCGGCCGAACAGC
fru-nde-2	CATATGTGCTCGTCCGACTGCG
fru-Sac2	CTGGCGCGCATGCTGGACAG
fru-Sc1	GAGCTCGGCGTGTAGACCTGGC

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