

1 **Induced ligno-suberin vascular coating and tyramine-derived hydroxycinnamic acid**  
2 **amides restrict *Ralstonia solanacearum* colonization in resistant tomato roots**

3

4 **Short title:** A pathogen-induced ligno-suberin vascular coating

5

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24

25 **Summary**

26

27 The soil borne pathogen *Ralstonia solanacearum* is the causing agent of bacterial wilt, a devastating  
28 disease affecting major agricultural crops. *R. solanacearum* enters plants through the roots and  
29 reaches the vasculature, causing rapid wilting. We recently showed that tomato varieties resistant to  
30 bacterial wilt restrict bacterial movement in the plant. In the present work we go a step forward by  
31 identifying the physico-chemical nature of the barriers induced in resistant tomato roots in response  
32 to *R. solanacearum*. We describe that resistant tomato specifically responds to infection by  
33 assembling *de novo* a structural barrier at the vasculature formed by a ligno-suberin coating and  
34 tyramine-derived hydroxycinnamic acid amides (HCAAs). On the contrary, susceptible tomato does  
35 not form these reinforcements in response to the pathogen but instead displays lignin structural  
36 changes compatible with its degradation. Further, we show that overexpressing genes of the ligno-  
37 suberin pathway in a commercial susceptible variety of tomato restricts *R. solanacearum* movement  
38 inside the plant and slows disease progression, enhancing resistance to the pathogen. We thus  
39 propose that the induced barrier in resistant plants does not only restrict the movement of the  
40 pathogen, but may also prevent cell wall degradation by the pathogen and confer anti-microbial  
41 properties.

42

43

44 **Key words:**

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46 Bacterial wilt, Feruloyltyramine, HCAAs, Lignin, *Ralstonia solanacearum*, Suberin,  
47 Tomato, Vascular coating

48

## 49 **Introduction**

50

51 In natural environments plants are constantly exposed to diverse microbiota, including  
52 pathogenic organisms. In addition to pre-existing structural cell barriers that act as a first  
53 line of defense (Serrano *et al.*, 2014; Falter *et al.*, 2015), pathogen perception results in  
54 activation of a complex, multi-layered immune system in plants (Jones and Dangl, 2006).  
55 As part of the suite of inducible defenses, *de novo* formation of physico-chemical barriers  
56 prevents pathogen colonization and spread inside the plant. Despite their importance, the  
57 exact composition of these barriers, as well as the mechanisms that lead to their formation  
58 in the plant upon pathogen invasion remain largely unknown.

59

60 The interaction between the soil-borne bacterial wilt pathogen *Ralstonia solanacearum* and  
61 tomato offers a paradigmatic scenario to study inducible physico-chemical barriers, because  
62 of its agro-economic impact, and the well-developed genetic and molecular tools available  
63 in both organisms. *R. solanacearum* enters the root system through wounds or at the points  
64 of emergence of lateral roots, where the epidermal barrier may be compromised, and both  
65 the endodermis and Casparian strip are either not fully differentiated or reoriented and  
66 endodermal suberin overlying the primordium is being degraded (Vasse *et al.*, 1995;  
67 Álvarez *et al.*, 2010; Ursache *et al.*, 2021) After entering the root, the bacterium moves  
68 centripetally towards the vasculature and once it reaches the xylem, it multiplies and  
69 spreads vertically within the vessels and horizontally to other vessels and the surrounding  
70 tissues (Digonnet *et al.*, 2012).

71

72 The xylem tissue is in fact a major battleground for the interaction between vascular wilt  
73 pathogens and their hosts, where the outcome of the infection is at stake (Yadeta and  
74 Thomma, 2013). To prevent the spread of pathogenic propagules, the xylem vasculature of  
75 resistant plants undergoes intense structural and metabolic modifications. Resistant plants  
76 form vertical barriers such as tyloses and gels inside the vessel lumen, which in some plant-  
77 pathogen interactions effectively slow down vertical progression of the pathogen, or even  
78 confine it to the infection site, preventing systemic infection (VanderMolen *et al.*, 1987;  
79 Rioux *et al.*, 2018). Further, resistant plants also reinforce the walls of xylem vessels, pit

80 membranes and surrounding xylem parenchyma cells in response to pathogens (Street *et*  
81 *al.*, 1986; Benhamou, 1995). This prevents pathogen colonization of the surrounding  
82 parenchyma cells, nearby vessels and inter-cellular spaces through degeneration of the  
83 vessel pit membranes or cell walls (Nakaho *et al.*, 2000; Digonnet *et al.*, 2012) caused by  
84 the pathogen's cell wall degrading enzymes (Liu *et al.*, 2005; Pérez-Donoso *et al.*, 2010;  
85 Lowe-Power *et al.*, 2018). In addition, deposits in the xylem cell walls act as a shield  
86 against pathogen-derived metabolites such as toxins and enzymes, and diminishes water  
87 and nutrient availability for pathogens, thereby impeding their growth (Araujo *et al.*, 2014).  
88 This vascular confinement is an effective strategy commonly found among plants resistant  
89 to vascular wilt pathogens such as *R. solanacearum*, which otherwise spread systemically  
90 once they reach the vasculature, clogging the vessels and causing irreversible damage and  
91 plant death (Potter *et al.*, 2011; Scortichini, 2020; Kashyap *et al.*, 2021;)

92

93 Among the various tomato germplasms, the cultivar Hawaii 7996 (H7996) is the most  
94 effective natural source of resistance against *R. solanacearum* (Nakaho *et al.*, 2004;  
95 Grimault *et al.*, 1994). In this cultivar, resistance to *R. solanacearum* is a complex  
96 polygenic trait. So far, two major (Bwr-12 and Bwr-6) and three minor (Bwr-3, Bwr-4, and  
97 Bwr-8) quantitative trait loci (QTLs) have been identified, although they only account for a  
98 portion of the observed phenotypic variation in resistance (2000; Thoquet *et al.*, 1996;  
99 Mangin *et al.*, 1999; Wang *et al.*, 2013). Our previous study using luminescent and  
100 fluorescent reporter strains of *R. solanacearum* identified four distinct spatiotemporal  
101 bottlenecks through which H7996 is able to limit bacterial spread *in planta* (Planas-  
102 Marquès *et al.*, 2019). In this resistant variety the pathogen encounters severe restriction in:  
103 i) root colonization, ii) vertical movement from roots to shoots, iii) circular invasion of the  
104 vascular bundle and iv) radial apoplastic spread from the vessels into the cortex. Vascular  
105 cell wall reinforcements seem to play a key role in confining *R. solanacearum* into the  
106 xylem vascular bundles of resistant tomato H7996. Ultra-microscopic studies in  
107 quantitatively resistant tomato cultivars showed that the pit membranes, as well as xylem  
108 vessel walls and parenchyma cells form a conspicuously thick coating in the form of an  
109 electron dense amorphous layer, as part of the defense response against *R. solanacearum*  
110 (Nakaho *et al.*, 2000; Kim *et al.*, 2016;). Thus, resistant H7996 plants have the ability to

111 effectively compartmentalize *R. solanacearum* into the lumen of xylem vascular bundles.  
112 However, the type of barriers and compounds involved in this interaction remain  
113 understudied.

114

115 Among the polymers constituting vascular coating structures, lignin is the most typically  
116 found, constituting an integral part of the secondary cell wall of the xylem vasculature.  
117 Lignin has been well studied as a common structural defense against vascular wilt  
118 pathogens (Novo *et al.*, 2017; Kashyap *et al.*, 2021). Suberin has also been reported to be  
119 deposited in vascular coatings as a defense response (Kashyap *et al.*, 2021), although the  
120 mechanisms regulating its synthesis, spatio-temporal dynamics and inducibility remain  
121 elusive. Interestingly, root microbiota has been recently shown to have the ability of  
122 shaping suberin deposits in the plant, highlighting its central role in plant-microbe  
123 interactions (Salas-González *et al.*, 2021). Suberin is a poly(acylglycerol)-derived polyester  
124 containing long and very long chain fatty acid compounds and derivatives and also some  
125 aromatics, mainly ferulic acid, which is a hydroxycinnamic acid. Cells that accumulate  
126 suberin also accumulate lignin, whose deposition has been described to precede that of  
127 suberin in phellem cells (Lulai and Corsini, 1998). This lignin is also known as a lignin-like  
128 polymer. The lignin-like polymer consists of hydroxycinnamates and monolignols linked  
129 by C-C and ether bounds (Graça, 2015). The ligno-suberin heteropolymer formed by the  
130 lignin-like polymer and suberin has been also referred to as the poly(aromatic) and  
131 poly(aliphatic) domains of suberin, respectively. Commonly, suberized cell walls also  
132 comprise free fatty acyl derived compounds, known as suberin-associated waxes, and  
133 phenolic soluble compounds, which share biosynthetic pathways with suberin and lignin,  
134 respectively (Bernards, 2002).

135

136 Ferulic acid present in the suberin and lignin-like fractions is proposed to link both  
137 polymers (Graça, 2010) and its continuous production has been demonstrated essential for  
138 suberin deposition (Andersen *et al.*, 2021). Ferulic acid amides, such as feruloyltyramine  
139 and feruloyloctopamine, have been described as structural components of the lignin-like  
140 polymer and in the phenolic soluble fraction of suberizing wound-healing potato tuber  
141 (Negrel *et al.*, 1996; Razem and Bernards, 2002). Ferulic acid amides belong to the

142 Hydroxycinnamic acid amide (HCAA) family, which present antimicrobial activity and are  
143 considered biomarkers during plant-pathogen interactions (Zeiss *et al.*, 2021). However, the  
144 molecular, biochemical and physiological role of HCAAs in plant defense remains to be  
145 elucidated (Macoy *et al.*, 2015). Besides their direct antimicrobial activity as soluble  
146 phenols, HCAAs have also been proposed to cross-link to cell wall structural polymers  
147 during infection, potentially contributing towards the formation of a phenolic barrier that  
148 can make the cell wall resilient to pathogenic degradation (Zeiss *et al.*, 2021).

149

150 In the present study, we conducted a detailed investigation of the inducibility, structure and  
151 composition of the xylem vascular wall reinforcements that restrict *R. solanacearum*  
152 colonization in resistant tomato. Using a combination of histological and live-imaging  
153 techniques, together with spectroscopy, gene expression analysis and gene activation we  
154 provide important new insights into the pathogen-induced formation of vascular coatings.  
155 In particular, we show that ligno-suberin vascular coating and tyramine-derived HCAAs  
156 contribute to restriction of *R. solanacearum* in resistant tomato. In addition, we demonstrate  
157 that genes in the ligno-suberin-associated pathways can be explored to engineer resistance  
158 against *R. solanacearum* into commercial susceptible varieties of tomato.

159

160

161 **Results:**

162

163 **Resistant H7996 tomato restricts *R. solanacearum* colonization and induces a vascular**  
164 **coating response involving wall-bound phenolics**

165

166 In order to understand the mechanisms underscoring restriction of *R. solanacearum* spread  
167 in resistant tomato varieties we used the resistant variety Hawaii 7996 (H7996) and  
168 compared it to the susceptible cultivar Marmande. In our assay conditions, most Marmande  
169 plants were wilted 10 days after inoculation with *R. solanacearum* GMI1000, while H7996  
170 plants remained largely asymptomatic (Fig. 1A, S1A and (Planas-Marquès *et al.*, 2019).  
171 Accordingly, bacterial loads in the taproot were drastically reduced in H7996 compared to  
172 Marmande, confirming the remarkable bacterial restriction ability of this cultivar (Fig. S1B  
173 and (Planas-Marquès *et al.*, 2019)).

174

175 To identify defense-associated anatomical and/or physico-chemical modifications in H7996  
176 after infection with *R. solanacearum* compared to Marmande we first analyzed ultraviolet  
177 (UV) autofluorescence of transverse taproot cross-sections, indicative of phenolic  
178 compounds (Donaldson, 2020). To focus on cell wall-deposited phenolic compounds,  
179 soluble phenolic compounds were removed with ethanol prior to observation as reported  
180 (Pouzoulet *et al.*, 2013; Araujo *et al.*, 2014). Infection with *R. solanacearum* induced a  
181 strong UV signal emitted from the walls of the vessels, and also from surrounding xylem  
182 parenchyma cells and tracheids in resistant H7996 (Fig. 1B). This enhanced  
183 autofluorescence was not observed in the susceptible variety Marmande nor in mock-  
184 treated samples (Fig. 1B).

185

186 **Spectroscopic analysis reveals *R. solanacearum*-induced deposition of suberin and**  
187 **accumulation of tyramine-derived amides in roots of resistant H7996 tomato and**  
188 **lignin structural modifications in roots of susceptible Marmande tomato**

189

190 In order to decipher the composition of the cell wall-deposited compounds we used two  
191 complementary spectroscopic techniques: Fourier transform infrared spectroscopy (FT-IR)

192 and two-dimensional heteronuclear single quantum correlation nuclear magnetic resonance  
193 (2D-HSQ NMR). Whereas FT-IR allows rapid analysis of the metabolic composition of a  
194 tissue at a given time (Türker-Kaya and Huck, 2017), 2D-HSQC NMR is considered one of  
195 the most powerful tools for plant cell wall structural analysis providing information on the  
196 composition and linkages in lignin/suberin polymers (Ralph and Landucci, 2010; Correia *et*  
197 *al.*, 2020).

198  
199 FT-IR confirmed that the most characteristic spectral features visible on the spectra of  
200 taproot vascular and paravascular tissue were connected with the presence of functional  
201 chemical groups of phenolic compounds (Fig. S2A and C). Calculation of relative  
202 absorbance ratios of the most diagnostic peaks showed a specific increase in phenolic  
203 compounds in resistant H7996 after infection with *R. solanacearum*, as can be seen by the  
204 higher phenolic –OH stretching value ( $\approx 3300\text{ cm}^{-1}$ ) when comparing to its mock control or  
205 susceptible Marmande plants (Fig. S2B).

206  
207 To deepen our understanding of the compounds involved, 2D-HSQC spectra of infected or  
208 mock-treated taproots of H7996 and Marmande tomato plants were obtained and the main  
209 lignin and suberin substructures identified are shown in Fig. 2, while the chemical shifts of  
210 the assigned cross-signals are detailed in Table S1. Importantly, the aliphatic region of the  
211 2D-HSQC spectra revealed that H7996 infected plants were more enriched in poly-aliphatic  
212 structures characteristic of suberin (magenta-colored signals), compared to its mock control  
213 (Fig 2A). Related to this, an olefinic cross-signal of unsaturated fatty acid structures (UF,  
214  $\delta_C/\delta_H$  129.4/5.31), typical of suberin, was also found to be increased in the HSQC spectrum  
215 of the infected H7996 tomato. A rough estimate based on the integration of lignin and  
216 suberin HSQC signals, revealed that the suberin/lignin ratio in *R. solanacearum*-infected  
217 H7996 plants was doubled compared to mock-treated plants, evidencing an increase in  
218 suberin deposition as a consequence of the bacterial infection. Interestingly, signals  
219 compatible with feruloylamides (FAM<sub>7</sub>;  $\delta_C/\delta_H$  138.6/7.31) and with tyramine-derived  
220 amides (Ty in orange;  $\delta_C/\delta_H$  129.3/6.92, 114.8/6.64, 40.5/3.29 and 34.2/2.62) were  
221 exclusively found in the spectrum of infected H7996 plants, suggesting the presence of  
222 feruloyltyramine exclusively in these samples (Fig. 2A). Since tyramines have been found



223 as structural components co-occurring with suberin (Bernards *et al.*, 1995; Bernards and  
224 Lewis, 1998), which generates physically and chemically resistant barriers (He and Ding,  
225 2020), our results substantiate the hypothesis of suberin as an important defense element  
226 against *R. solanacearum* infection in resistant tomato plants. On the contrary, the 2D-  
227 HSQC spectra of the lignin/suberin fractions isolated from the Marmande variety did not  
228 display notable variations between mock and infected plants in the signals corresponding to  
229 suberin, tyramine-related structures nor feruloylamides (Fig 2A).

230

231 Interestingly, 2D-HSQC NMR spectra also revealed significant structural modifications in  
232 the composition of lignin and the distribution of linkages in tomato plants after infection.  
233 Lignins with lower S/G ratios are more branched (condensed) and recalcitrant towards  
234 pathogen attack (Iiyama *et al.*, 2020). Therefore, lignin in H7996, with an S/G ratio of 1.0  
235 should be, a priori, more resistant than the lignin in Marmande plants (S/G ratio of 1.5).  
236 2D-HSQC analysis revealed that the infection of susceptible Marmande plants resulted in  
237 an increase of the S/G ratio (from 1.5 to 1.8) and a clear reduction of all major lignin  
238 linkages ( $\beta$ -O-4',  $\beta$ -5' and  $\beta$ - $\beta'$ ; reduction in roughly 9%, 43% and 46%, respectively),  
239 evidencing that a lignin depolymerization process took place (Fig. 2A). In contrast, infected  
240 H7996 tomato roots displayed a slight decrease of the S/G ratio (from 1 to 0.8) (Fig. 2A),  
241 and only  $\beta$ -O-4' linkages (the easiest to degrade in the lignin polymer) were significantly  
242 reduced (in roughly 10%), while the  $\beta$ -5' and  $\beta$ - $\beta'$  were not so affected as in the case of  
243 Marmande plants. In this context, the major reduction in lignin linkages observed in  
244 Marmande after infection could explain, at least in part, its higher susceptibility to the  
245 pathogen.

246

247 **Histochemical analysis reveals the formation of structural vascular coatings**  
248 **containing suberin and ferulate/feruloylamide in resistant H7996 tomato roots in**  
249 **response to *R. solanacearum* infection**

250

251 To confirm our spectroscopic data, we histochemically analyzed taproot samples of mock  
252 and infected H7996 and Marmande tomato plants. Observation of Phloroglucinol-HCl  
253 stained sections under brightfield microscopy (Wiesner staining) (Pomar *et al.*, 2002;

254 Pradhan Mitra and Loqué, 2014) , showed that mock and infected H9776 (resistant) as well  
255 as mock Marmande (susceptible) samples showed a red-purple color characteristic of the  
256 reaction of phloroglucinol-HCl in vessels and fibers, indicative of lignin (Fig. 3A). In  
257 contrast, infected Marmande taproot sections exhibited reduced phloroglucinol-HCl  
258 staining, primarily concentrated in the xylem with less staining in the interfascicular fibers,  
259 suggesting a change in composition of xylem lignin upon infection, especially in the cell  
260 walls of fibers (Fig. 3A). This observation is in agreement with the structural changes  
261 specifically detected in the lignin structure of infected Marmande plants by 2D-HSQC  
262 NMR (Fig. 2A), which suggest lignin depolymerization and may partly underscore the high  
263 susceptibility of this tomato variety to *R. solanacearum*.

264  
265 Ultraviolet (UV) illumination of phloroglucinol-HCl-stained samples allows quenching the  
266 autofluorescence from lignin and hence detect residual cell wall autofluorescence, which  
267 has been associated with suberin deposits (Baayen and Elgersma, 1985; Rioux *et al.*, 1998;  
268 Pouzoulet *et al.*, 2013). Under these conditions the increased autofluorescence observed in  
269 the vascular coating regions of infected H7996 tomato plants was not quenched in  
270 phlorogucionol-HCl stained samples (Fig. 3A, B). A more detailed observation revealed  
271 that this not-quenched autofluorescence was localized in specific regions compatible with  
272 (i) intervessel and vessel-parenchyma pit membranes or pit chamber walls and (ii)  
273 parenchyma coatings with fluorescent signals enriched in intracellular spaces (Fig 3C).

274  
275 Since phenolic autofluorescence from walls that cannot be quenched by phloroglucinol-HCl  
276 treatment could be attributed to the suberin polymer (Biggs, 1984; Pouzoulet *et al.*, 2013),  
277 we analyzed whether the pathogen-induced coating of vessels observed in H7996 correlated  
278 also with an increase in ferulates, a major suberin component. We performed KOH  
279 treatment of plant tissues, which specifically shifts the UV fluorescence of  
280 ferulate/feruloylamide to green, allowing its detection (Carnachan and Harris, 2000; Harris  
281 and Trethewey, 2010; Donaldson and Williams, 2018). UV autofluorescence of vascular  
282 coatings in response to *R. solanacearum* infection in resistant H7996 shifted from blue to a  
283 strong green color upon treatment with alkali (1N KOH) (Fig. S3A). This indicated that the  
284 *R. solanacearum*-induced xylem vasculature feruloylation was specific to resistant H7996,

285 as the fainter blue autofluorescence observed in mock-treated resistant H7996 or  
286 susceptible Marmande tissues did not change to green at high pH in either early (Fig. S3A,  
287 B) or late (Fig. S3C) stages of infection.

288

289 To corroborate that the ferulate/feruloylamide accumulation in infected H7996 tomato was  
290 related with vascular suberization, we combined the ferulate-specific UV-alkali treatment  
291 described above with Sudan IV staining, which binds aliphatic components of suberin to  
292 produce a reddish-brown coloration. This revealed suberization in the taproot of *R.*  
293 *solanacearum*-infected H7996 plants, xylem vessel walls as well as the layers of vessels,  
294 parenchyma cells and tracheids in the immediate vicinity (reddish-brown signal from Sudan  
295 IV, Fig. 4). In the periphery of suberized cells, a green signal from UV-alkali was observed  
296 (Fig. 4), which may indicate ferulate/feruloylamide deposition indicative of a preceding  
297 stage towards suberization in this cell layer. In comparison, no positive Sudan IV or UV-  
298 alkali staining was detected in infected Marmande or mock-treated tomato plants. Together,  
299 suberized and feruloylated layers of parenchyma cells, vessels and tracheids might form a  
300 “suberization zone” creating a strong physico-chemical barrier to limit *R. solanacearum*  
301 spread from the colonized xylem vessel lumen.

302

303 ***R. solanacearum* infection activates the biosynthesis of aliphatic suberin precursors**  
304 **and feruloylamide, and aliphatic esterification of ferulic acid in the vasculature of**  
305 **resistant H7996**

306

307 Since a differential accumulation of suberin-compatible compounds was specifically  
308 observed in infected H7996, we surmised that genes related to suberin and feruloylamide  
309 synthesis, as well as ferulic acid esterification to aliphatics may be upregulated in resistant  
310 tomato in response to *R. solanacearum* invasion. To test this hypothesis, we analyzed: i)  
311 expression of genes in the phenylpropanoid and suberin biosynthesis pathways, which  
312 provide the necessary precursors for the ligno-suberin heteropolymer; ii) the feruloyl  
313 transferase FHT (ASFT/HHT in Arabidopsis), which is involved in the formation of  
314 ferulate esters of fatty acyl compounds necessary to form suberin and soluble waxes  
315 (Molina *et al.*, 2009; Gou *et al.*, 2009; Serra *et al.*, 2010); and iii) N-hydroxycinnamoyl

316 transferases (*THT*), which are involved in the synthesis of HCAAs such as  
317 feruloyltyramine, previously found both as part of the lignin-like polymer and in the  
318 soluble phenolic fraction of some suberized tissues (Negrel *et al.*, 1993; Schmidt *et al.*,  
319 1999) .

320

321 Quantitative RT-PCR from taproot xylem vascular tissue of *R. solanacearum*- or mock-  
322 treated H7996 and Marmande plants showed specific upregulation of all genes analyzed  
323 from the suberin biosynthetic pathway in H7996 infected plants compared to the mock  
324 controls (Fig. 5, S4). These included essential suberin biosynthesis genes such as *CYP86A1*  
325 and *CYP86B1* (fatty acid oxidation), *FAR* (primary alcohol generation), *KCSs* (fatty acid  
326 elongases) and *GPAT5* (acylglycerol formation). In addition, feruloyl transferase FHT  
327 (ASFT/HHT in Arabidopsis), was also strongly upregulated in infected H7996 plants (Fig.  
328 5 and S5). Regarding THT, in tomato we identified five putative homologs (Fig S6A), all  
329 induced by infection in the vascular tissue of H7996 (Fig. 5 and S6B). Among them,  
330 *SITHT1-3* showed the strongest upregulation in H7996 after infection, although a slight  
331 upregulation could also be observed in Marmande (Fig. 5 and S6B). In comparison, *R.*  
332 *solanacearum* infection had only a modest effect in genes related to phenylpropanoid  
333 pathway as only upregulation was detected in the first enzyme of the pathway (PAL) (Fig. 5  
334 and S7).

335

336 Together, these data indicate that upregulation of genes involved in the formation of  
337 aliphatic suberin precursors, ferulic acid esterification to aliphatics (FHT) and production of  
338 HCAAs, such as feruloyltyramine (THT), constitute a very specific response of H7996  
339 plants that takes place in the vasculature upon *R. solanacearum* infection. Further, these  
340 data are in agreement with NMR data of infected H7996, which showed a specific increase  
341 in insoluble fatty acid structures typical of suberin as well as the appearance of signals from  
342 structural tyramine-derived amides and feruloylamides (Fig. 2a). The higher expression of  
343 all these genes in H7996 upon infection may also result in the overproduction of soluble  
344 waxes and soluble HCAAs.

345

346 **Overexpression of *SITHTI-3* in a susceptible tomato cultivar confers resistance to *R.***  
347 ***solanacearum***

348

349 Based on our results, we set to determine whether overexpressing genes involved in ferulic  
350 acid esterification to suberin aliphatics and feruloylamide biosynthesis, such as *SIFHT* and  
351 *SITHTI-3*, respectively, would increase resistance against *R. solanacearum* in a susceptible  
352 tomato background. First, we obtained transgenic tomato lines stably overexpressing  
353 *SIFHT* on a susceptible Marmande background (Fig. S8) and analyzed symptom  
354 progression and bacterial colonization. *SIFHT* overexpression lines showed a slight delay in  
355 disease progression (Fig. 6a) and moderately milder symptoms. The taproot and hypocotyl  
356 of *SIFHT* overexpressors displayed a slight reduction in bacterial loads after soil-soak  
357 inoculation in comparison to Wt tomato (Fig. 6b).

358

359 Regarding *SITHTI-3*, the corresponding tomato overexpressing line was readily available  
360 on a Moneymaker background (Campos *et al.*, 2014). This line overaccumulates soluble  
361 HCAA such as feruloyltyramine. Using this *SITHTI-3* overexpressing line and the  
362 corresponding Moneymaker wild type, we performed a variety of *R. solanacearum*  
363 infection assays. As expected, the Moneymaker tomato cultivar showed similar  
364 susceptibility to *R. solanacearum* as Marmande (Fig. 1a, b and Fig. 7a, b). In contrast,  
365 overexpression of *SITHTI-3* resulted in a dramatic increase of resistance against *R.*  
366 *solanacearum*, with disease progressing remarkably slower in this line compared to the Wt  
367 Moneymaker (Fig. 7a, b). Importantly, bacterial loads were significantly lower in the  
368 taproot and hypocotyl of the *SITHTI-3* overexpressor after soil inoculation in comparison  
369 to Wt tomato (Fig. 7c). Similarly, direct leaf inoculation also showed severe bacterial  
370 growth restriction in the *SITHTI-3* overexpressing line (Fig. S9a). Further, we monitored the  
371 colonization patterns of a *R. solanacearum* GFP reporter strain after stem inoculation of the  
372 *SITHTI-3* overexpressing line compared to Wt. In transverse stem cross-sections of 6 dpi  
373 plants, bacteria stayed confined near the inoculation point in the *35S::SITHTI-3* line  
374 whereas they spread unrestrictedly in susceptible wild type stems from the inoculation point  
375 and at least 3 cm up and downwards (Fig. 7d and S9b). Quantification of the GFP signal  
376 confirmed that bacterial growth was drastically reduced in *SITHTI-3* overexpressing plants

377 in comparison to Wt (Fig. 7e). Together, our data clearly show that *StTHT1-3* ectopic  
378 expression provides a very effective resistance mechanism against *R. solanacearum* -  
379 potentially mediated by accumulation of elevated amounts of HCAAs such as  
380 feruloyltyramine-, which drastically restricts vascular colonization, preventing bacterial  
381 spread and blocking the onset of disease.

382

383

384

385 **Discussion**

386 **Ligno-suberin deposits in vascular cell walls and feruloyltyramine accumulation acts**  
387 **as a resistance mechanism restricting *R. solanacearum* colonization in resistant tomato**

388

389 The root xylem vasculature is one of the first sites of multiplication of *R. solanacearum*  
390 inside the host (Vasse *et al.*, 1995; Álvarez *et al.*, 2010; Digonnet *et al.*, 2012).  
391 Colonization of the xylem vasculature is critical, as in this particular tissue the pathogen  
392 multiplies and moves vertically to the stem alongside the xylem fluid. In susceptible hosts,  
393 the pathogen also spreads horizontally from colonized vessels to the healthy neighboring  
394 tissues, including vessels and surrounding parenchyma cells (Nakaho *et al.*, 2000). To  
395 facilitate this process *R. solanacearum* secretes an array of cell wall degrading enzymes  
396 (Liu *et al.*, 2005; Lowe-Power *et al.*, 2018) In parallel, plant cell walls also act as dynamic  
397 barriers against pathogens, acting as first line of defense by undergoing remodeling or  
398 strengthening upon pathogen recognition (Underwood, 2012). However, the precise role of  
399 cell walls in defense responses is far from being understood and has been mostly studied in  
400 the leaves.

401

402 In our study, resistant tomato (H7996) was observed to react aggressively to *R.*  
403 *solanacearum* infection by reinforcing the walls of vessels and the surrounding parenchyma  
404 cells with phenolic deposits, which can be observed as UV autofluorescence (Fig. 1). An  
405 increase in autofluorescence had been previously reported in another resistant tomato  
406 variety, LS-89, although its composition was not precisely defined (Ishihara *et al.*, 2012).  
407 Histochemical analysis of vascular coatings in resistant tomato upon *R. solanacearum*  
408 infection showed that the strong UV autofluorescence emitted from xylem vessel walls and  
409 the surrounding parenchyma cells observed in resistant H7996 against *R. solanacearum*  
410 (Fig. 1) could not be quenched by phloroglucinol-HCl, suggesting the presence of suberin  
411 deposits (Fig. 3) (Biggs, 1984; Rittinger *et al.*, 1986; Pouzoulet *et al.*, 2013). Detailed  
412 observation revealed that this suberin-associated autofluorescence was prominent in vessel-  
413 parenchyma and intervessel pit membranes and/or chambers and in parenchyma  
414 intercellular spaces (Fig. 3C). In line with this, previous reports using TEM showed  
415 thickening of the pit membranes accumulating electron dense material in tomato plants

416 resistant to *R. solanacearum* (Nahako *et al.*, 2000 and 2004). The suberin nature of these  
417 coatings was further supported by the positive Sudan IV staining of vessels and  
418 surrounding parenchyma cells of H7996 roots upon infection (Fig. 4). These results are in  
419 agreement with the previous suberin coatings detected in tomato plants resistant to  
420 *Verticillium albo-atrum*. Upon infection or after treatment with the suberin hormone  
421 inducer abscisic acid, suberin was chemically detected in petioles (Robb *et al.*, 1991), and  
422 suberin as well as lignin coatings, were both deposited in intercellular spaces between  
423 parenchyma cells adjoining a xylem vessel or infusing and occluding pit membranes  
424 coatings (Robb *et al.*, 1991; Street *et al.*, 1996). Besides, inhibition of the phenylpropanoid  
425 pathway by blocking PAL enzyme inhibited the formation of both lignin and suberin  
426 coatings (Street *et al.*, 1996), in agreement with the ferulic acid requirement to correctly  
427 deposit suberin (Andersen *et al.*, 2021) and reinforcing our observations of the presence of  
428 a ferulate/feruloylamide-derived polymer detected in H7996 *R. solanacearum* KOH-treated  
429 samples observed under UV light (Fig. 4). In line with this, 2D-HSQC NMR data of  
430 resistant H7996 tomato vascular tissue revealed the presence of tyramine-derived amides  
431 and feruloylamides incorporated into the cell wall and also an enrichment in poly-aliphatic  
432 structures characteristic of suberin (Fig. 2). An increase in feruloyltyramine has been  
433 detected associated with suberization (Graça, 2015; Legay *et al.*, 2016; Figueiredo *et al.*,  
434 2020) and is compatible with the lignin coatings seen in conjunction with suberin coatings  
435 in *V. albo-atrum* infected tomatoes (Robb *et al.*, 1991).

436

437 Interestingly, in the periphery of the suberized (Sudan IV-stained) layers surrounding the  
438 vasculature after infection in resistant tomato we could observe cells with intense  
439 accumulation of phenolics where Sudan IV did not bind. In these peripheral areas, the  
440 strong blue-to-green color conversion upon alkali treatment (Carnachan and Harris, 2000;  
441 Harris and Trethewey, 2010) revealed the presence of wall-bound ferulic acid, potentially  
442 derived from ester hydrolysis of suberin aliphatics (ferulates). However, since amides can  
443 also be hydrolyzed as esters in basic solution (Robert and Caserio, 1977), ferulic acid could  
444 alternatively be derived from wall-bound feruloylamides, such as the feruloyltyramines  
445 shown to accumulate in resistant tomato by 2D-HSQC NMR (Figure 2A) (Fig. 4, S3),  
446 which may act as further reinforcements against the pathogen. Importantly, in tomato



447 vasculature infected with *V. albo-atrum* lignin deposits were detected preceding those of  
448 suberin (Robb *et al.*, 1991).

449

450 Beyond histochemistry and spectroscopic signature detections of suberin, lignin and ether  
451 linked feruloyltyramine or related amides, further evidence supporting the nature of these  
452 ligno-suberin coatings as responsible of the resistance observed in H7796 to *R.*  
453 *solanacearum* was unequivocally provided transcriptionally using transcriptional gene  
454 markers. Tissues undergoing suberization have to go through a complex genetic and  
455 metabolic reprogramming involving a network of metabolic pathways, in order to produce  
456 the precursors of the polymer and subsequently their polymerization into the matrix  
457 (Lashbrooke *et al.*, 2016). Transcriptional reprogramming associated to suberin  
458 biosynthesis was clearly observed in the root vascular tissue of resistant H7996 tomato  
459 upon infection with *R. solanacearum*. Specific upregulation in the xylem of resistant  
460 tomato of genes that are specific and key for suberin monomer biosynthesis was observed,  
461 including KCS elongases, FAR reductases, CYP86  $\omega$ -hydroxylases, GPAT5 acyltransferase  
462 and FHT/ASFT feruloyltransferase (Fig. 5). In contrast, only moderate differences were  
463 found in transcripts of phenylpropanoid pathway genes. Interestingly, PAL, which showed  
464 modest upregulation in resistant H7996, had been previously defined as a rate-limiting  
465 enzyme of phenylpropanoid pathway (Faragher and Brohier, 1984; Howles *et al.*, 1996).  
466 Considering this, the observed upregulation could provide more tyramine and feruloyl-  
467 CoA, which together with the upregulation of *THT* would be in agreement with the  
468 increased presence of feruloyltyramine detected by 2D-HSQC NMR (Figure 2A). The  
469 slight upregulation in resistant H7996 and downregulation in susceptible Marmande of  
470 other phenylpropanoid pathway genes upon infection corroborates previous reports  
471 (Ishihara *et al.*, 2012).

472

473 2D-HSQC NMR also revealed differences in the composition and structure of lignin  
474 between resistant and susceptible tomato cultivars after infection. The amounts and the  
475 level of lignin of a particular tissue affect wall strength, degradability and pathogen  
476 resistance (Mnich *et al.*, 2020) and phenolic reinforcements in the xylem vasculature can  
477 act as a shield against pathogen-derived metabolites such as toxins and enzymes, and make

478 water and nutrients inaccessible for pathogens, thereby impeding their growth (Araujo *et*  
479 *al.*, 2014). Susceptible Marmande, which do not develop ligno-suberized coatings,  
480 presented predominance of S-type lignin, while the lower S/G ratio observed for the  
481 infected H7996 resistant cultivar, revealed a higher presence of a G-type lignin, which has  
482 also been related with the lignin accompanying suberized tissues (Graça, 2015). S-lignin is  
483 relatively unbranched and has a lower condensation degree than G lignin, which is more  
484 difficult to hydrolyze because it contains a higher proportion of condensed carbon-carbon  
485 linkages (Novaes *et al.*, 2010). The observed lignin structural differences between varieties  
486 after infection were corroborated by phloroglucinol-HCl staining observed under bright  
487 field: in H7996 the G lignin together with the higher cross-linking resulted in strong red-  
488 purple positive staining, while S lignin, less cross-linking of cell wall polymers and a  
489 certain degree of degradation of the vascular root tissue resulted in less reaction to the stain  
490 (Kutscha and Gray, 1972; Hao *et al.*, 2014) (Fig. 2 and 3). Together, these data indicate that  
491 i) under basal conditions the two tomato varieties used in this study display differences in  
492 the composition and structure of lignin and ii) *R. solanacearum* infection affects very  
493 differently the lignin fraction in the two varieties: resistant H7996 tomato shows only a  
494 slight decrease in the S/G ratio that may be linked to an accumulation of the ligno-suberin  
495 heteropolymer, while susceptible Marmande undergoes pronounced depolymerization that  
496 correlates with a decrease in phloroglucinol-HCl staining (Fig. 3A). Although the *R.*  
497 *solanacearum* has not been shown to be able to specifically depolymerize lignin, the  
498 pathogen secretes enzymes that can degrade cell wall polysaccharides and could participate  
499 in the observed Marmande stem collapse phenotype (Fig. 1A). In resistant H7996, on the  
500 other hand, vascular suberin-containing coatings would allow to create a hydrophobic  
501 barrier to prevent enzymes from accessing the cell wall substrates and at the same time  
502 create reinforcements, leading to resistance to the pathogen. The fact that these  
503 reinforcements are rich in tyramine or feruloyltyramine as shown by HSQC-NMR, may  
504 further reinforce the structural barrier formed in H7996 in response to *R. solanacearum*,  
505 providing rigidity, hampering cell wall digestibility by the pathogen's hydrolytic enzymes  
506 (Macoy *et al.*, 2015; Zeiss *et al.*, 2020).  
507

508 Overall, our data indicate that vascular coating with wall-bound ligno-suberized  
509 compounds may restrict horizontal spread of the bacterium at early stages of bacterial  
510 colonization (starting at  $\sim 10^5$  CFU g<sup>-1</sup> taproot tissue), before the plant shows any visible  
511 wilting symptom (Fig 1). In comparison, susceptible tomato (Marmande) is either not able  
512 to induce such vascular coating upon *R. solanacearum* infection or induces a very weak and  
513 late response (Figs. 1, 3), potentially predisposing its vascular walls to disruption by the  
514 pathogen's cell wall degrading enzymes. In absence of ligno-suberin reinforcements, the  
515 bacterium multiplies and colonizes abundantly moving out from vessel lumen into  
516 surrounding parenchyma cells and apoplastic spaces. Based on the above observations, we  
517 propose a model whereby resistant H7996 tomato undergoes vascular suberization upon *R.*  
518 *solanacearum* infection as follows (Fig. 8). When reaching the xylem vessels of resistant  
519 H7996, *R. solanacearum* multiplies and tries to invade the surrounding healthy vessels and  
520 parenchyma cells by degradation of the xylem pit membranes and walls. Resistant tomato  
521 plants respond to vascular invasion by the pathogen depositing feruloyltyramine and other  
522 HCAA-tyramine derived compounds, and suberin. These deposits would block the pit  
523 membrane access and serve as coatings of the vessel walls and parenchyma cells present in  
524 the immediate vicinity of colonized vessels, compartmentalizing the infection. These ligno-  
525 suberized layers of parenchyma cells, vessels and tracheids together form a “zone of ligno-  
526 suberization” creating a strong physico-chemical barrier to limit *R. solanacearum* spread  
527 from colonized xylem vessel lumen.

528

### 529 **Engineering tomato resistance against *R. solanacearum* by inducing the tyramine-** 530 **HCAA pathway**

531

532 Considering the observed accumulation of lignosuberin and cell wall-linked  
533 feruloyltyramine in resistant H7996 tomato in response to *R. solanacearum* infection, we  
534 sought to understand the implications of overexpressing genes involved in the synthesis of  
535 these compounds in susceptible tomato cultivars upon *R. solanacearum* infection. We  
536 focused on FHT and THT and because their corresponding transcripts are upregulated in  
537 the xylem vasculature of resistant tomato upon *R. solanacearum* infection (Fig. 5) and they

538 are the enzymes related with the synthesis of suberin ferulates and ether linked  
539 feruloyltyramine, respectively.

540

541 *SIFHT* overexpression had a small effect on the responses of susceptible tomato against *R.*  
542 *solanacearum*, showing a slight delay in wilting symptoms together with a slight decrease  
543 of bacterial loads in the plant (Fig. 6). In resistant H7996 tomato, *SIFHT* was highly  
544 induced in and around the vasculature upon *R. solanacearum* expression, and this was  
545 accompanied by a strong upregulation in fatty acid biosynthesis genes (Fig. 5, S5), which  
546 provide key precursors to form suberin. The fact that increasing the levels of FHT in  
547 Marmande does only result in a marginal increase in resistance might be linked to a  
548 shortfall of aliphatic precursors in this variety (Fig. 5), which constrain a subsequent  
549 increase in suberin synthesis.

550

551 In contrast, transgenic tomato overexpressing *SITHT1-3* in a susceptible MoneyMaker  
552 background was highly resistant to *R. solanacearum*. Wilting symptoms and *in planta*  
553 bacterial loads were drastically reduced and colonization was dramatically restricted (Fig.  
554 7). Importantly, this transgenic line was previously shown to accumulate elevated amounts  
555 of soluble HCAAs such as feruloyltyramine upon infection with the bacteria *Pseudomonas*  
556 *syringae* pv. *tomato* (*Pto*) (Campos *et al.*, 2014). Disease susceptibility towards *Pto* was  
557 slightly reduced in the *SITHT1-3* overexpressing lines (Campos *et al.*, 2014). The fact that  
558 overexpressing *SITHT1-3* in tomato confers strong resistance against *R. solanacearum*, but  
559 only a marginal increase in resistance against *Pto* indicates that enhanced production of  
560 tyramine-derived HCAAs constitute an important defense strategy against vascular  
561 pathogens, while for foliar pathogens other mechanisms are in place. There are several  
562 evidences that feruloyltyramine exhibit antimicrobial activity (Fattorusso *et al.*, 1999; Novo  
563 *et al.*, 2017) and that they can be involved in plant priming or an adaptive strategy where  
564 plants are in a physiological state with improved defensive capacity (Zeiss *et al.*, 2021).  
565 These tyramine-derived HCAAs overproduced in *SITHT1-3* overexpressing tomato lines  
566 may interfere with *R. solanacearum* colonization by i) becoming incorporated into the  
567 vascular and perivascular cell walls, providing a stronger cross-linking and restricting the  
568 movement of the pathogen inside the plant and/or ii) remaining soluble and acting as direct

569 antimicrobial agents against the pathogen. The fact that *R. solanacearum* possesses a  
570 hydroxycinnamic acid degradation pathway, and mutants that cannot degrade  
571 hydroxycinnamic acids are less virulent on tomato (Zhang *et al.*, 2019) clearly underscores  
572 the importance of HCAAs in the arms race taking place in this pathosystem.

573

574 In conclusion, we have provided evidence of the formation of a “ligno-suberization zone”  
575 enriched in ether linked feruloyltyramine and possibly related amides as an effective  
576 strategy to confine *R. solanacearum* into infected vessels of resistant tomato plants,  
577 preventing horizontal spread of the pathogen into healthy tissues and delaying disease  
578 symptoms. Resistance against *R. solanacearum* can be attained in susceptible tomato  
579 background by stably overexpressing *THT*, potentially contributing to the formation of a  
580 lignin physico-chemical barrier and/or through a direct anti-microbial effect. Still, many  
581 questions remain to be answered. In the future, it will be interesting to investigate the  
582 contribution of HCAAs and suberin to resistance against the pathogen, the mechanisms  
583 whereby *R. solanacearum* perception leads to the formation of a ligno-suberin coatings  
584 around the vasculature in resistant tomato varieties. Increasing the spatio-temporal  
585 resolution of the tomato-*R. solanacearum* interaction will be instrumental to reach a deeper  
586 insight into structural resistance mechanisms. Also, since vascular confinement has been  
587 reported in different plant species as a means of resistance against various vascular wilt  
588 pathogens (De Ascensao and Dubery, 2000; Martín *et al.*, 2008; Xu *et al.*, 2011; Sabella *et*  
589 *al.*, 2018), the level of conservation of vascular ligno-suberin deposition as a constituent of  
590 vascular coatings and part of a resistance mechanism remains to be determined.

591

## 592 **Materials and Methods**

### 593 **Plant materials and growth conditions**

594 The tomato (*Solanum lycopersicum*) varieties used in this study were the susceptible  
595 commercial variety Marmande and the quantitatively resistant public open-pollinated  
596 breeding line Hawaii 7996. We also used the tomato variety Moneymaker wild-type and  
597 *35s::THT 1-3*, generated by Campos *et al.*, (2014). Seeds were germinated and plants were  
598 grown in pots consisting of soil (Substrate 2, Klasmann- Deilmann GmbH) mixed with  
599 perlite and vermiculite (30:1:1) in controlled growth chambers at 60% humidity and 12 h

600 day/night with light intensity of 120–150  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Temperature was set at 27°C when  
601 using LED lighting and at 25°C when using fluorescent lighting.

602

### 603 ***Ralstonia solanacearum* strains and growth conditions**

604 All assays in tomato were performed using *R. solanacearum* GMI1000 strain (Phylotype I,  
605 race 1 biovar 3). Luminescent and fluorescent reporter strains of *R. solanacearum*  
606 GMI1000 were used in the study containing constructs *PpsbA::LuxCDABE* and  
607 *PpsbA::GFPuv*, respectively (Cruz *et al.*, 2014; Planas-Marquès *et al.*, 2019).

608

### 609 **DNA constructs**

610 For generation *35S::FHT-HA* construct the *FHT* (Solyc03g097500) coding sequence was  
611 amplified from tomato H7996 cDNA using the forward primer (part7FHTF1), having a  
612 flanking *SmaI* restriction enzyme digestion site at 5' end and reverse primer  
613 (part7FHTHAR1), including the sequence of hemagglutinin (HA) epitope tag and a *BamHI*  
614 restriction enzyme digestion site at the 5' end. The amplified product was cloned into the  
615 pJET1.2/blunt cloning vector using CloneJet PCR cloning kit (ThermoFisher) and then  
616 digested by *SmaI* and *BamHI*. The digested products were purified using NZYGelpure  
617 (Nzytech) followed by ligation into the pART7 and later to pART27 vector (Gleave, 1992).

618

### 619 **Stable transformation of tomato**

620 *35S::FHT-HA* were transformed into Marmande. For this, the construct was transformed  
621 into *Agrobacterium tumefaciens* strain C58C1. *A. tumefaciens* was used for co-culture with  
622 tomato cotyledons. Explant preparation, selection, and regeneration followed the methods  
623 described by (Mazier *et al.*, 2011). Transformants were selected on kanamycin-containing  
624 medium. Accumulation of FHT-HA protein was assayed by immunoblot with a monoclonal  
625 HA antibody (GenScript).

626

### 627 **Bacterial inoculation in plants**

628 Four to five week-old tomato plants were inoculated through roots with *R. solanacearum*  
629 using the soil drenching method. For this, roots were wounded by making four holes in the  
630 corners of the pot with a 1 ml pipette tip and inoculated with a to  $1 \times 10^7$  CFU  $\text{ml}^{-1}$  ( $\text{OD}_{600} =$

631 0.01) suspension of bacteria (Planas-Marquès *et al.*, 2018). Inoculated plants were kept in a  
632 growth chamber at 27°C. For tomato leaf infiltration, plants were vacuum-infiltrated by  
633 submerging the whole aerial part in a  $\sim 10^5$  CFU ml<sup>-1</sup> (OD<sub>600</sub> = 0.0001) *R. solanacearum*  
634 suspension as described in Planas-Marquès *et al.*, (2018). For inoculation directly onto the  
635 stem vasculature, 10 µl (5 µl at a time) of  $10^5$  CFU ml<sup>-1</sup> (OD<sub>600</sub> = 0.0001) *R. solanacearum*  
636 suspension was placed at the node of the petiole and pin-inoculated using a sterile 0.3×13  
637 mm needle (30G×½", BD Microlance, Becton Dickinson).

638

### 639 ***R. solanacearum* pathogenicity assays and quantification of bacterial growth *in planta***

640 Infected plants were scored for wilting symptoms using a scale from 0 to 4: 0=healthy plant  
641 with no wilt, 1=25%, 2=50%, 3=75%, and 4=100% of the canopy wilted as described by  
642 Planas-Marquès *et al.*, (2019). The relative light units per second (RLU·s<sup>-1</sup>) readings were  
643 converted to CFU·g<sup>-1</sup> tissue as described in Planas-Marquès *et al.*, (2019). For bacterial  
644 colonization assays using GFP reporter strain, transverse stem cross-sections were made at  
645 the inoculation point as well as at a distance of 0.5 cm, 1 cm, 2 cm and 3 cm in both upward  
646 and downward direction, using a sterile razor blade. The sections were photographed using  
647 an Olympus SZX16 stereomicroscope with a UV fluorescent lamp (BP330-385 BA420  
648 filter) and equipped with a DP71 camera system (Olympus). Quantification of mean green  
649 fluorescence from xylem vascular ring and pith parenchyma was done using ImageJ  
650 software (Planas-Marquès *et al.*, 2019). For leaf *in planta* multiplication assays, 3 leaf discs  
651 of 0.8 cm<sup>2</sup> size were homogenized in 200 µl of sterile distilled water. CFU cm<sup>-2</sup> leaf tissue  
652 were calculated after dilution plating of samples with appropriate selection antibiotics and  
653 CFU counting 24 hours later.

654

### 655 **Histological methods**

656 Taproots of *R. solanacearum*-soil drench inoculated or water-treated plants were used for  
657 obtaining thin transverse cross-sections with a sterile razor blade. Inoculated plants were  
658 either sectioned at 9 dpi or when bacterial colonization level reached  $10^5$  CFU g<sup>-1</sup> taproot  
659 tissue, as indicated in the figure legend where only H7996 sections showed a localized  
660 browning at one xylem pole indicative of infection and defense reactions at xylem. Sections  
661 were kept in 70 % ethanol at room temperature for 5-7 days and examined using

662 fluorescence microscopy using a Leica DM6B-Z microscope under UV illumination (340-  
663 380 nm excitation and 410-450 nm barrier filters). Autofluorescence emitted from phenolic  
664 deposits was recorded using a Leica-DFC9000GT-VSC07341 camera and the signal was  
665 pseudo-colored green.

666

667 Sections were also stained with a Phloroglucinol-HCl solution (100 mg phloroglucinol in 8  
668 ml of ethanol 95% and 8 ml of hydrochloric acid 37 %) for the detection of lignin and  
669 observed under bright field (Pomar *et al.*, 2004). Photographs were taken with a DP71  
670 Olympus color digital camera. Since Phloroglucinol binds to lignin and quenches the  
671 autofluorescence emitted from it (Martín *et al.*, 2005), the cross-sections were then  
672 observed under UV microscopy to detect the remaining autofluorescence which would  
673 correspond to non-lignin phenolic sources such as suberin (Pouzoulet *et al.*, 2013). A  
674 Leica-DM6B-Z microscope (340-380 nm excitation and 410-450 nm barrier filters) was  
675 used. Autofluorescence was recorded using a Leica-DFC9000GT-VSC07341 digital  
676 camera and the signal was pseudo-colored green. Detailed observations (Fig. 3c) were done  
677 using a Olympus AH2 Vanox-T microscope, exciting at 330-380 nm and collecting  
678 emission wavelengths from 420 nm. Images were recorded using the Olympus XC-50 color  
679 digital camera.

680 Auto-fluorescence from ferulic acid bound to the cell wall shows a pH-dependent blue to  
681 green color conversion (Harris and Trethewey, 2010; Carnachan and Harris, 2000;  
682 Donaldson and Williams, 2018). Autofluorescence in the xylem vascular tissue was  
683 visualized by mounting cross-sections in 70 % ethanol (neutral pH) and illuminating them  
684 with UV using a Leica DM6B-Z microscope to observe blue auto-fluorescence (340-380  
685 nm excitation and 410-450 nm barrier filters). Images were recorded using a Leica MC190-  
686 HD-0518131623 digital camera. These same sections were subsequently mounted in 1N  
687 KOH (pH above 10) to observe green auto-fluorescence from ferulic acid using the same  
688 settings.

689

690 To visualize suberin aliphatics, sections were treated with 5 % Sudan IV, dissolved in 70 %  
691 ethanol and illuminated with UV light to produce the typical reddish-brown coloration.  
692 These sections were subsequently treated with 1N KOH to detect ferulic acid as described



693 in the previous paragraph. For both ferulic acid and suberin, the HC PL APO or HC PL  
694 FLUOTAR objectives of the Leica DM6B-Z microscope were used and images were  
695 captured using a Leica MC190-HD-0518131623 color digital camera.

696

697 The UV auto-fluorescence signal from xylem vessel walls and surrounding layer of  
698 parenchyma cells, tracheids was measured using the LAS X Leica software. Change in  
699 ferulate accumulation was quantified from mean fluorescence in the green channels using  
700 ImageJ software by selecting area of xylem vessel walls and surrounding layer of  
701 parenchyma cells, tracheids showing autofluorescence.

702

### 703 **FT-IR**

704 Dried taproot cross-sections of H7996 and Marmande plants, water-treated or *R.*  
705 *solanacearum*-inoculated by soil soak and containing bacteria  $10^5$  CFU g<sup>-1</sup> taproot tissue  
706 were analyzed using a FT-IR spectrophotometer Jasco 4700 with ATR accessory on the  
707 range of 300-4000 cm<sup>-1</sup>. The area analyzed was adjacent to the vasculature. All spectra  
708 were smoothed to minimize noise and baseline corrected, and the peak due to  
709 atmospheric CO<sub>2</sub> at 2300 cm<sup>-1</sup> was eliminated for clarity using OriginPro software.  
710 Assignment of vibration bands allowed peak identification (Lopes *et al.*, 2000; Dorado *et*  
711 *al.*, 2001; Martín *et al.*, 2005; Lahlali *et al.*, 2017), Relative absorbance ratios of peaks of  
712 significant importance were calculated by using the absorbance at 1236 cm<sup>-1</sup> as a reference.

713

### 714 **2D-NMR**

715 The samples of a pool of 15 tomato plant tap roots, water treated or having a bacterial load  
716 of  $10^5$  CFU.g<sup>-1</sup> were milled and extracted sequentially with water (3 x 30 mL), 80% ethanol  
717 (3 x 30 mL), and with acetone (2 x 40 mL), by sonicating in an ultrasonic bath during 30  
718 min each time, centrifuging (9000 rpm, 25 min) and eliminating the supernatant. Then,  
719 lignin/suberin fraction was enzymatically isolated by hydrolyzing the carbohydrates  
720 fraction with Cellulysin (Calbiochem), as previously described (Rico *et al.* 2014).  
721 Approximately 20 mg of enzymatic lignin/suberin (ELS) preparation was dissolved in 0.6  
722 mL of DMSO-*d*<sub>6</sub>. Heteronuclear single quantum coherence (HSQC) spectra were acquired  
723 on a Bruker AVANCE III 500 MHz spectrometer equipped with a 5 mm TCI cryoprobe,

724 using the experimental conditions previously described (Rico *et al.*, 2014). HSQC cross-  
725 signals were assigned and quantified as described elsewhere (Rencoret *et al.*, 2018; del Río  
726 *et al.*, 2018; Mahmoud *et al.*, 2020) . In the aromatic region, the correlation signals of G<sub>2</sub>  
727 and S<sub>2,6</sub> were used to estimate the content of the respective G- and S-lignin units. The  
728 C<sub>α</sub>/H<sub>α</sub> signals of the β-O-4' ethers (A<sub>α</sub>), phenylcoumarans (B<sub>α</sub>), and resinols (C<sub>α</sub>) in the  
729 linkages region were used to estimate their relative abundances, whereas the C<sub>γ</sub>/H<sub>γ</sub> signal  
730 was used in the case of cinnamyl alcohol end-units (I<sub>γ</sub>).

731

### 732 **RNA extraction, cDNA synthesis and quantitative RT-PCR analysis**

733 Tomato H7996 and Marmande plants were water-treated or inoculated with *R.*  
734 *solanacearum* by soil soak. Plants with a taproot inoculum of 10<sup>5</sup> CFU g<sup>-1</sup> were selected  
735 for RNA extraction. With a sharp razor blade, taproot sections of ~ 0.5 mm thickness were  
736 obtained and the xylem vascular tissues (vascular bundles and surrounding parenchyma  
737 cells) were collected and kept in liquid nitrogen. Each sample comprised taproot xylem  
738 tissues of 6 plants. RNA was extracted using the Maxwell RSC Plant RNA Kit (Promega)  
739 according to the manufacturer's recommendations. Extracted RNA was treated with  
740 RNase-free DNase provided in the kit. cDNA was synthesized from 2 µg RNA using High  
741 Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA). For each reaction  
742 2.5 µl of cDNA (1:20 dilution), 1 µl forward and reverse primer mix (10 µM/µl), 5 µl  
743 SYBR Green PCR master mix (Roche) and ultrapure water up to 10 µl was used and  
744 analyzed using the LightCycler 480 System (Roche). The amplification program was  
745 performed as follows: 10 min at 95°C, followed by 45 cycles of 95°C for 10 sec, 60°C for  
746 30 sec and 72°C for 30 sec. The Elongation Factor 1 alpha housekeeping gene (*eEF1 α*,  
747 *Solyc06g005060*) was used as a reference. All reactions were run in triplicate for each  
748 biological replicates. Melting curves and relative quantification of target genes were  
749 determined using the software LightCycler V1.5 (Roche). The level of expression relative  
750 to the reference gene was calculated using the formula  $2^{-\Delta CT}$ , where  $\Delta CT = (CT \text{ RNA target}$   
751  $- CT \text{ reference RNA})$ .

752

### 753 **Statistical analysis**

754 Statistical analyses were performed using Statgraphics software. All statistical tests are  
755 indicated in the respective figure legends.

756

757

758 **Supplemental data:**

759

760 **Table S1:** Assignments of the correlation signals in the 2D HSQC spectra.

761 **Table S2:** List of primers used in this study.

762 **Figure S1:** H7996 plants show mild symptoms upon challenge inoculation of *R.*  
763 *solanacearum*.

764 **Figure S2:** FT-IR showed significantly high induction of phenolics in the xylem  
765 vasculature of resistant H7996.

766 **Figure S3:** *R. solanacearum*-induced xylem vascular ferulic acid deposition occurs in  
767 resistant H7996, but not in susceptible Marmande.

768 **Figure S4:** Expression of suberin biosynthetic genes in xylem vasculature of taproots upon  
769 infection of *R. solanacearum*.

770 **Figure S5:** Phylogeny of Feruloyl transferase (FHT) orthologues in different plant species  
771 and expression of the putative tomato FHT ortholog in response to *Ralstonia solanacearum*  
772 infection.

773 **Figure S6:** Phylogeny of tyramine hydroxycinnamoyl transferase (THT) orthologues in  
774 different plant species and expression of the tomato THT gene family members in response  
775 to *R. solanacearum* infection.

776 **Figure S7:** Expression of phenylpropanoid pathway genes in xylem vasculature of taproots  
777 upon invasion of *R. solanacearum*.

778 **Figure S8:** Immunoblot of SIFHT-HA in independent Marmande tomato lines expressing  
779 *35S::SIFHT-HA* (Marmande).

780 **Figure S9:** Overexpression of *SITHT1-3* in tomato results in restricted colonization by *R.*  
781 *solanacearum*.

782

783 **Acknowledgements**

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799

#### 800 **Author Contribution**

801 AK designed and performed experiments, interpreted data and wrote the manuscript.

802 MC performed experiments, interpreted data and reviewed the manuscript.

803 WZ performed experiments.

804 SS conducted the FT-IR experiments and reviewed the manuscript.

805 JR isolated the lignin/suberin fractions and conducted the 2D-HSQC NMR analysis,  
806 including data interpretation.

807 AG isolated the lignin/suberin fractions and conducted the 2D-HSQC NMR analysis,  
808 including data interpretation.

809 AL conducted the FT-IR experiments and reviewed the manuscript.

810 OS conducted histopathology staining experiments, interpreted data and reviewed the  
811 manuscript.

812 MF interpreted data and reviewed the manuscript.

813 MV designed experiments, interpreted data and review the manuscript.

814 NSC conceptualized the research, designed experiments, interpreted data and wrote the  
815 manuscript.

816

#### 817 **Data Availability**

818 The data that support the findings of this study are available from the corresponding author  
819 upon reasonable request.

820

## 821 **Figure Legends**

822

823 **Figure 1: Resistant H7996 tomato restricts *R. solanacearum* colonization and induces**  
824 **a vascular coating response with wall bound phenolics.** Susceptible (Marmande) and  
825 resistant (H7996), 5-week old tomato plants were inoculated through roots by soil-soak  
826 with  $\sim 1 \times 10^7$  CFU/ml of *R. solanacearum* GMI1000 and incubated at 28°C. **(A)** At 12 days  
827 post-inoculation (dpi) most Marmande plants showed severe wilting symptoms, whereas  
828 H7996 remained mostly symptomless. **(B)** Taproot cross-sections were obtained at 9 days  
829 post-infection (dpi). UV microscopy showed a strong autofluorescence signal emitted from  
830 the walls of vessels and surrounding parenchyma cells in infected H7996 plants compared  
831 to Marmande or the mock controls. Fluorescence signal in white was green colored. Images  
832 from a representative experiment out of 3 with  $n=5$  plants per cultivar. Scale bar = 500  $\mu\text{m}$ .

833

834 **Figure 2: Feruloylamides, tyramine-derived amides and suberin-compatible**  
835 **compounds are specifically enriched in resistant H7996 tomato after infection with *R.***  
836 ***solanacearum*.** **(A)** 2D-HSQC NMR spectra of enzymatically isolated lignin/suberin  
837 fractions from mock-treated and *R. solanacearum*-infected taproots of H7996 and  
838 Marmande tomato plants. **(B)** Main lignin/suberin structures identified:  $\beta$ -O-4' alkyl aryl  
839 ethers (A),  $\beta$ -5' fenylicoumarans (B),  $\beta$ - $\beta'$  resinols (C), cinnamyl alcohols end-groups (I),  
840 feruloylamides (FAM), tyramine-derived amides (Ty), guaiacyl lignin units (G), syringyl  
841 lignin units (S), as well as unassigned aliphatic signals from suberin. The structures and  
842 contours of the HSQC signals are color coded to aid interpretation.  $^1\text{H}$  and  $^{13}\text{C}$  NMR  
843 chemical shifts of the assigned signals are detailed in Table S1. To detect FAM<sub>7</sub> signal, the  
844 spectrum scaled-up to 2-fold ( $\times 2$ ) intensity. The abundances of the main lignin linkages (A,  
845 B and C) and cinnamyl alcohol end-groups (I) are referred to as a percentage of the total  
846 lignin units (S + G = 100%).

847

848 **Figure 3: Resistant H7996 tomato shows vascular autofluorescence not-quenched with**  
849 **phloroglucinol and susceptible Marmande shows a decrease in phloroglucinol-HCl**  
850 **lignin signal.** Susceptible (Marmande) and resistant (H7996) 5-week-old tomato plants

851 were root-inoculated with a *R. solanacearum* GMI1000 strain at a concentration of  $\sim 1 \times 10^7$   
852 CFU/ml or water mock. **(A)** Taproot cross-sections containing  $10^5$  CFU  $g^{-1}$  of *R.*  
853 *solanacearum* were stained with phloroglucinol-HCl and observed under UV to visualize  
854 other autofluorescent compounds different from lignin (not quenched with phloroglucionol-  
855 HCl) (left) and under brightfield to visualize lignin deposition (right). In infected H7996  
856 strong UV autofluorescence could be observed in the walls of xylem vessels surrounding  
857 xylem parenchyma cells and tracheids, indicating reinforcement of walls of vascular tissue  
858 with phenolics formed *de novo* upon infection. In infected Marmande the red phlorogucinol  
859 stain was reduced especially in the intervessel areas. **(B)** The UV auto-fluorescence signal  
860 in (A) was measured using the LAS X Leica software after the Phloroglucinol-HCl  
861 treatment. **(C)** Detailed observation of infected H7996 xylem after the Phloroglucinol-HCl  
862 treatment shows the strong UV fluorescence concentrated in specific areas possibly  
863 corresponding to intervessel and vessel-parenchyma bordered pit membranes and/or pit  
864 chambers (yellow and white arrows, respectively). Fluorescence was also observed in  
865 parenchyma cells, specially enriched at intercellular cell corners (green arrow). (B)  
866 correspond to a representative experiment out of 3 each with  $n=6$  plants per variety.  
867 Different letters indicate statistically significant differences ( $\alpha=0.05$ , Fisher's least  
868 significant difference test). (A) and (C) were representative images. Scale bars = 100  $\mu m$  in  
869 (A, left), 500  $\mu m$  in (A, right) and 50  $\mu m$  in (C).

870

871 **Figure 4: Resistant H7996 tomato shows cell wall ferulic acid and suberin deposition**  
872 **in restricted zones of vascular tissue upon *R. solanacearum* infection.** Susceptible  
873 Marmande or resistant H7996 tomato plants were soil-inoculated with a  $\sim 1 \times 10^7$  CFU/ml  
874 suspension of *Ralstonia solanacearum* GMI1000 or mock-inoculated with water and  
875 incubated at 28°C. Cross-sections were obtained from taproot tissue containing  $10^5$  CFU  $g$   
876  $^{-1}$  of *R. solanacearum*. Sections were stained with Sudan IV to visualize suberin aliphatics  
877 and subsequently treated with 1N KOH (pH above 10) to visualize ferulic acid bound to  
878 cell wall. Sudan IV positive staining (reddish-brown coloration) was observed around  
879 xylem vessels specifically in infected H7996, indicating accumulation of suberin aliphatics.  
880 Accumulation of ferulic acid bound to cell wall (blue-green coloration) appears also  
881 specifically in infected H7996 resistant tomato, surrounding sudan IV-stained areas. White

882 arrowheads indicate the sites of accumulation of ferulates and aliphatic compounds.  
883 Representative images from one experiment out of three with  $n=6$  plants each were taken.

884

885 **Figure 5: Genes of the ligno-suberin heteropolymer biosynthesis pathway are**  
886 **specifically induced in the xylem vasculature of resistant H7996 tomato upon *R.***

887 *solanacearum*. The levels of expression of genes belonging to metabolic pathways relevant  
888 for suberin, lignin and feruloyltyramine and related amides biosynthesis were analyzed by  
889 qPCR of taproot vascular tissue in infected or mock-treated H7996 or Marmande tomato  
890 plants. Plants containing an *R. solanacearum* inoculum of  $10^5$  CFU  $g^{-1}$  were selected and  
891 taproot xylem vascular tissue, comprising of metaxylems and surrounding parenchyma  
892 cells was collected for RNA extraction and cDNA synthesis. In parallel, xylem tissue was  
893 collected from mock plants. Heatmaps show  $\log_2$  fold change RTA (relative transcript  
894 abundance) values of infected vs. mock for Marmande (left) and Hawaii (right). The tomato  
895 gene encoding for the alpha-subunit of the translation elongation factor 1 (*SlEF1  $\alpha$* ) was  
896 used as endogenous reference. Three biological replicates ( $n=3$ ) were used, and taproots of  
897 6 plants were used in each replicate. The scheme represents the phenylpropanoid and  
898 suberin biosynthesis pathways providing lignin-like and suberin precursors for the ligno-  
899 suberin heteropolymer. Abbreviations: PAL: Phenylalanine ammonia-lyase; C4H:  
900 Cinnamate-4-hydroxylase; C3H: Coumarate 3-hydroxylase; 4CL: 4-Coumarate-CoA  
901 ligase; HCT: Hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase;  
902 COMT: Caffeic acid 3-O-methyltransferase; CCoAOMT: Caffeoyl CoA 3-O-  
903 methyltransferase; CYP86A1 and CYP86B1: cytochrome P450 fatty acid  $\omega$ -hydroxylases;  
904 KCS1/2: 3-ketoacyl-CoA synthase; FAR 1/3/4: Fatty acyl-CoA reductase; GPAT5:  
905 glycerol-3-phosphate acyltransferase 5; THT: Tyramine hydroxycinnamoyl transferase;  
906 TyDC: Tyrosine decarboxylase; FHT: feruloyl transferase. The question mark (?) denotes a  
907 hypothetical reaction.

908

909 **Figure 6: Overexpression of *SIFHT-HA* in susceptible tomato slightly restricts**  
910 **colonization by *R. solanacearum*. (A, B) A pathogenicity assay was performed comparing**  
911 **Wt and 3 independent 35S::*SIFHT-HA* Marmande tomato lines (A, C and D) after infection**  
912 **with *R. solanacearum* GMI1000 lux reporter strain. Five-week-old plants were soil-soak**



913 inoculated with  $\sim 1 \times 10^7$  CFU/ml or mock and grown at 28°C. **(A)** Wilting progress was  
914 monitored by rating plants daily on a 0 to 4 disease index scale where 0 = healthy and 4  
915 =100% wilted. Plotted values correspond to means  $\pm$  standard error of 24 independent  
916 plants (n=24) from a representative experiment out of a total of 3. Asterisks indicate  
917 statistically significant differences between Wt and each of the *35S::FHT-HA* analyzed  
918 using a paired Student's t-test (\* p<0.05). **(B)** The level of *R. solanacearum* colonization in  
919 the taproot and hypocotyl was calculated as colony forming units per gram of fresh taproot  
920 tissue (CFU·g<sup>-1</sup>) at 12 dpi. Data presented are of a representative experiment out of a total  
921 of 3 experiments. Asterisks indicate statistically significant differences between wild type  
922 and *35S::FHT-HA* tomato lines in a paired Student's t-test (\* corresponds to a p-value of p  
923 <0.05 and \*\*\* to p < 0.001).

924

925 **Figure 7: Overexpression of *SITHT1-3* in susceptible tomato confers resistance to *R.***  
926 ***solanacearum*.** **(A, B)** A pathogenicity assay was performed comparing Wt and  
927 *35S::SITHT1-3* tomato lines (Moneymaker background) after infection with *R.*  
928 *solanacearum* lux reporter strain of GMI1000. Five-week-old plants were soil-soak  
929 inoculated with  $\sim 1 \times 10^7$  CFU/ml and grown at 28°C. **(A)** Wilting progress was monitored  
930 by rating plants daily on a 0 to 4 disease index scale where 0 = healthy and 4 =100%  
931 wilted. Plotted values correspond to means  $\pm$  standard error of 24 independent plants  
932 (n=24) from a representative experiment out of a total of 3. Asterisks indicate statistically  
933 significant differences between Wt and *35S::SITHT1-3* using a paired Student's t-test (\*  
934 p<0.05, \*\* p<0.01 and \*\*\* p<0.001). **(B)** Pictures were taken 12 days post-infection. Wt  
935 plants were arranged according to the degree of symptom severity (from 4 to 0). **(C)**  
936 Transgenic *35S::SITHT1-3* tomato significantly restricted *R. solanacearum* colonization in  
937 both the taproot and hypocotyl to Wt. Five-week-old tomato plants were root-inoculated  
938 with a *R. solanacearum* GMI1000 luciferase reporter strain at a concentration of  $\sim 1 \times 10^7$   
939 CFU/ml or water mock. The level of *in planta* colonization by *R. solanacearum* was  
940 calculated as colony forming units per gram of fresh taproot tissue (CFU·g<sup>-1</sup>) at 12dpi.  
941 Box-and-whisker plots show data from a single representative experiment out of 3 (n =14  
942 to 16). **(D)** Transverse stem cross-sections of Wt and transgenic *35S::SITHT1-3* tomato  
943 lines were imaged under a confocal microscope 6 days after infection with a *R.*

944 *solanacearum* GMI1000 GFP reporter strain. *R. solanacearum* at a concentration of  $10^5$   
945 CFU ml<sup>-1</sup> was injected directly into the xylem vasculature of the first internode through  
946 the petiole. Representative images of *R. solanacearum* colonization progress at the point of  
947 inoculation are shown. (E) Mean green fluorescence of the GFP signal emitted from *R.*  
948 *solanacearum* at cross-sections obtained as described in (D) at the point of inoculation (0),  
949 below the point of inoculation (-0.5 cm) and above the point of inoculation (+0.5 cm) was  
950 measured using ImageJ. Data from a representative experiment out of a total of 3, with  $n=5$   
951 plants per condition. Asterisks indicate statistically significant differences between wild  
952 type and *35S::THT1-3* tomato plants in a paired Student's t-test (\* corresponds to a p-value  
953 of  $p < 0.05$ , \*\* to  $p < 0.01$  and \*\*\* to  $p < 0.001$ ).

954

955 **Figure 8: Schematic representation of the vascular ligno-suberization process**  
956 **potentially taking place in infected vessels of resistant H7996 tomato upon *R.***  
957 ***solanacearum* infection.** Colonization of the vasculature by *R. solanacearum* in resistant  
958 tomato plants induces a ligno-suberization process in the walls of the infected vessel (V)  
959 and of the adjacent tracheids (T) and parenchyma cells (XP) (red). The lignin-like polymer  
960 accompanying suberin would be enriched in structural feruloyltyramine and related amides.  
961 The signal of structural ferulic acid (ester or as amide) would extend to the walls of  
962 peripheral parenchyma cells, vessels and tracheids (green), indicating a stage preceding  
963 suberization or a final layered pattern, still to be resolved. Together, the red and green  
964 areas, would form a “zone of ligno-suberization” (black dashed line) potentially creating a  
965 physico-chemical barrier to limit *R. solanacearum* spread from the colonized xylem vessel  
966 lumen.

**A**

MARMANDE (S)

H7996 (R)



**B**

MARMANDE (S)

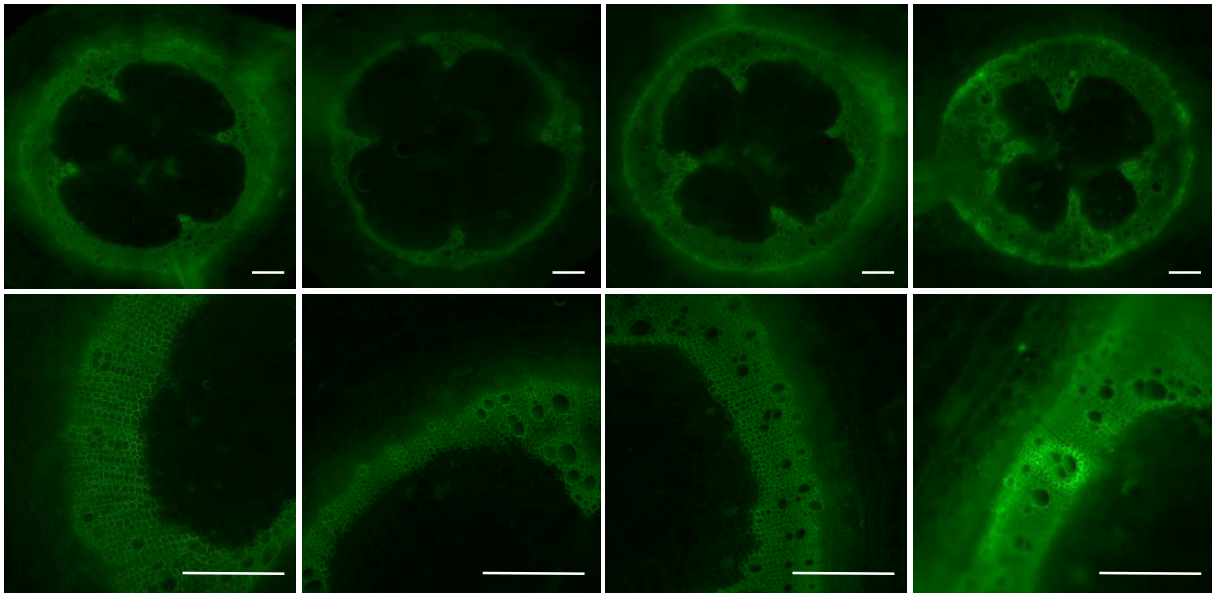
H7996 (R)

Mock

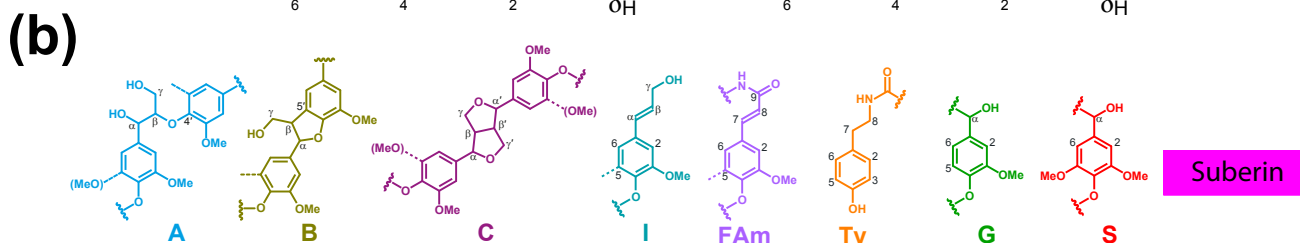
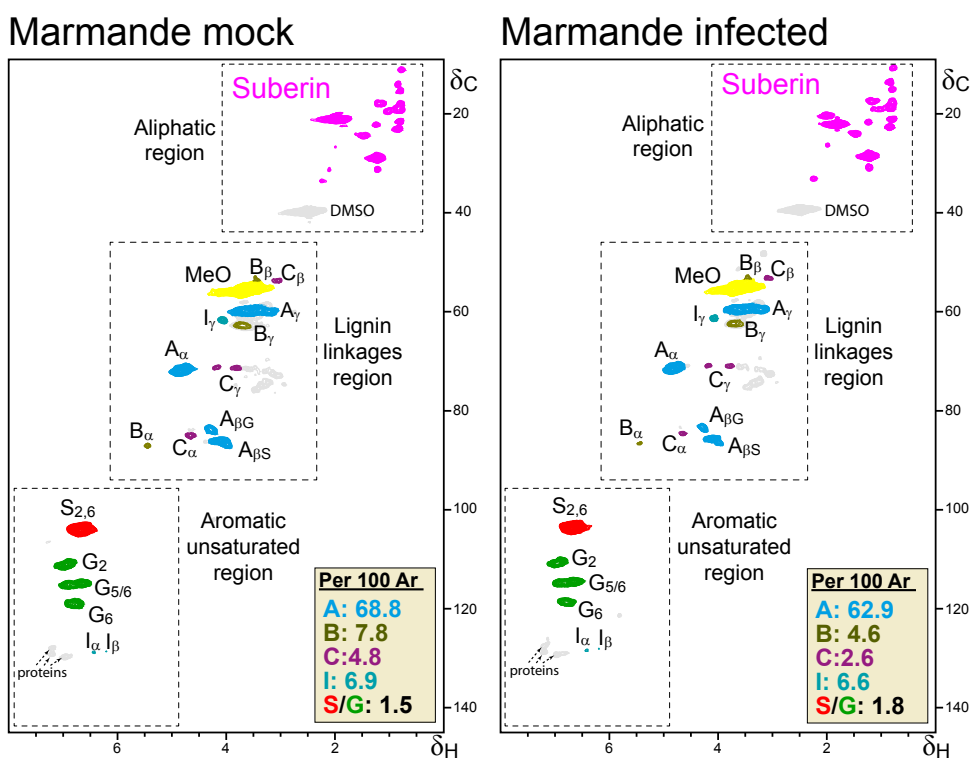
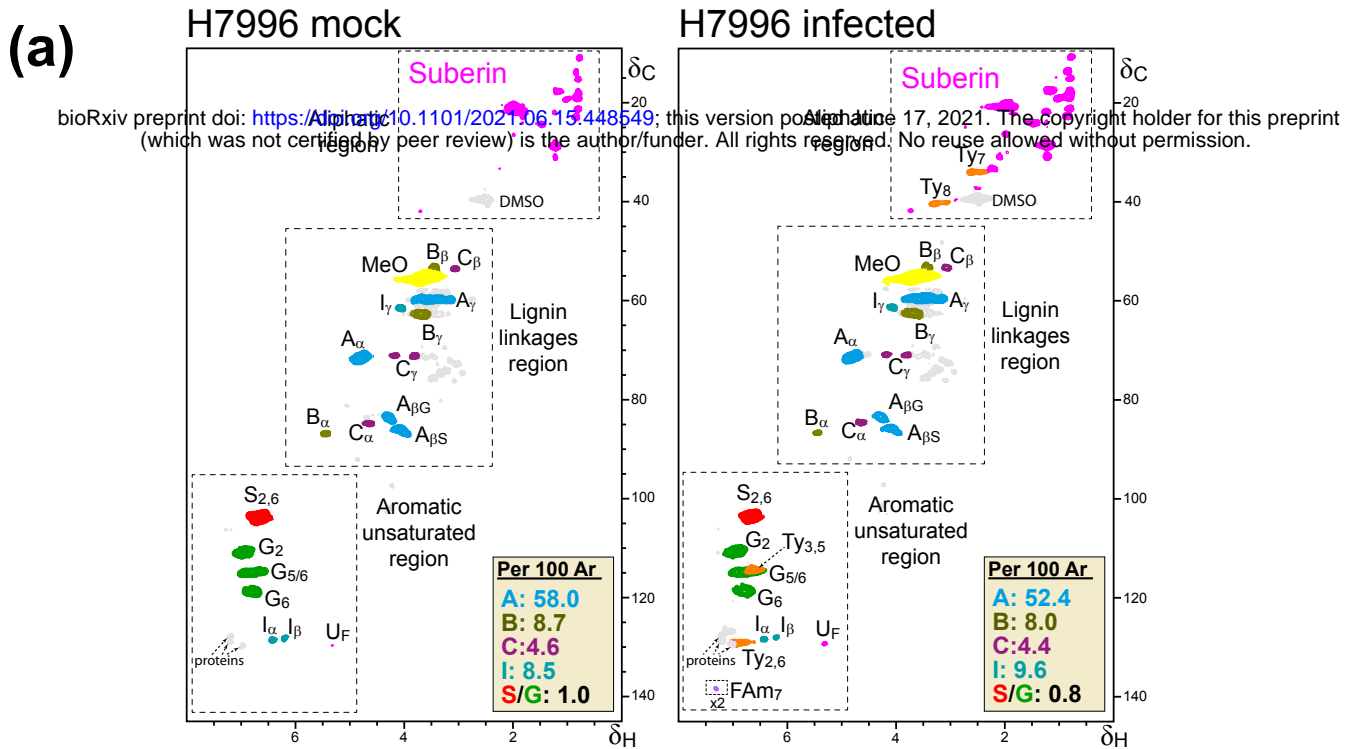
Infected

Mock

Infected



**Figure 1: Resistant H7996 tomato restricts *R. solanacearum* colonization and induces a vascular coating response with wall bound phenolics.** Susceptible (Marmande) and resistant (H7996), 5-week old tomato plants were inoculated through roots by soil-soak with  $\sim 1 \times 10^7$  CFU/ml of *R. solanacearum* GMI1000 and incubated at 28°C. **(A)** At 12 days post-inoculation (dpi) most Marmande plants showed severe wilting symptoms, whereas H7996 remained mostly symptomless. **(B)** Taproot cross-sections were obtained at 9 days post-infection (dpi). UV microscopy showed a strong autofluorescence signal emitted from the walls of vessels and surrounding parenchyma cells in infected H7996 plants compared to Marmande or the mock controls. Fluorescence signal in white was green colored. Images from a representative experiment out of 3 with  $n=5$  plants per cultivar. Scale bar = 500  $\mu$ m.



**Figure 2.** (a) 2D-HSQC spectra of enzymatically isolated lignin/suberin fractions from mock-treated and *R. solanacearum*-infected taproots of H7996 and Marmande tomato plants. (b) Main lignin/suberin structures identified:  $\beta$ -O-4' alkyl aryl ethers (A),  $\beta$ -5' phenylcoumarans (B),  $\beta$ - $\beta'$  resinols (C), cinnamyl alcohols end-groups (I), feruloyl amides (FAm), amides of tyramine (Ty), guaiacyl lignin units (G), syringyl lignin units (S), as well as unassigned aliphatic signals from suberin. The structures and contours of the HSQC signals are color coded to aid interpretation.  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts of the assigned signals are detailed in Table S1. To detect FAM<sub>7</sub> signal, the spectrum scaled-up to 2-fold ( $\times 2$ ) intensity. The abundances of the main lignin linkages (A, B and C) and cinnamyl alcohol end-groups (I) are referred to as a percentage of the total lignin units ( $S + G = 100\%$ ).

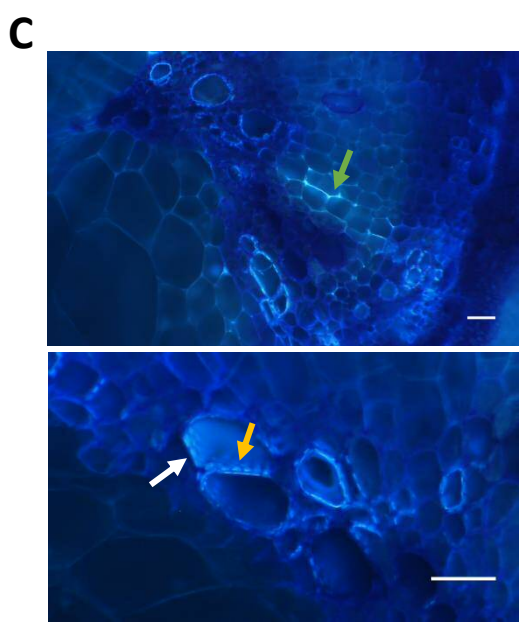
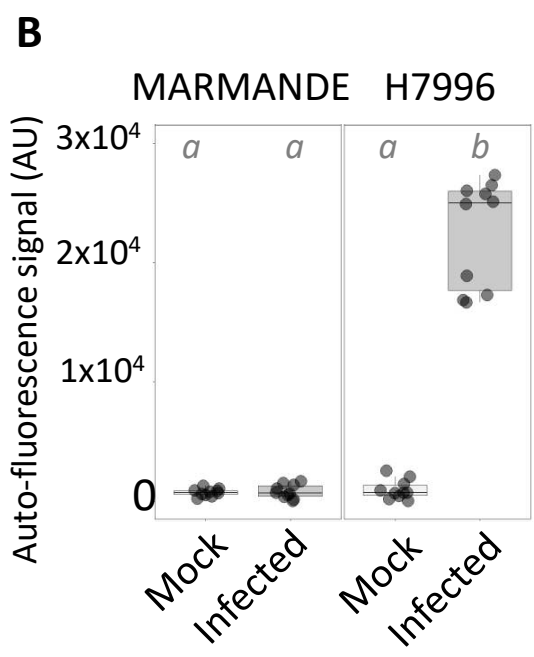
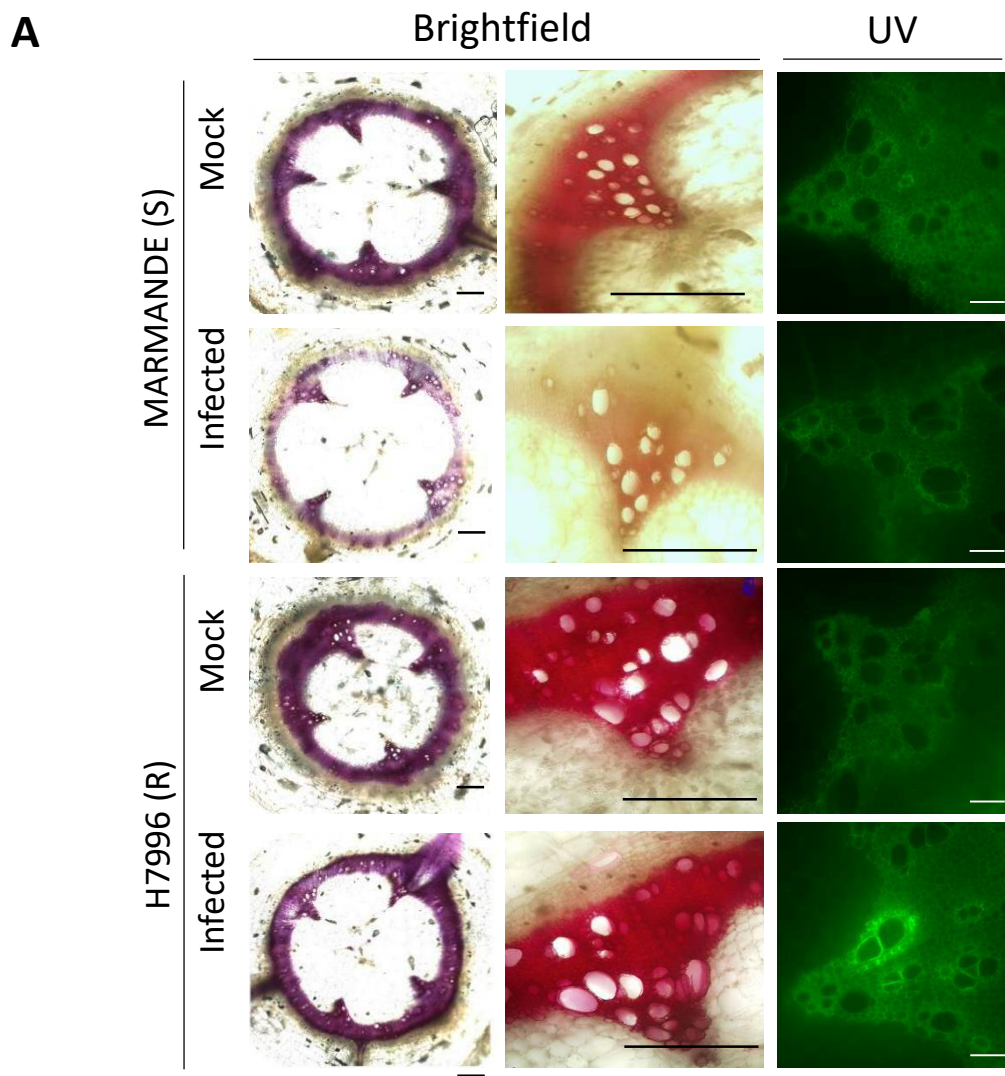
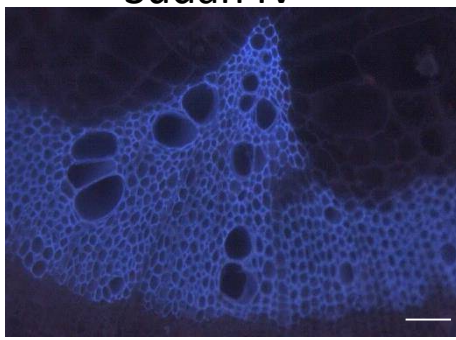


Figure 3: Legend on the next page

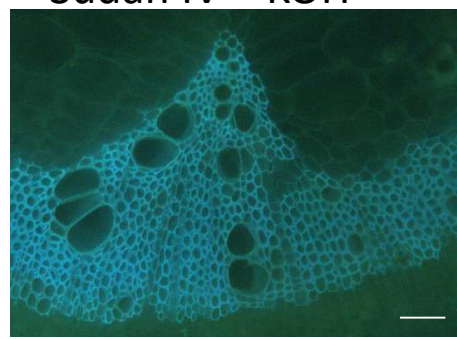
**Figure 3: Resistant H7996 tomato shows vascular autofluorescence not-quenched with phloroglucinol and susceptible Marmande shows a decrease in phloroglucinol-HCl lignin signal.** Susceptible (Marmande) and resistant (H7996) 5-week-old tomato plants were root-inoculated with a *R. solanacearum* GM11000 strain at a concentration of  $\sim 1 \times 10^7$  CFU/ml or water mock. **(A)** Taproot cross-sections containing  $10^5$  CFU  $g^{-1}$  of *R. solanacearum* were stained with phloroglucinol-HCl and observed under UV to visualize other autofluorescent compounds different from lignin (not quenched with phloroglucinol-HCl) (left) and under brightfield to visualize lignin deposition (right). In infected H7996 strong UV autofluorescence could be observed in the walls of xylem vessels surrounding xylem parenchyma cells and tracheids, indicating reinforcement of walls of vascular tissue with phenolics formed *de novo* upon infection. In infected Marmande the red phloroglucinol stain was reduced especially in the intervessel areas. **(B)** The UV auto-fluorescence signal in (A) was measured using the LAS X Leica software after the Phloroglucinol-HCl treatment. **(C)** Detailed observation of infected H7996 xylem after the Phloroglucinol-HCl treatment shows the strong UV fluorescence concentrated in specific areas possibly corresponding to intervessel and vessel-parenchyma bordered pit membranes and/or pit chambers (yellow and white arrows, respectively). Fluorescence was also observed in parenchyma cells, specially enriched at intercellular cell corners (green arrow). (B) correspond to a representative experiment out of 3 each with  $n=6$  plants per variety.

MARMADE (S)

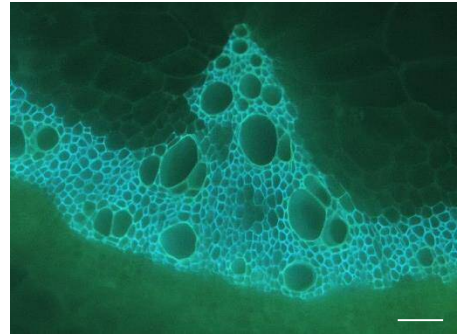
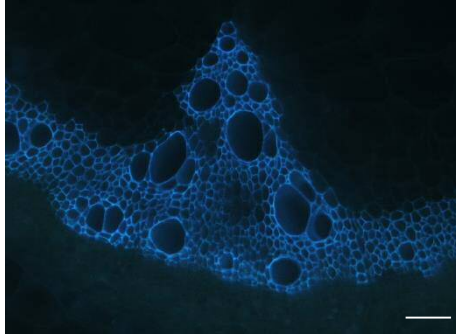
Mock



Sudan IV + KOH

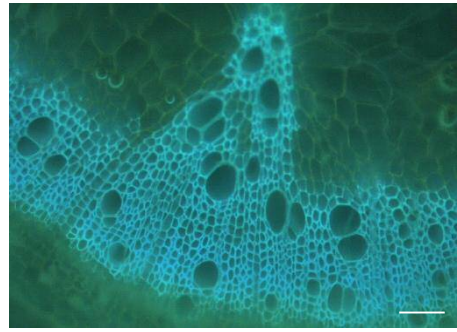
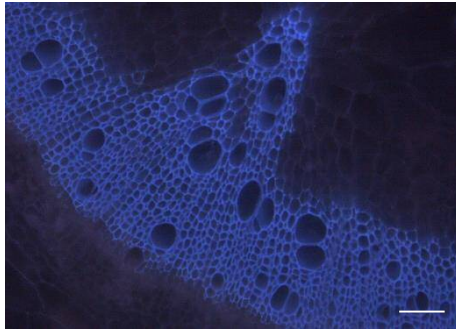


Infected

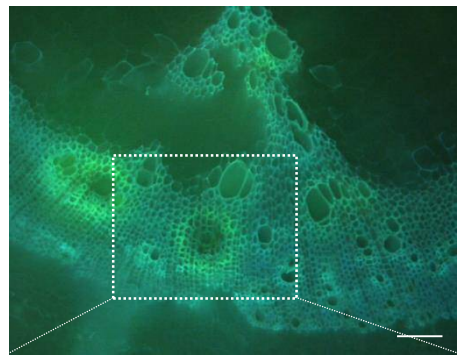
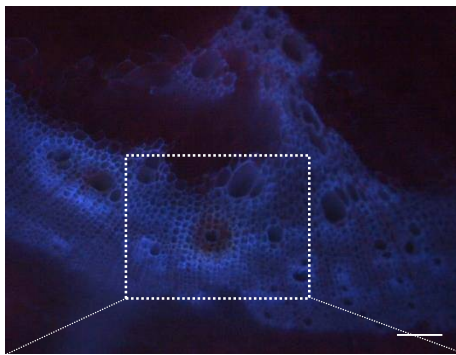


H7996 (R)

Mock



Infected



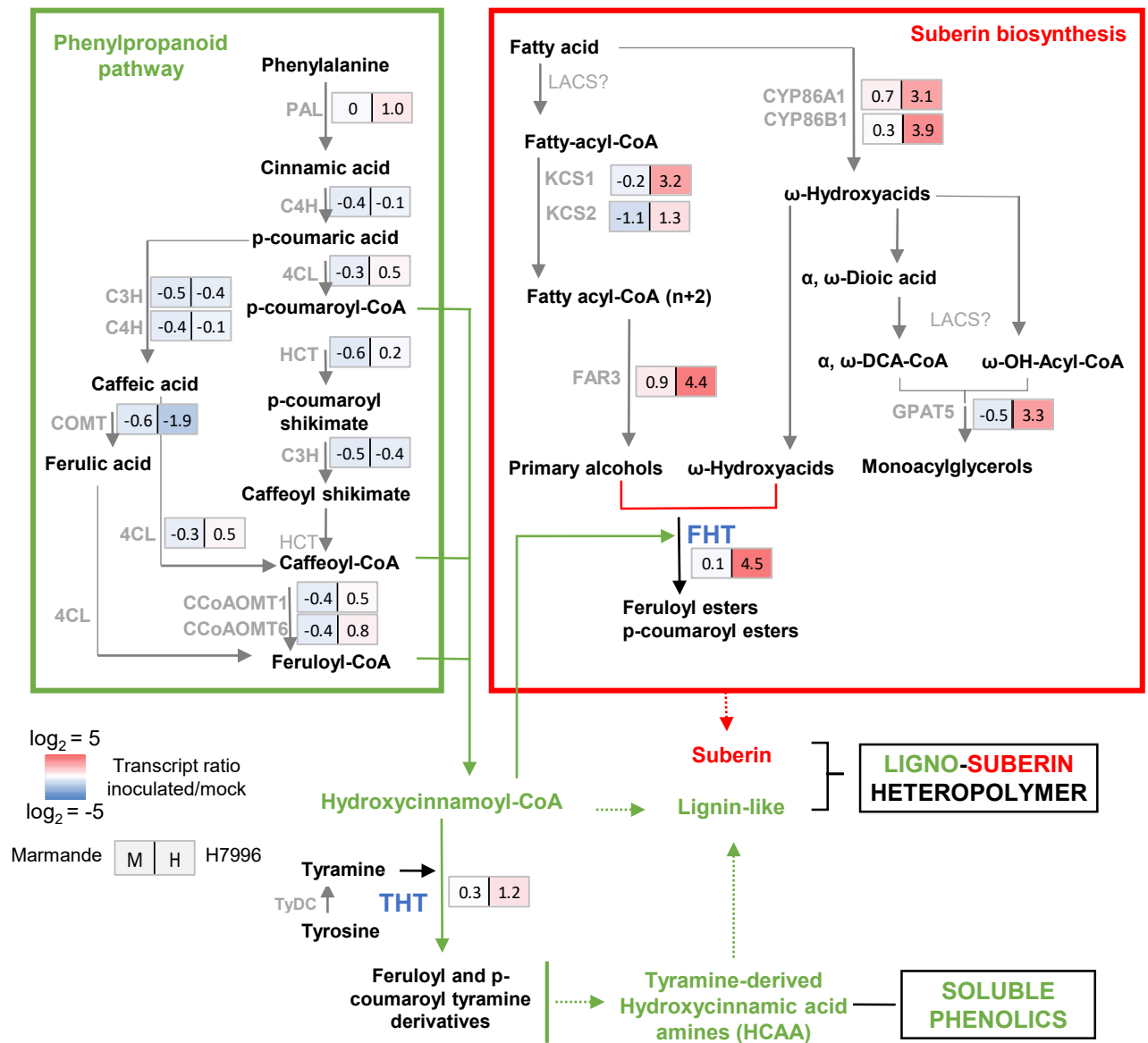
Ferulates ▶ ◀ Aliphatics

Ferulates ▶ ◀ Aliphatics

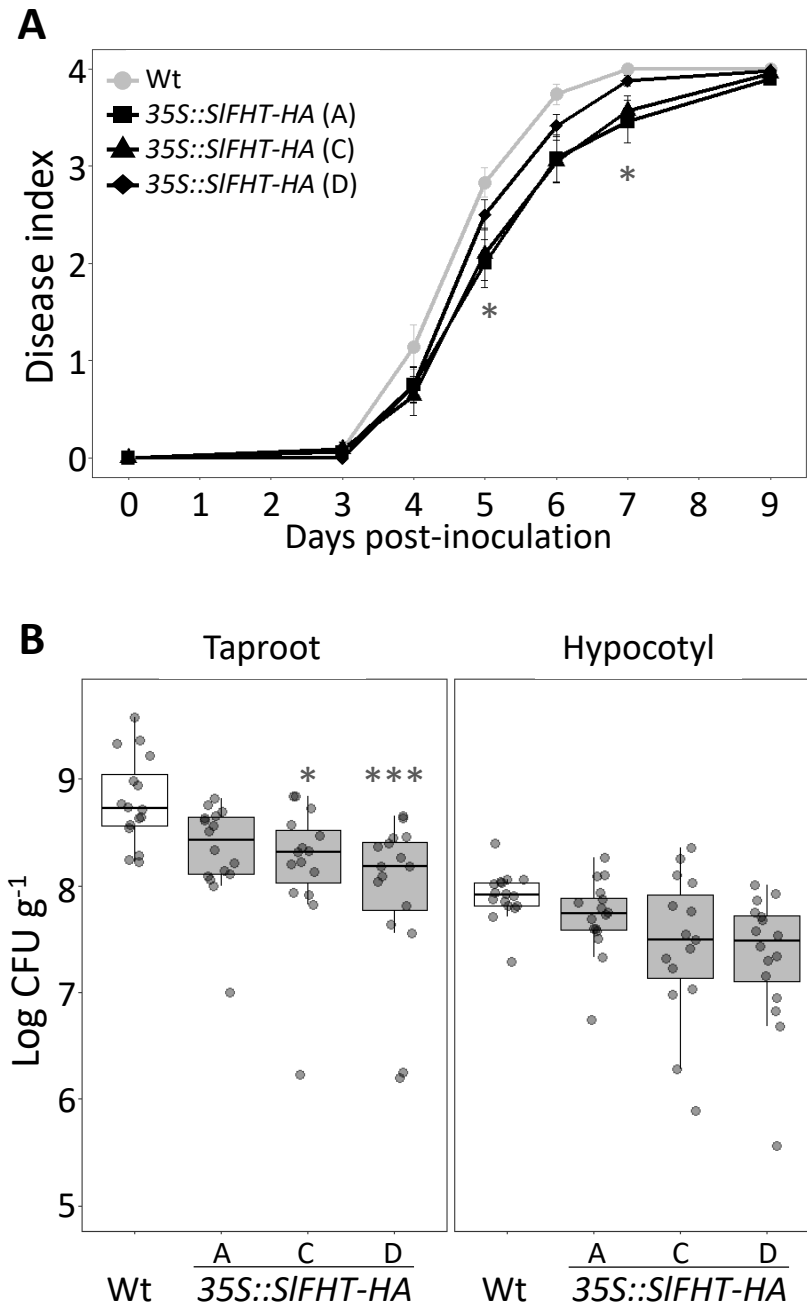
Figure 4: Legend on the next page

**Figure 4: Resistant H7996 tomato shows cell wall ferulic acid and suberin deposition in restricted zones of vascular tissue upon *R. solanacearum* infection.** Susceptible Marmande or resistant H7996 tomato plants were soil-inoculated with a  $\sim 1 \times 10^7$  CFU/ml suspension of *Ralstonia solanacearum* GMI1000 or mock-inoculated with water and incubated at 28°C. Cross-sections were obtained from taproot tissue containing  $10^5$  CFU  $g^{-1}$  of *R. solanacearum*. Sections were stained with Sudan IV to visualize suberin aliphatics and subsequently treated with 1N KOH (pH above 10) to visualize ferulic acid bound to cell wall. Sudan IV positive staining (reddish-brown coloration) was observed around xylem vessels specifically in infected H7996, indicating accumulation of suberin aliphatics. Accumulation of ferulic acid bound to cell wall (blue-green coloration) appears also specifically in infected H7996 resistant tomato, surrounding sudan IV-stained areas. White arrowheads indicate the sites of accumulation of ferulates and aliphatic compounds. Representative images from one experiment out of three with  $n=6$  plants each were taken.

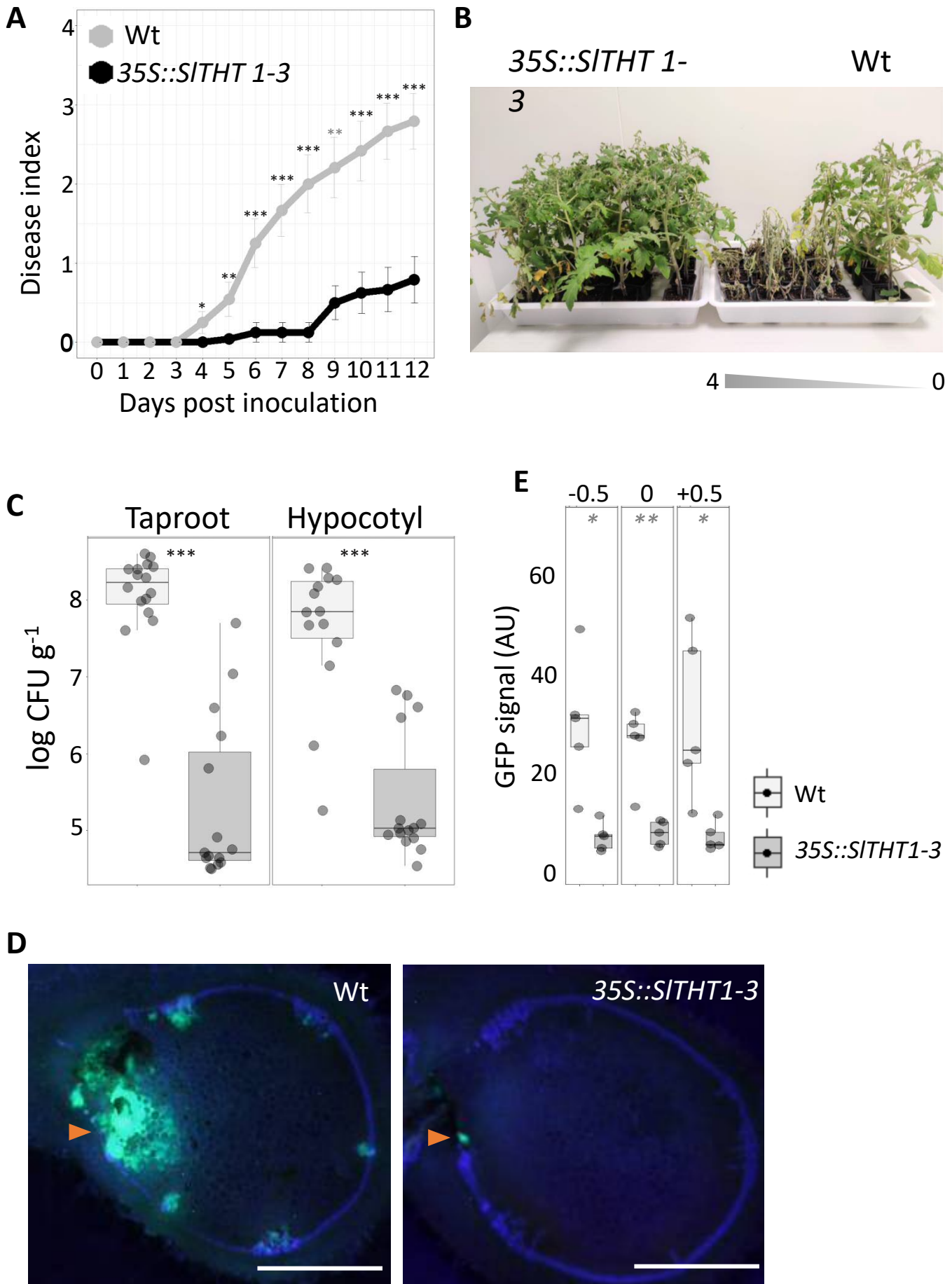




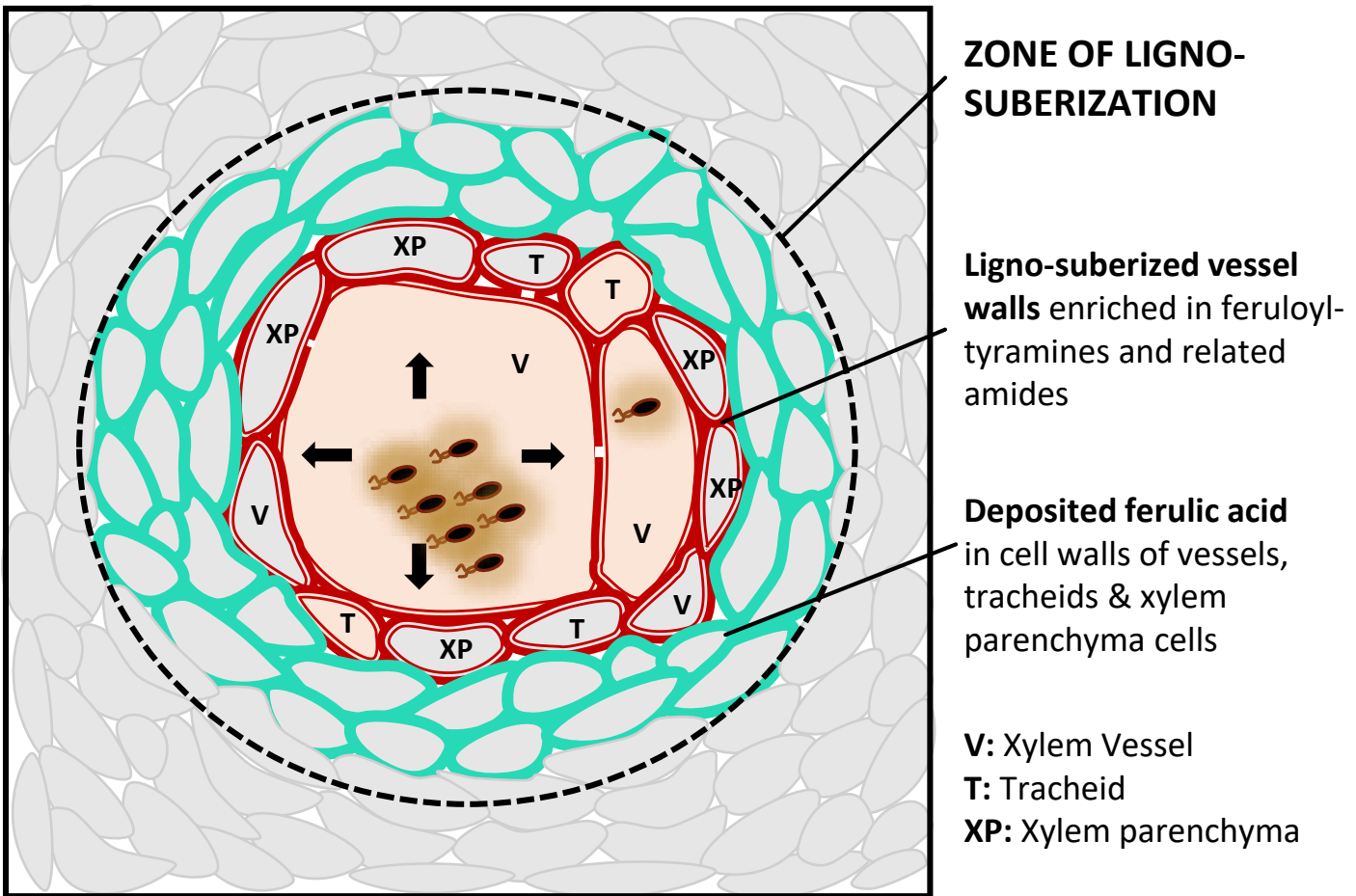
**Figure 5: Genes of the ligno-suberin heteropolymer biosynthesis pathway are specifically induced in the xylem vasculature of resistant H7996 tomato upon *R. solanacearum*.** The levels of expression of genes belonging to metabolic pathways relevant for suberin, lignin and feruloyltyramine and related amides biosynthesis were analyzed by qPCR of taproot vascular tissue in infected or mock-treated H7996 or Marmande tomato plants. Plants containing an *R. solanacearum* inoculum of  $10^5$  CFU  $g^{-1}$  were selected and taproot xylem vascular tissue, comprising of metaxylems and surrounding parenchyma cells was collected for RNA extraction and cDNA synthesis. In parallel, xylem tissue was collected from mock plants. Heatmaps show log<sub>2</sub> fold change RTA (relative transcript abundance) values of infected vs. mock for Marmande (left) and Hawaii (right). The tomato gene encoding for the alpha-subunit of the translation elongation factor 1 (*SleEF1*  $\alpha$ ) was used as endogenous reference. Three biological replicates (n=3) were used, and taproots of 6 plants were used in each replicate. The scheme represents the phenylpropanoid and suberin biosynthesis pathways providing lignin-like and suberin precursors for the ligno-suberin heteropolymer. Abbreviations: PAL: Phenylalanine ammonia-lyase; C4H: Cinnamate-4-hydroxylase; C3H: Coumarate 3-hydroxylase; 4CL: 4-Coumarate-CoA ligase; HCT: Hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase; COMT: Caffeic acid 3-O-methyltransferase; CCoAOMT: Caffeoyl CoA 3-O-methyltransferase; CYP86A1 and CYP86B1: cytochrome P450 fatty acid  $\omega$ -hydroxylases; KCS1/2: 3-ketoacyl-CoA synthase; FAR 1/3/4: Fatty acyl-CoA reductase; GPAT5: glycerol-3-phosphate acyltransferase 5; THT: Tyramine hydroxycinnamoyl transferase; TyDC: Tyrosine decarboxylase; FHT: feruloyl transferase. The question mark (?) denotes a hypothetical reaction.



**Figure 6: Overexpression of *SIFHT-HA* in susceptible tomato slightly restricts colonization by *R. solanacearum*.** (A, B) A pathogenicity assay was performed comparing Wt and 3 independent *35S::SIFHT-HA* Marmande tomato lines (A, C and D) after infection with *R. solanacearum* GMI1000 lux reporter strain. Five-week-old plants were soil-soak inoculated with  $\sim 1 \times 10^7$  CFU/ml or mock and grown at 28°C. (A) Wilting progress was monitored by rating plants daily on a 0 to 4 disease index scale where 0 = healthy and 4 = 100% wilted. Plotted values correspond to means  $\pm$  standard error of 24 independent plants (n=24) from a representative experiment out of a total of 3. Asterisks indicate statistically significant differences between Wt and each of the *35S::FHT-HA* analyzed using a paired Student's t-test (\* p<0.05). (B) The level of *R. solanacearum* colonization in the taproot and hypocotyl was calculated as colony forming units per gram of fresh taproot tissue (CFU·g<sup>-1</sup>) at 12 dpi. Data presented are of a representative experiment out of a total of 3 experiments. Asterisks indicate statistically significant differences between wild type and *35S::FHT-HA* tomato lines in a paired Student's t-test (\* corresponds to a p-value of p < 0.05 and \*\*\* to p < 0.001).



**Figure 7: Overexpression of *SITH1-3* in susceptible tomato confers resistance to *R. solanacearum*.** (A, B) A pathogenicity assay was performed comparing Wt and *35S::SITH1-3* tomato lines (Moneymaker background) after infection with *R. solanacearum* lux reporter strain of GMI1000. Five-week-old plants were soil-soak inoculated with  $\sim 1 \times 10^7$  CFU/ml and grown at 28°C. (A) Wilting progress was monitored by rating plants daily on a 0 to 4 disease index scale where 0 = healthy and 4 = 100% wilted. Plotted values correspond to means  $\pm$  standard error of 24 independent plants ( $n=24$ ) from a representative experiment out of a total of 3. Asterisks indicate statistically significant differences between Wt and *35S::SITH1-3* using a paired Student's t-test (\*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ ). (B) Pictures were taken 12 days post-infection. Wt plants were arranged according to the degree of symptom severity (from 4 to 0). (C) Transgenic *35S::SITH1-3* tomato significantly restricted *R. solanacearum* colonization in both the taproot and hypocotyl to Wt. Five-week-old tomato plants were root-inoculated with a *R. solanacearum* GMI1000 luciferase reporter strain at a concentration of  $\sim 1 \times 10^7$  CFU/ml or water mock. The level of *in planta* colonization by *R. solanacearum* was calculated as colony forming units per gram of fresh taproot tissue ( $\text{CFU} \cdot \text{g}^{-1}$ ) at 12dpi. Box-and-whisker plots show data from a single representative experiment out of 3 ( $n=14$  to 16). (D) Transverse stem cross-sections of Wt and transgenic *35S::SITH1-3* tomato lines were imaged under a confocal microscope 6 days after infection with a *R. solanacearum* GMI1000 GFP reporter strain. *R. solanacearum* at a concentration of  $10^5$  CFU  $\text{ml}^{-1}$  was injected directly into the xylem vasculature of the first internode through the petiole. Representative images of *R. solanacearum* colonization progress at the point of inoculation are shown. (E) Mean green fluorescence of the GFP signal emitted from *R. solanacearum* at cross-sections obtained as described in (D) at the point of inoculation (0), below the point of inoculation (-0.5 cm) and above the point of inoculation (+0.5 cm) was measured using ImageJ. Data from a representative experiment out of a total of 3, with  $n=5$  plants per condition. Asterisks indicate statistically significant differences between wild type and *35S::SITH1-3* tomato plants in a paired Student's t-test (\* corresponds to a p-value of  $p < 0.05$ , \*\* to  $p < 0.01$  and \*\*\* to  $p < 0.001$ ).



**Figure 8: Schematic representation of the vascular ligno-suberization process potentially taking place in infected vessels of resistant H7996 tomato upon *R. solanacearum* infection.** Colonization of the vasculature by *R. solanacearum* in resistant tomato plants induces a ligno-suberization process in the walls of the infected vessel (V) and of the adjacent tracheids (T) and parenchyma cells (XP) (red). The lignin-like polymer accompanying suberin would be enriched in structural feruloyltyramine and related amides. The signal of structural ferulic acid (ester or as amide) would extend to the walls of peripheral parenchyma cells, vessels and tracheids (green), indicating a stage preceding suberization or a final layered pattern, still to be resolved. Together, the red and green areas, would form a “zone of ligno-suberization” (black dashed line) potentially creating a physico-chemical barrier to limit *R. solanacearum* spread from the colonized xylem vessel lumen.

1 **SUPPLEMENTAL DATA:**

2

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4 **Induced ligno-suberin vascular coating and tyramine-derived hydroxycinnamic acid**  
5 **amides restrict *Ralstonia solanacearum* colonization in resistant tomato roots**

6

7 **Short title:** A pathogen-induced ligno-suberin vascular coating

8

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**Table S1.** Assignments of the correlation signals in the 2D HSQC spectra.

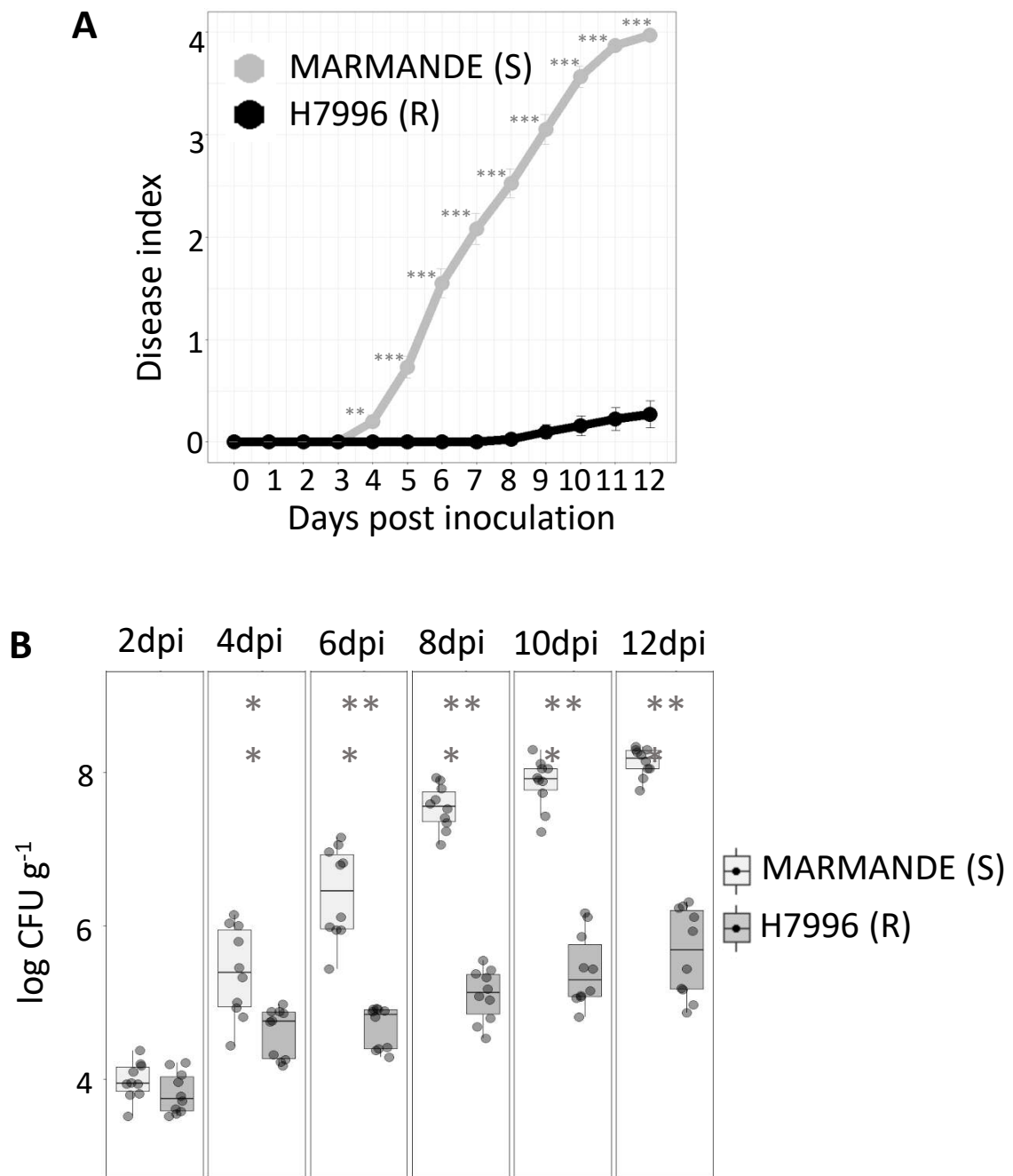
<b>Label</b>	<b><math>\delta_C/\delta_H</math> (ppm)</b>	<b>Assignment</b>
Ty <sub>7</sub>	34.2/2.62	C <sub>7</sub> /H <sub>7</sub> in amides of tyramine ( <b>Ty</b> )
Ty <sub>8</sub>	40.5/3.29	C <sub>8</sub> /H <sub>8</sub> in amides of tyramine ( <b>Ty</b> )
B <sub>β</sub>	53.3/3.43	C <sub>β</sub> /H <sub>β</sub> in phenylcoumarans ( <b>B</b> )
C <sub>β</sub>	53.5/3.05	C <sub>β</sub> /H <sub>β</sub> in β-β' resinols ( <b>C</b> )
MeO	55.3/3.72	C/H in aromatic methoxy group
A <sub>γ</sub>	59.7/3.23, 3.58	C <sub>γ</sub> /H <sub>γ</sub> in β-O-4' alkyl-aryl ethers ( <b>A</b> )
I <sub>γ</sub>	61.5/4.06	C <sub>γ</sub> /H <sub>γ</sub> in cinnamyl alcohol end-groups ( <b>I</b> )
B <sub>γ</sub>	62.6/3.70	C <sub>γ</sub> /H <sub>γ</sub> in phenylcoumarans ( <b>B</b> )
C <sub>γ</sub>	71.1/3.80, 4.17	C <sub>γ</sub> /H <sub>γ</sub> in β-β' resinols ( <b>B</b> )
A <sub>α</sub>	71.3/4.79	C <sub>α</sub> /H <sub>α</sub> in β-O-4' alkyl-aryl ethers ( <b>A</b> )
A <sub>βG</sub>	83.9/4.27	C <sub>β</sub> /H <sub>β</sub> in β-O-4' alkyl-aryl ethers ( <b>A</b> ) linked to a G unit
C <sub>α</sub>	84.9/4.67	C <sub>α</sub> /H <sub>α</sub> in β-β' resinols ( <b>C</b> )
A <sub>βS</sub>	83.6/4.28	C <sub>β</sub> /H <sub>β</sub> in β-O-4' alkyl-aryl ethers ( <b>A</b> ) linked to a S unit
B <sub>α</sub>	86.9/5.45	C <sub>α</sub> /H <sub>α</sub> in phenylcoumarans ( <b>B</b> )
S <sub>2,6</sub>	104.0/6.68	C <sub>2</sub> /H <sub>2</sub> and C <sub>6</sub> /H <sub>6</sub> in syringyl units ( <b>S</b> )
S' <sub>2,6</sub>	106.3/7.29	C <sub>2</sub> /H <sub>2</sub> and C <sub>6</sub> /H <sub>6</sub> in C <sub>α</sub> -oxidized syringyl units ( <b>S'</b> )
G <sub>2</sub>	111.1/6.97	C <sub>2</sub> /H <sub>2</sub> in guaiacyl units ( <b>G</b> )
Ty <sub>3,5</sub>	114.8/6.64	C <sub>3</sub> /H <sub>3</sub> and C <sub>5</sub> /H <sub>5</sub> in amides of tyramine ( <b>Ty</b> )
G <sub>5/6</sub>	114.9/6.79	C <sub>5</sub> /H <sub>5</sub> and C <sub>6</sub> /H <sub>6</sub> in guaiacyl units ( <b>G</b> )
G <sub>6</sub>	119.0/6.76	C <sub>6</sub> /H <sub>6</sub> in guaiacyl units ( <b>G</b> )
I <sub>β</sub>	128.2/6.21	C <sub>β</sub> /H <sub>β</sub> in cinnamyl alcohol end-groups ( <b>I</b> )
I <sub>α</sub>	128.6/6.43	C <sub>α</sub> /H <sub>α</sub> in cinnamyl alcohol end-groups ( <b>I</b> )
Ty <sub>2,6</sub>	129.3/6.92	C <sub>2</sub> /H <sub>2</sub> and C <sub>6</sub> /H <sub>6</sub> in amides of tyramine ( <b>Ty</b> )
U <sub>F</sub>	129.4/5.31	-CH=CH- in unsaturated fatty acid structures ( <b>U<sub>F</sub></b> )
FAm <sub>7</sub>	138.6/7.31	C <sub>7</sub> /H <sub>7</sub> in feruloyl amides ( <b>FAm</b> )

**Table S2: List of primers used for cloning and qPCR analysis**

Gene	Gene ID	Primers	Sequence	Usage	Origin
FHT	Solyc03g097500	part7FHTF1	GGCCCCGGGATGGAGAATGGTAAACA CAGTGTTC	Cloning	This paper
		part7FHTHAR1	GGGGATCCTTAAGCGTAGTCTGGGAC GTCGTATGGGTAGATCTCCATAAGTT CCTC		
FHT	Solyc03g097500	qSIFHT F1	GGTGGCTCAGGTGACAAAGT	qPCR	This paper
		qSIFHT R1	CCTCTCGCAATTCACCCCA		
THT 1-3	Solyc08g068730	qTHT1-3F1	CCCCTTTTGACGAACCTAAA	qPCR	(Campos <i>et al.</i> , 2014)
		qTHT1-3R1	TTTGGATCGGAATTCCTCAA		
EF	Solyc06g005060	qeEF1 $\alpha$ F1	CCACCTCGAGATCCTAATGG	qPCR	(Campos <i>et al.</i> , 2014)
		qeEF1 $\alpha$ R1	ACCCTCACGTATGCTTCCAG		
PAL1	Solyc09g007920	qSIPAL1 F1	TACGTGTTTGCCTATGCTGATG	qPCR	(Rahim <i>et al.</i> , 2019)
		qSIPAL1 R1	CGGCCTTTAATTCGTCCTC		
COMT	Solyc03g080180	qSICOMT F1	GGTGGTGAACAGGGGCTACT	qPCR	(Rahim <i>et al.</i> , 2019)
		qSICOMT R1	TAAACAATGCTCATCGCTCCAATC		
CCoAOMT1	Solyc02g093270	qSICCOAOMT1 F1	GAGAGCCTGAAGCCATGAAAGAGC	qPCR	(Rahim <i>et al.</i> , 2019)
		qSICCOAOMT1 R1	GAGCCATGGCAGTAGCAAGCAGAG		
CCoAOMT6	Solyc01g107910	qSICCOAOMT6 F1	ATTTTCGAGAGGGCCCTGCTTTAC	qPCR	(Rahim <i>et al.</i> , 2019)
		qSICCOAOMT6 R1	ATCCGATCACACCACCAACTTTCA		
HCT	Solyc03g117600	qSIHCT F1	CCCTCCTCCGTGCTCGTGA	qPCR	(Rahim <i>et al.</i> , 2019)
		qSIHCT R1	CCCGGGTTAGTTTGAAGATTGACA		
C3H	Solyc01g096670	qSIC3H F1	CTGCAATGCGTGGCCAAGGAAGC	qPCR	(Rahim <i>et al.</i> , 2019)
		qSIC3H R1	TCGCGAGCAACAGCCAGACATT		
4CL	Solyc12g094520	qSL4CL F1	CGA GCA TGG AAG GGA AAA TTG	qPCR	(Rigano <i>et al.</i> , 2016)
		qSL4CL R1	TCA GAG TCT AGA GTG GAA GCA G		
C4H	TC93956	qSIC4H F1	CTAGCTAACAACCCCGCCCA	qPCR	(Zhang <i>et al.</i> ,



		qSIC4H R1	AACTCCTCCTGCCAACACCG		2019)
THT 7-8	Solyc08g068780	qSLTHT 7-8 F1	GGAAACTGATAAGGAGAAGGTGG	qPCR	This paper
		qSLTHT 7-8 R1	GTTTGCACGGCGTATGGAG		
THT like 4	Solyc08g068710	qSLTHT4 F1	AGTTTAGGTATGGCAAATTGCATGG	qPCR	This paper
		qSLTHT4 R1	AAGAAAACACACAGTAGCTAACAGC		
THT like 5	Solyc08g068690	qSLTHT6 F1	TCAGTCGATGGAATAGTAGCAGTT	qPCR	This paper
		qSLTHT6 R1	TCCTCAATTTCCCCCTTGTTATG		
CYP86 A1	Solyc06g076800	qSICYP86A1 F1	GGTCTACTGGTGTATCCGCA	qPCR	This paper
		qSICYP86A1 R1	CCTTTAGGATAGTTATCGAACCTGG		
KCS1	Solyc10g009240	qSIKCS1 F1	GTCGTAGGGGTGCTACTAGC	qPCR	This paper
		qSIKCS1 R1	GTCATGAAAAACCTGAATTGCTCAG		
GPAT5	Solyc04g011600	qSIGPAT5 F2	CCCTAGGCCAATGTATGAGGTAAC	qPCR	This paper
		qSIGPAT5 R2	GTTGCTGCCAAAATCCTCTGG		
DAISY/ KCS2	Solyc05g009280	qDAISY F1	TCCGAGTTCATCCCAAGTCG	qPCR	This paper
		qDAISY R1	AACAGTATGGCTGCACCTCC		
FAR3	Solyc06g074390	qSIFAR3 F1	TGGTGCTACTGGATTTCTTGC	qPCR	This paper
		qSIFAR3 R1	TGCCACAGCCTCATTGTTGA		
THT 7-1	Solyc08g068700	qSITHT7-1 F1	GCTTGAACGCTTGGTTAGTGG	qPCR	This paper
		qSITHT7-1 R1	AGTCCTCCTTAGAGGGCTTGC		
CY986 B1	Solyc02g014730	qSICYP86B1 F1	TCCGTTGATTTCAAGCCAGC	qPCR	This paper
		qSICYP86B1 R1	TCGTCTCAACAACCTCTTTGTG		



**Figure S1: H7996 plants show mild symptoms upon challenge inoculation of *R. solanacearum*.** Susceptible (Marmande) and resistant (H7996), 5-week-old tomato plants were inoculated through roots by soil-soak with  $\sim 1 \times 10^7$  CFU/ml of *R. solanacearum* GMI1000 and incubated at 28°C. **(A)** Wilting progress was assayed in both cultivars by rating plants daily on a 0 to 4 disease index scale where 0 = healthy and 4 = 100% wilted. Data presented are means  $\pm$  SE of a representative experiment with  $n=20$  plants for each cultivar, out of a total of 3 experiments. **(B)** The level of in planta colonization by *R. solanacearum* was calculated as colony forming units per gram of fresh taproot tissue (CFU-g<sup>-1</sup>) at the indicated days post-infection (dpi). Data presented are of a representative experiment with  $n=10$  plants for each time point each cultivar out of a total of 3 experiments. Asterisks indicate statistically significant difference between Marmande and H7996 in a paired Student's t-test (\*\* p-value of  $p < 0.01$  and \*\*\* p-value of  $p < 0.001$ ). Supports Figure 1 of the main manuscript.

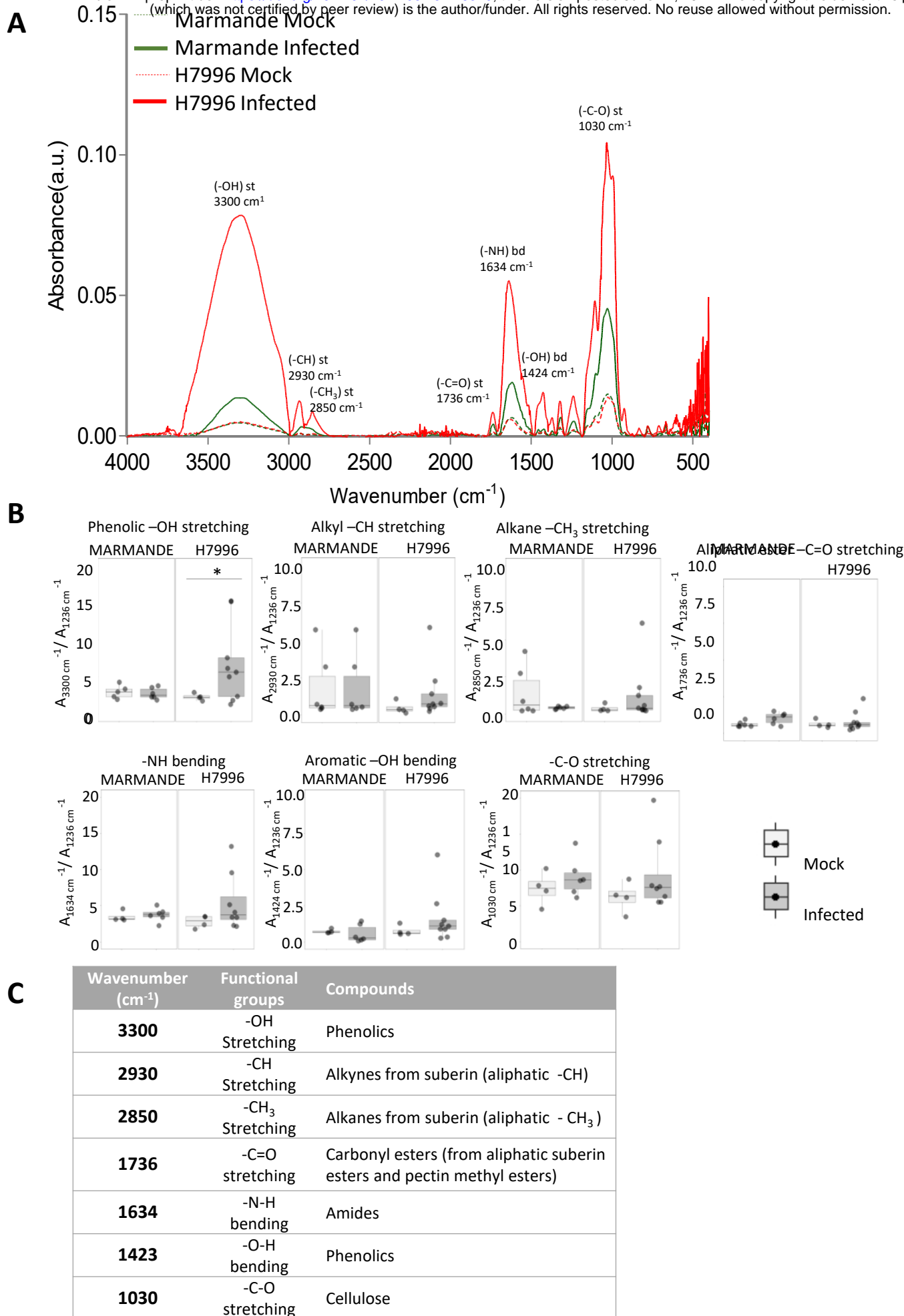


Figure S2: Legend on the next page

**Figure S2: FT-IR showed significantly high induction of phenolics in the xylem vasculature of resistant H7996.** Taproot cross-sections of H7996 and Marmande plants, water-treated or *R. solanacearum*-inoculated by soil soak and containing bacteria  $10^5$  CFU  $g^{-1}$  were analyzed using a FT-IR spectrophotometry in areas adjacent to the vasculature. **(A)** Average absorbance in the range of 500-4000  $cm^{-1}$  is shown for both cultivars water treated or infected with *R. solanacearum*. **(B)** The relative absorbance ratios of the most prominent peaks in (A) were calculated for phenolic -OH stretching ( $\approx 3300$   $cm^{-1}$ ), alkyl -OH stretching ( $\approx 2930$   $cm^{-1}$ ), alkane -CH<sub>3</sub> stretching ( $\approx 2850$   $cm^{-1}$ ), aliphatic ester -C=O stretching ( $\approx 1736$   $cm^{-1}$ ), -NH bending ( $\approx 1634$   $cm^{-1}$ ), aromatic -OH bending ( $\approx 1424$   $cm^{-1}$ ) and -C-O stretching ( $\approx 1030$   $cm^{-1}$ ) by using the absorbance at 1236  $cm^{-1}$  as a reference. The asterisk (\*) indicates statistically significant differences ( $\alpha=0.05$ , Student's t-test). **(C)** Correspondence between wavenumbers (mean of vibrational range), functional groups and compounds. Assignments based on the literature (Dorado *et al.*, 2001; Martín *et al.*, 2005, 2008; Lahlali *et al.*, 2017). Supports Figure 3 of the main manuscript.

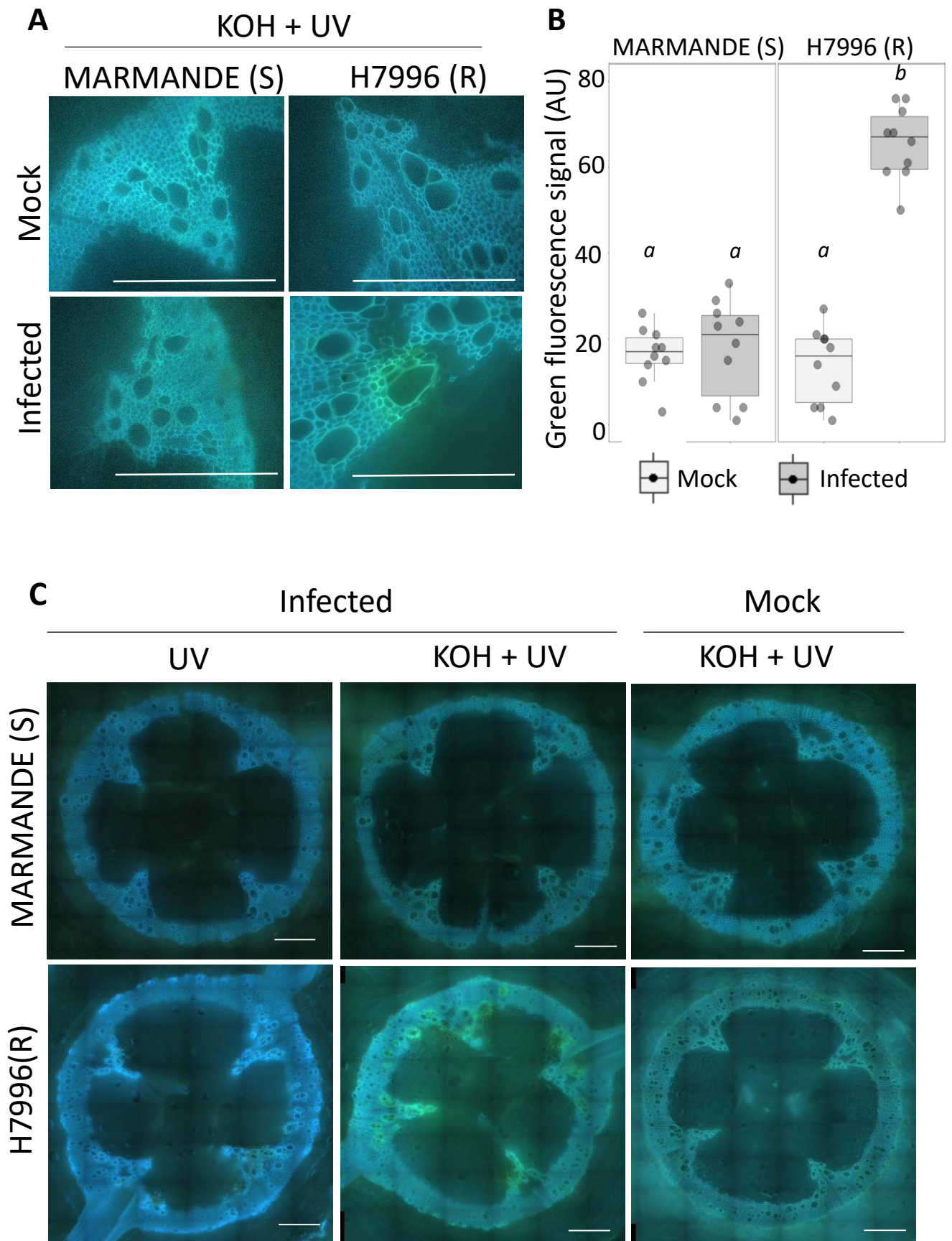


Figure S3: Figure legend on the next page

**Figure S3: *R. solanacearum*-induced xylem vascular ferulic acid deposition occurs in resistant H7996, but not in susceptible Marmande.** Cell wall-bound ferulic acid can be detected by emission of a blue fluorescence with UV excitation at neutral pH, which characteristically changes to stronger green emission under conditions of high pH such as in the presence of alkali. Five-week-old Marmande and H7996 plants were inoculated with  $\sim 1 \times 10^7$  CFU/ml of *R. solanacearum* GMI1000 and incubated at 28°C. Taproot cross-sections were obtained at 9 dpi **(A)** or in plants containing a bacterial load of approximately  $10^5$  CFU g<sup>-1</sup> of *R. solanacearum* **(B, C)**. **(A)** Autofluorescence emitted from taproot cross-section from mock-treated and infected Marmande and H7996 plants was visualized at 9 dpi under UV before and after treatment with KOH alkali (high pH above 10). In infected H7996 a green/turquoise color appears in vessel walls and surrounding xylem parenchyma cells, indicative of ferulic acid deposition in the cell walls. Scale bars = 500  $\mu$ m. **(B)** The same as in (A) but cross-sections were obtained from plants containing a bacterial load of approximately  $10^5$  CFU g<sup>-1</sup> of *R. solanacearum*. Scale bars = 500  $\mu$ m. **(C)** Green fluorescence from ferulate deposits in the xylem and surrounding parenchyma cells was measured using ImageJ. Box-and-whisker plots show data from a single representative experiment (n =6) out of a total of 3. Different letters indicate statistically significant differences ( $\alpha=0.05$ , Fisher's least significant difference test). Supports Figure 4 of the main manuscript.

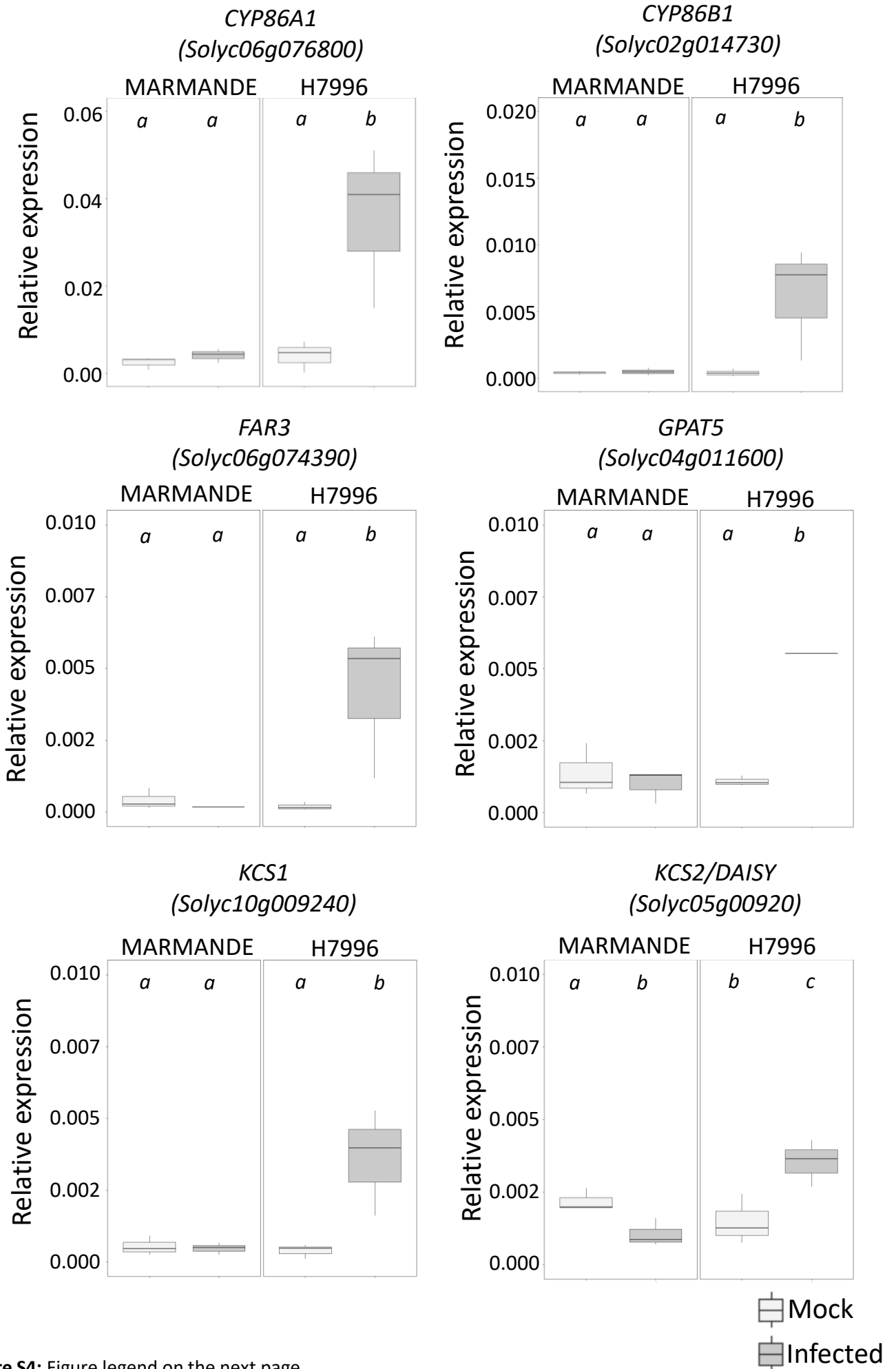
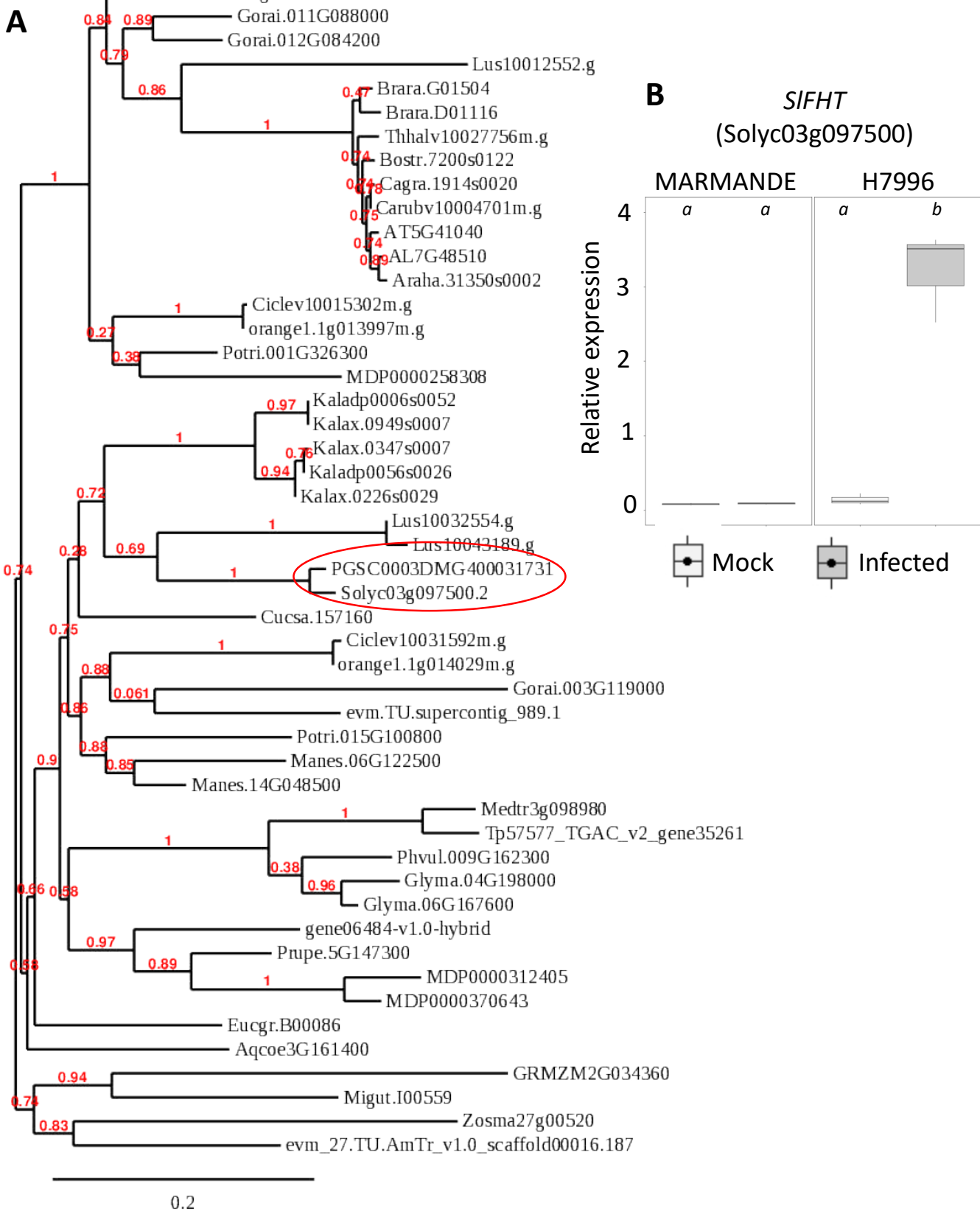


Figure S4: Figure legend on the next page

**Figure S4: Expression of suberin biosynthetic genes in xylem vasculature of taproots upon infection of *R. solanacearum*.** Expression levels of tomato putative orthologs of the suberin fatty acid pathway were analyzed by qPCR in H7996 and Marmande plants infected with *R. solanacearum* or mock-treated. Xylem vascular tissue comprising of metaxylems and surrounding parenchyma cells was collected from infected plants with a *R. solanacearum* inoculum of  $10^5$  CFU  $g^{-1}$  in the taproot or mock-inoculated plants of a similar age. Relative expression values were calculated using the Elongation Factor 1 alpha (eEF1  $\alpha$ ) gene as reference. Three biological replicates (n=3) were used, and taproots of 6 plants were used in each replicate. Different letters indicate statistically significant differences ( $\alpha=0.05$ , Fisher's least significant difference test). Supports Figure 5 of the main manuscript.





**Figure S5: Phylogeny of Feruloyl transferase (FHT) orthologues in different plant species and expression of the putative tomato FHT ortholog in response to *Ralstonia solanacearum* infection. (A)** Protein homologs of potato *FHT* gene (*PGSC0003DMG400031731*) were obtained from [www.phytozome.jgi.doe.gov](http://www.phytozome.jgi.doe.gov) and matches with more than 80 % similarity were used for phylogenetic analysis using [www.phylogeny.fr](http://www.phylogeny.fr). **(B)** Gene expression of the putative tomato *FHT* ortholog (*Solyc03g097500*) was analyzed by qPCR. Relative expression levels were calculated using the Elongation Factor 1 alpha (*eEF1  $\alpha$* , *Solyc06g005060*) as the reference gene. H7996 and Marmande plants, containing a *R. solanacearum* inoculum of  $10^5$  CFU  $g^{-1}$  in the taproot were selected. Xylem vascular tissue, comprising of metaxylems and surrounding parenchyma cells was collected from taproots for RNA extraction and cDNA synthesis. Similarly, xylem tissue was collected from Marmande mock plants and H7996 mock plants. Three biological replicates ( $n=3$ ) were used, and taproots of 6 plants were used in each replicate. Different letters indicate statistically significant differences ( $\alpha=0.05$ , Fisher's least significant difference test). Supports Figure 5 of the main manuscript.

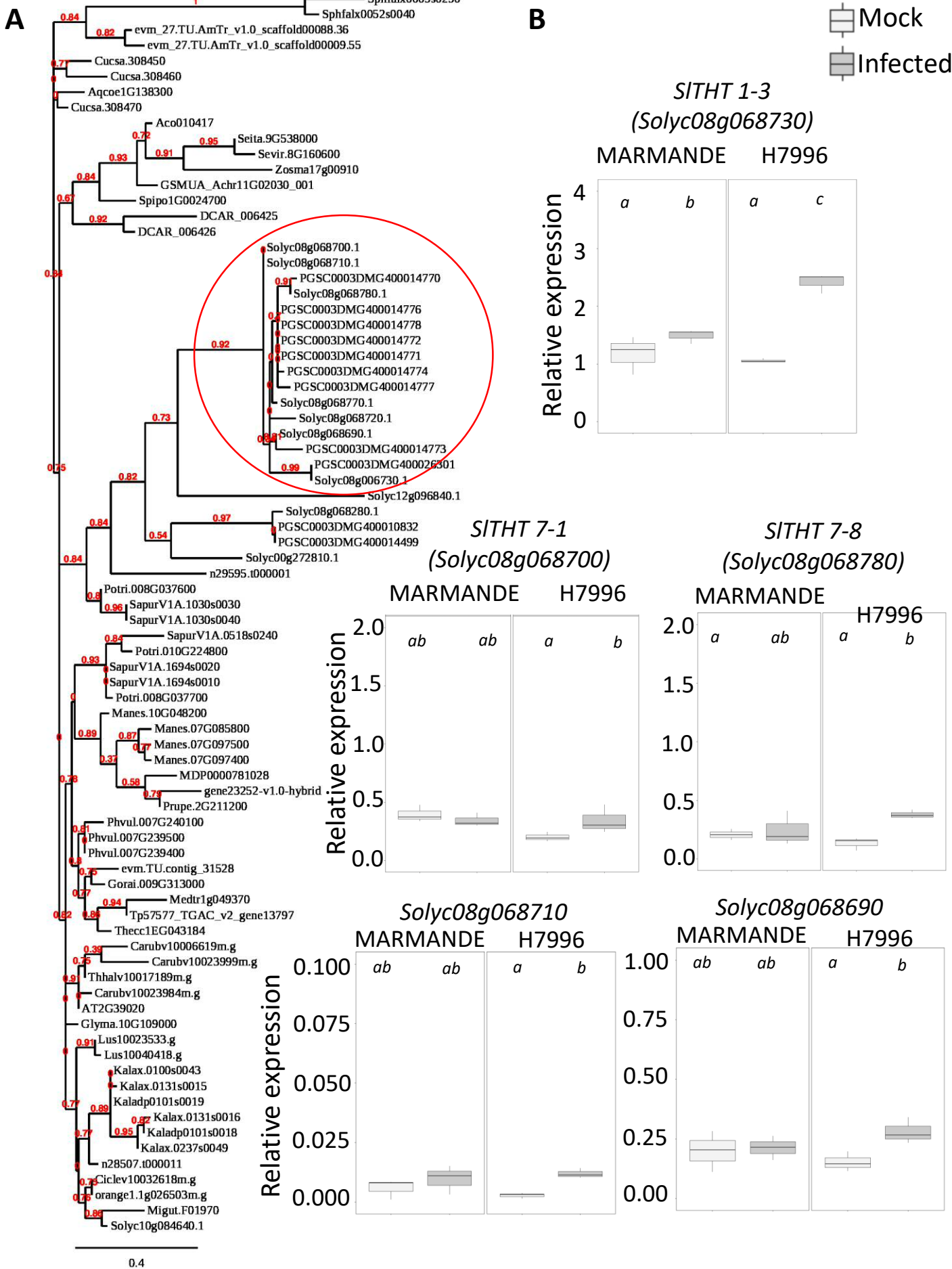


Figure S6: Figure legend on the next page

**Figure S6: Phylogeny of tyramine hydroxycinnamoyl transferase (THT) orthologues in different plant species and expression of the tomato THT gene family members in response to *R. solanacearum* infection. (A)** Protein homologs of tomato *THT1-3* gene (*Solyc08g068730*) were obtained from [www.phytozome.jgi.doe.gov](http://www.phytozome.jgi.doe.gov) and matches with more than 60 % similarity were used for phylogenetic analysis using the webpage [www.phylogeny.fr](http://www.phylogeny.fr). **(B)** Gene expression of the tomato *THT* gene family members was analyzed by qPCR. Relative expression levels were calculated using the Elongation Factor 1 alpha (*eEF1 α*, *Solyc06g005060*) as the reference gene. H7996 and Marmande plants, containing a *R. solanacearum* inoculum of  $10^5$  CFU  $g^{-1}$  in the taproot were selected. Xylem vascular tissue, comprising of metaxylems and surrounding parenchyma cells was collected from taproots for RNA extraction and cDNA synthesis. Similarly, xylem tissue was collected from Marmande mock plants and H7996 mock plants. Three biological replicates (n=3) were used, and taproots of 6 plants were used in each replicate. Different letters indicate statistically significant differences ( $\alpha=0.05$ , Fisher's least significant difference test). Supports Figure 5 of the main manuscript.

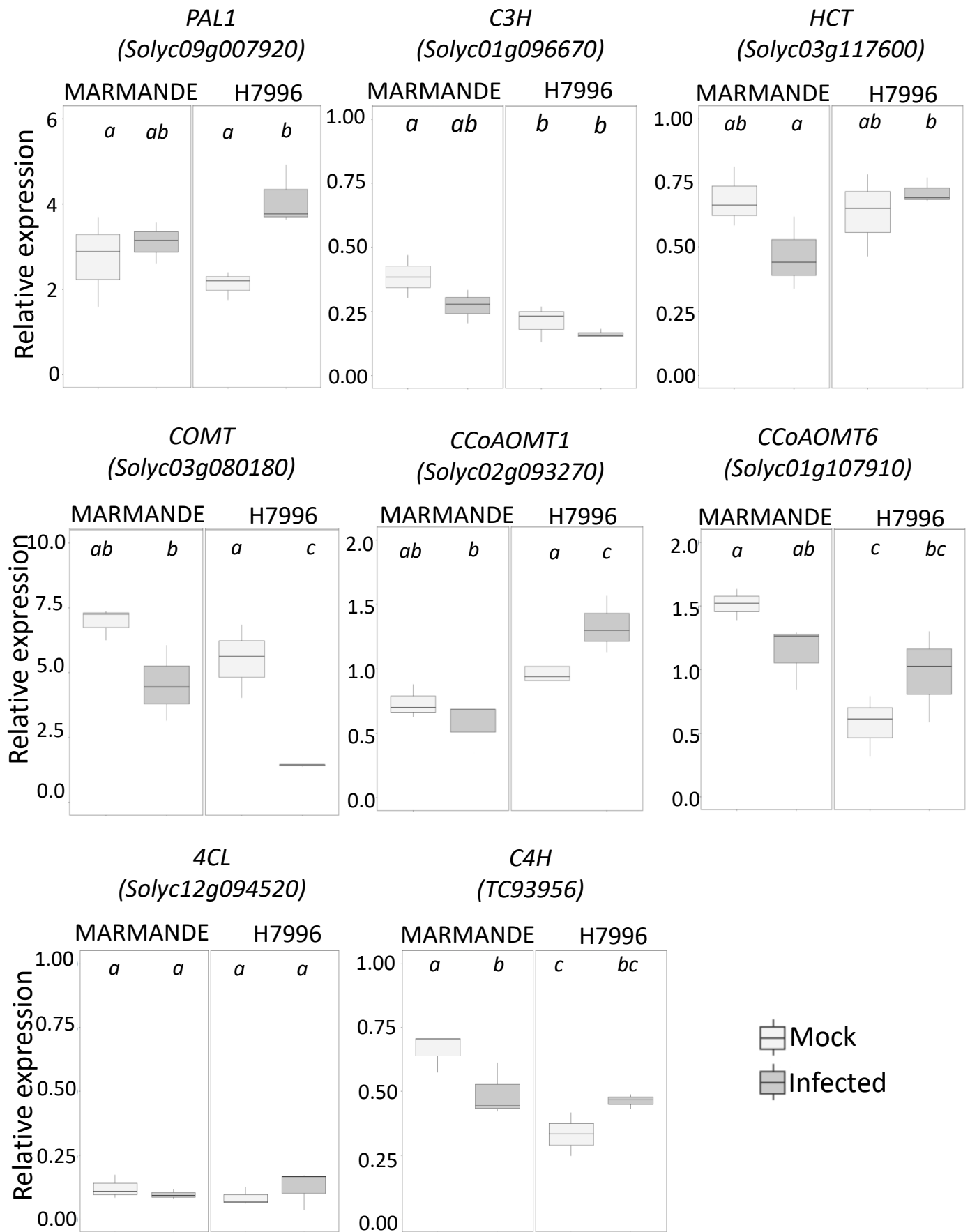
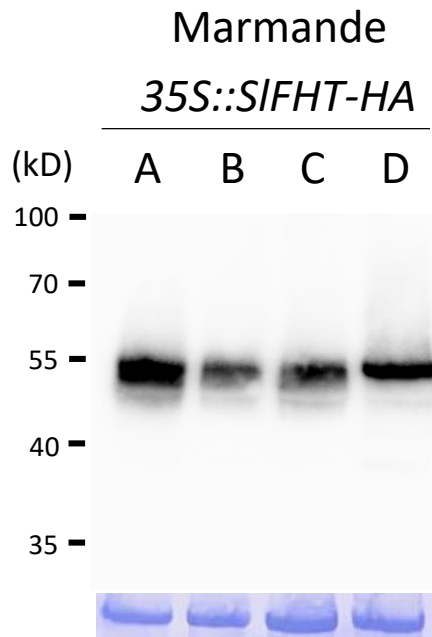


Figure S7: Figure legend on the next page

**Figure S7: Expression of phenylpropanoid pathway genes in xylem vasculature of taproots upon invasion of *R. solanacearum*.** Expression levels of tomato putative orthologs of the phenylpropanoid pathway were analyzed by qPCR in H7996 and Marmande plants infected with *R. solanacearum* or mock-treated. Xylem vascular tissue comprising of metaxylems and surrounding parenchyma cells was collected from infected plants with a *R. solanacearum* inoculum of  $10^5$  CFU g<sup>-1</sup> in the taproot or mock-inoculated plants of a similar age. Relative expression values were calculated using the Elongation Factor 1 alpha (*eEF1  $\alpha$* ) gene as reference. Three biological replicates (n=3) were used, and taproots of 6 plants were used in each replicate. Different letters indicate statistically significant differences ( $\alpha=0.05$ , Fisher's least significant difference test). Supports Figure 5 of the main manuscript.



**Figure S8: Immunoblot of SIFHT-HA in independent Marmande tomato lines expressing *35S::SIFHT-HA* (Marmande).** Immunoblot using anti-HA antibody showing SIFHT-HA protein (predicted protein size: 49kDa) levels of 4 independent transgenic lines (A, B, C D) stably overexpressing *SIFHT-HA* on a susceptible Marmande background (*35S::SIFHT-HA*). In the bottom panel Coomassie blue staining showing similar protein load in all the lanes is shown. Supports Figure 6 of the main manuscript.

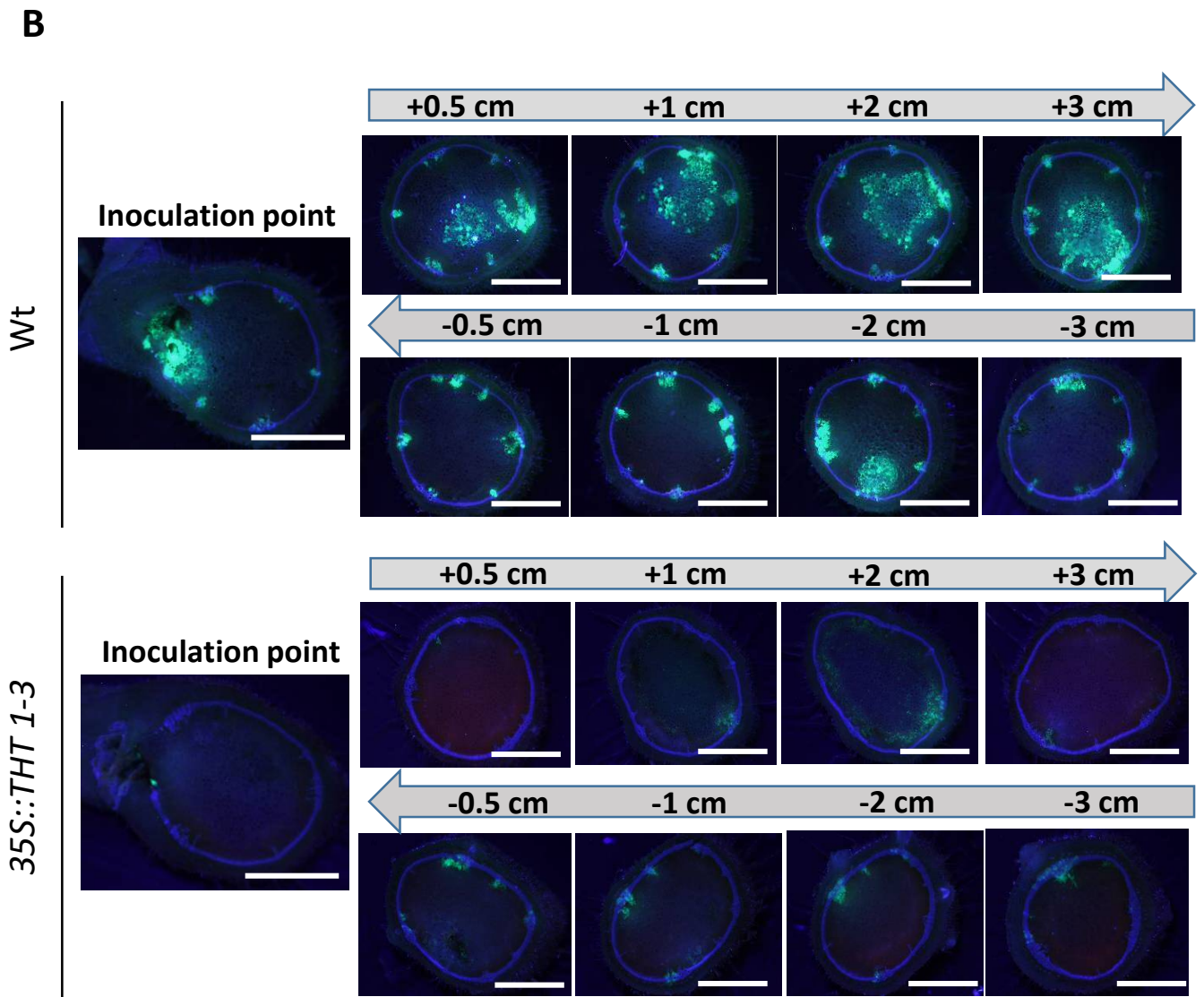
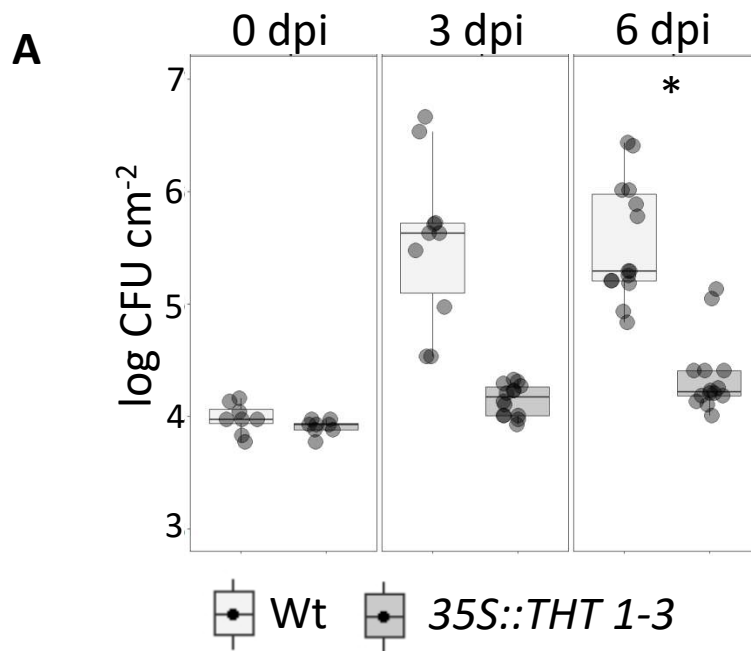


Figure S9: Figure legend on the next page

**Figure S9: Overexpression of *SITH1-3* in tomato results in restricted colonization by *R. solanacearum*.** **(A)** Growth of *R. solanacearum* GMI1000 in planta was monitored in leaves of *35S::THT1-3* transgenic plant compared with Wt MoneyMaker tomato lines over time. The bacterium was vacuum infiltrated into the leaves at a concentration of  $\sim 1 \times 10^5$  CFU/ml and growth was recorded at 0, 3 and 6 dpi. Box-and-whisker plots show data of 6 to 8 independent plants ( $n=6-8$ ) from a representative experiment out of 3. Asterisk indicates statistically significant difference between Wt and overexpression line in a paired Student's t-test (\* corresponds to p-value of  $p < 0.05$ ). **(B)** Representative images of tomato stem cross-sections showing colonization by the *R. solanacearum* GMI1000 GFP reporter strain at 6 dpi. *R. solanacearum* was directly injected into the xylem vasculature of the first internode through the petiole at a concentration of  $10^5$  CFU ml<sup>-1</sup>. Colonization progress was analyzed at the point of inoculation, at higher (+0.5, +1, +2 and +3 cm) and lower (-0.5, -1, -2 and -3 cm) sections. Images from a representative experiment out of 3 with  $n=5$  plants each. Scale bar = 2 mm. Supports Figure 7 of the main manuscript.



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