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Tomato response traits to pathogenic *Pseudomonas* species. Does nitrogen limitation matter?

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Abstract

Induced chemical defence is a cost-efficient protective strategy, whereby plants induce the biosynthesis of defence-related compounds only in the case of pest attack. Plant responses that are pathogen specific lower the cost of defence, compared to constitutive defence. As nitrogen availability (N) in the root zone is one of the levers mediating the concentration of

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defence-related compounds in plants, we investigated its influence on response traits of
tomato to two pathogenic bacteria, growing plants hydroponically at low or high N supply.
Using two sets of plants for each level of N supply, we inoculated one leaf of one set of plants
with *P. syringae*, and inoculated the stem of other set of plants with *P. corrugata*. Tomato
response traits (growth, metabolites) were investigated one and twelve days after inoculation.
In infected areas, *P. syringae* decreased carbohydrate concentrations whereas they were
increased by *P. corrugata*. *P. syringae* mediated a redistribution of carbon within the
phenylpropanoid pathway, regardless of N supply: phenolamides, especially
caffeoylputrescine, were stimulated, impairing defence-related compounds such as
chlorogenic acid. Inoculation of *P. syringae* produced strong and sustainable systemic
responses. By contrast, inoculation of *P. corrugata* induced local and transient responses. The
effects of pathogens on plant growth and leaf gas exchanges appeared to be independent of N
supply. This work shows that the same genus of plant pathogens with different infection
strategies can mediate contrasted plant responses.

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Key words: Tomato-*Pseudomonas* pathogenic interactions; N limitation; carbohydrates;
phenolics; elicitation; systemic response

43 44 45 46 47 48 49 50 51 52 53 **Highlights**

- 43 • Inoculation of *P. syringae* produced strong and sustainable systemic responses
- 44 • By contrast, inoculation of *P. corrugata* induced local and transient responses
- 45 • *P. syringae* mediated a redistribution of carbon within the phenylpropanoid pathway
- 46 • The study provides novel assessments of *P. corrugata*-tomato pathogenic interaction

54 55 56 57 58 59 60 61 62 63 64 65 **Abbreviations**

CO₂. carbon dioxide

DAI. days after inoculation

1 DAS. days after sowing

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3 DW. dry weight

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6 HN. high nitrogen

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8
9 LN. low nitrogen

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11 PPF. photosynthetic photon flux density

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14 [X]. concentration of X

1. Introduction

During the process of evolution, plants have acquired several natural defence mechanisms and specific barriers that act as protection against pests. This inherent protection, however, does not provide adequate defence in field crop production, as can be inferred from the regular practice of pesticide application required in conventional agriculture. In the current context of "green" agriculture, there is therefore an important challenge for agronomists to strengthen natural defence mechanisms of crop plants. Lowering the use of nitrogen (N) fertilisers and pesticides by crops forms a major part of the emerging concept of ecological intensification, which seeks to maximise primary production per unit area without compromising the ability of the system to sustain its productive capacity [1, 2]. It is well established that nitrogen availability influences the resistance of plants to pathogens [3-6] and that N limitation or deficiency enhances the concentration of certain defence-related compounds in plant tissues [7-11]. It is reasonable to postulate, therefore, that controlling N fertilisation may offer some practical agronomic leverage to strengthen plant defence by enhancing the composition and concentrations of defensive compounds in plant tissues. This approach accords with hypotheses that secondary metabolism is stimulated when nutrient resources in the environment are limited, thereby raising plant defence at the expense of plant growth [12]. For example, it has been reported that the susceptibility of tomato plants to *Pseudomonas syringae* decreases under low N fertilisation rates as compared to high rates [4] although the opposite effect has also been reported for other pathogens. This is the case for the susceptibility of tomato to *Botrytis cinerea*, which has been shown to decrease under increasing N fertilisation rates [3, 13, 14]. The effect of N on plant defence, therefore, appears to be pathogen-dependent, illustrating the complexity of plant-pathogen interactions. Plant defence involves a great diversity of mechanisms operating at molecular, cellular and whole-plant levels. Untangling this complexity of plant-enemy interactions is thus a major

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26 challenge for research. Work in this area, is often focused on restricted aspects of plant
27 defence. However, additional studies are required to identify the traits of plant response to
28 pathogens. These include, for example, changes in plant architecture and in plant metabolism
29 such as the balance between primary and secondary pathways. A greater knowledge of these
30 traits would be expected to allow mediation of plant defence through crop/plant management
31 or plant breeding.

32 In this study we investigated the response of adequately N-supplied vs N-limited tomato
33 plants to two bacteria of the genus *Pseudomonas* (*P. syringae* pv. *tomato* and *P. corrugata*),
34 that are capable of depressing crop yields of tomatoes produced either in open fields or
35 greenhouses. *P. syringae* pv. *tomato* is the causal agent of bacterial speck (dark brown
36 necrotic spots surrounded by a yellow halo) which preferentially attacks the leaves [15]. *P.*
37 *corrugata* is the causal agent of pith necrosis (brown to black necrotic areas along the stem
38 that may extend over several centimetres), which exclusively attacks the stems. On a
39 longitudinal stem section, the pith appears brown, necrotic and ultimately decomposes [16,
40 17]. Very few studies have described the responses of both primary and secondary
41 metabolism of tomato to *Pseudomonas* infection [18, 19]. Some studies have focused on the
42 plant growth response and concentrations of primary metabolites (amino acids,
43 carbohydrates) [15, 20]. Others have reported on defence mechanisms, including synthesis of
44 defence metabolites [21-23]. As far as we are aware, nothing is known concerning changes in
45 the metabolic profile of tomato tissues in response to the pathogen *P. corrugata*.

46 The main objective of this work was to assess the importance of N nutrition on chemical
47 plant defence in the context of healthy versus pseudomonas-attacked tomato plants. In this
48 respect we investigated the metabolic and ecophysiological changes of young hydroponically
49 grown plants occurring during the first few hours and days following infection by these two
50 *Pseudomonas* species. We tested the hypothesis that N limitation restricts the development of

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51 symptoms by strengthening the tomato defensive responses. We also tested the hypothesis
52 that tomato responses to *P. syringae* and to *P. corrugata* differ both as a consequence of
53 elicitation of different metabolic pathways and differences in pathogen behaviour towards
54 available resources (carbohydrates, nitrogen). The main traits monitored included net CO₂
55 assimilation rate, non-structural carbohydrates, and organ concentrations of phenolic
56 compounds and tomatine.

57 The specialized metabolites targeted correspond to two major metabolic pathways - the
58 ubiquitous phenylpropanoid pathway derived from phenylalanine (rutin, chlorogenic acid,
59 kaempferol rutinoside, caffeoylputrescine...) and the glycoalkaloid pathway, which typically
60 produces tomatine in tomato. All these metabolites are present in tomato [11] where they are
61 considered as putative defence-related compounds [23, 24] and their concentrations are
62 mediated by N availability [7, 9, 11].

63 We investigated these plant traits 24 h and 12 days after infection, in order to identify short-
64 term responses, to evaluate their sustainability and eventually to assess longer-term
65 processes. We considered that after 12 days there was a settlement in the reaction cascade
66 following infection, including impacts on the carbon economy (C assimilation, regulation of
67 source-sink interaction, partitioning of C to reserve compounds, [25]), on the concentration of
68 specific metabolites elicited and probably on the dry matter partitioning.

69 **2. Materials and methods**

70 ***2.1 Plant material, growth conditions and experimental design***

71 Tomato plants *Solanum lycopersicum* L. cv Better Bush (Park Seed Co., Hodges, SC, USA -
72 resistant to *Verticillium* wilt and *Fusarium* wilt) were raised on a hydroponic system set up in

73 a growth chamber maintained at 25°C and 85% humidity. Germinating seeds were retained in
74 the dark for the first three days then illuminated with a 16h photoperiod at 60% humidity.
75 Ten days after sowing (DAS), seedlings were transferred to two independent Nutrient Film
76 Technique (NFT) systems set up in twin growth chambers (16h photoperiod, 25°C in air and
77 nutrient solutions, 60% air humidity). Nineteen DAS (i.e. seven days before *Pseudomonas*
78 inoculation), the ambient conditions were changed to 21°C and 90% humidity. All
79 temperature sensors were calibrated in a water bath with 0.01°C precision (Haake model
80 C35/F6, Karlsruhe, Germany).
81 Two contrasting N nutrition regimes were imposed. Half of the plants were fed with a N
82 supply previously shown to be non-limiting for tomato growth [10, 24, 26] and presently
83 referred to as "High N" (HN). This consisted of a complete nutrient solution described below,
84 with N provided as NO₃⁻ at a concentration continuously regulated at 1.5 mM N. The second
85 regime, previously shown to limit plant growth [9, 27] and referred to as "Low N" (LN),
86 contained 0.15 mM N at the start of the experiment. It was adjusted every day using an
87 automatic system that limited the daily N uptake of the LN plants to only 30% of that of HN
88 plants [10, 24, 28]. The nitrate uptake rate calculations followed the unbiased procedure
89 described by Adamowicz and Le Bot [29].
90 The complete HN nutrient solution was prepared with demineralised water and analytically
91 pure salts at the following concentrations: KNO₃ (0.6 mM), KH₂PO₄ (1 mM), K₂SO₄ (0.7
92 mM), Ca(NO₃)₂ (0.3 mM), CaSO₄ (3.2 mM), Mg(NO₃)₂ (0.15 mM) and MgSO₄ (1.4 mM).
93 The LN solution at 0.15 mM NO₃ was composed of KNO₃ (0.06 mM), KH₂PO₄ (1 mM),
94 K₂SO₄ (0.97 mM), Ca(NO₃)₂ (0.03 mM), CaSO₄ (3.47 mM), Mg(NO₃)₂ (0.13 mM) and
95 MgSO₄ (1.49 mM). Sulphate was chosen as the compensating anion for nitrate variation so
96 that in both solutions the concentrations of cations and H₂PO₄⁻ were the same and the anionic
97 charge [NO₃⁻] + 2 × [SO₄²⁻] remained constant and equal to 12 mM.

1 98 Micronutrients were provided as a "ready to use" commercial mixture of Kanieltra (Hydro
2 99 Azote, France) formula 6 Fe (0.1 l m^{-3}) and EDTA-Fe ($43 \text{ }\mu\text{M}$). Solution volumes, pH and
3
4 100 NO_3^- concentration were measured, monitored and regulated automatically by the Totomatix
5
6 101 system [28]. Nitrate adjustments were made by automatic additions of 1mM stock solution of
7
8 102 NO_3 containing (in mol l^{-1}) 0.408 KNO_3 , 0.204 $\text{Ca}(\text{NO}_3)_2$ and 0.092 $\text{Mg}(\text{NO}_3)_2$ [28].
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12 103 The experimental design comprised six randomised blocks equally distributed between the
13
14 104 twin growth rooms. In each block the plants were fed with the complete nutrient solutions
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16 105 containing the HN and the LN regimes. For each N regime, the plants were inoculated with
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18 106 either water (as a mock inoculation) or a suspension of the bacterium (*P. syringae* or *P.*
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20 107 *corrugata*).
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26 108 ***2.2 Plant inoculation procedure using pathogenic Pseudomonas***

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29 109 We used strain DC3000 of *P. syringae*, initially isolated in 1960 in Guernsey [30] and strain
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31 110 CFBP 5307 of *P. corrugata*, initially isolated in 1980 in France and kindly provided by Dr.
32
33 111 Cindy Morris (INRA, Avignon).
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36 112 The *P. syringae* complex is composed of numerous genetic lineages of strains from both
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38 113 agricultural and environmental habitats (including habitats closely linked to the water cycle).
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40 114 DC3000 strain is part of Phylogroup 1 which contains many isolates from diseased plants
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42 115 [31].
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46 116 *P. corrugata* has the capacity to adapt to a large diversity of habitats (soil, plants). This
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48 117 bacterium is pathogenic on tomato (pith necrosis) but not of other plants such alfalfa [32]. It
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50 118 is more closely related to *Pseudomonas fluorescens* and it is outside the *P. syringae* species
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52 119 complex [33].
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55 120 Inoculations were performed 26 DAS, either on the leaves or on the stems, depending on the
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57 121 pathogen. Inoculations of *P. syringae* consisted in infiltrating 100 μl aliquots of bacterial
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122 suspension within the terminal leaflet of the fourth leaf (L4 - numbered from the cotyledon
123 leaves) with a needleless syringe. Mock inoculations were carried out similarly with sterilised
124 ultrapure water on control plants. The contours of infiltrated areas were traced with a felt pen.
125 Inoculations with *P. corrugata* consisted in injecting with 10 µl aliquots of a bacterial
126 suspension into the internode between leaves L4 and L5. The inoculum was set at the centre
127 of the stem, using a 20 µl pipette tip. The same procedure was applied to control plants with
128 the same volume of sterilised ultrapure water.

129 Both bacterial inocula (at a final concentration of approximately 10^8 cells ml⁻¹) were prepared
130 from 48h cultures on KBC medium at 22-25°C [34]. We deliberately applied a high-titer
131 inoculation (as made in other studies, for instance [35]), in order to maximize the chances of
132 induction of a defence reaction from the plant, although such a high dose does not always
133 induce a response ([30, 36]). Our objective was not to assess the evolution of the symptoms
134 but to induce the hypersensitive-like reaction allowing ecophysiological and phytochemical
135 investigations of plant responses.

136 **2.3 Net CO₂ exchange rates**

137 The CO₂ exchange rates (CER) were measured 27 DAS (*i.e.* one day after inoculation, DAI)
138 on leaves of plants, which were later to be sampled at 38 DAS, using a portable
139 photosynthesis system (Li-Cor 6400, Li-Cor, Lincoln Nebraska, USA) equipped with a LED
140 light source (6400-02B red/blue diodes, Li-Cor, Lincoln Nebraska, USA). Temperature,
141 [CO₂] and humidity were set at growth chamber ambience. Each measurement comprised two
142 steps. Firstly, the LED illuminator was switched on and adjusted at the growth chamber
143 PPF (190 µmol photons m⁻² s⁻¹) in order to measure net photosynthesis. Secondly, the
144 illuminator was switched off in order to measure respiration in the dark. The measurements
145 were made on the terminal leaflet of the fourth true leaf (L4) using 6 plants per treatment. For

146 plants inoculated at leaf L4 with *P. syringae*, measurements were made on tissue close to (but
147 not including) the inoculated area.

148 ***2.4 Plant sampling and sample preparation***

149 To minimise the risks of mechanical stress and putative elicitation of metabolic pathways,
150 plants were handled with caution throughout the experiment. Plant samples were collected 27
151 and 38 DAS (i.e. 24 hours and 12 days after inoculation, DAI). The inoculated organs and
152 those adjacent (not in contact with *Pseudomonas*) were sampled separately to assess whether
153 the chemical responses to the pathogen were localised or systemic. For plants inoculated with
154 *P. syringae* and for the water-inoculated control plants, the inoculated leaf (L4) was sampled
155 separately. The two adjacent leaves (L3 and L5) were combined and stems were also
156 sampled. For plants inoculated with *P. corrugata* and the corresponding control plants, the
157 inoculated internode was sampled separately and the two adjacent leaves (L4 and L5) were
158 combined. In all cases, the remaining leaves and stems were pooled for shoot biomass
159 determination. The whole inoculated organ (L4, internode) was sampled for analyses, the
160 inoculated area representing only a small fraction of the whole organ (see Fig. 2).

161 At sampling dates, the leaf area of each plant was measured (Li-3100 C area meter, Li-Cor,
162 Lincoln Nebraska, USA). The sampled organs were immediately frozen in liquid nitrogen,
163 then stored at -20°C until freeze-drying. The dry samples were weighed, ground to a fine
164 powder and further stored at -20°C.

165 ***2.5 Assessment of plant reaction***

166 At the second sampling (12 DAI and 38 DAS), photographs were taken of the inoculated and
167 mock-inoculated leaves and stems (cut in half lengthwise) in order to assess whether a
168 hypersensitive-like response or an invasive infection had been established (Fig. 2 a & b).

169 **2.6 Chemical analyses**

170 **2.6.1 Non-structural carbohydrates**

171 Soluble carbohydrates were extracted (10 mg dry weight (DW) / 1 ml 80% ethanol) for 30
172 min at 80°C and centrifuged (10 min, 12100 g). This operation was repeated twice and the
173 supernatant was collected and evaporated to dryness (Speedvac, Savant, USA). The residue
174 was dissolved in 1.5 ml of ultrapure water. About 10 mg of polyvinylpyrrolidone were
175 added to the extract and blended every 5 min for 15 min at 4°C in order to eliminate phenols
176 and proteins. After centrifugation at 16100 g for 10 min at 4°C, the soluble proteins were
177 eliminated by adding 450 µl of Courtonne reagent. After mixing, the extract was allowed to
178 stand for 5 min at 4°C. A spatula tip of Na₂HPO₄ (*c.a.* 4 to 5 mg) was added to precipitate
179 lead in excess. After centrifugation (10 min, 7500 g), the extract was filtered with a 0.22 µm
180 mesh and frozen until analysis [37].

181 An internal standard of rhamnose solution (20 µl at 10 mg l⁻¹, Sigma, Saint Quentin Fallavier,
182 France) was added to 10-fold diluted samples. Glucose, fructose and sucrose were separated
183 and detected on a HPLC-MS (LTQ-MS, Thermo Scientific, San Jose, CA, USA). Separation
184 was performed on a Polyamine II column (250 × 4.6 mm, YMC, Dinslaken, Germany) fitted
185 with a guard column (7.5 × 4.6 mm, YMC, Dinslaken, Germany) following the protocol of
186 Ricochon *et al.* [38].

187 The pellet from the extraction of soluble carbohydrates was vacuum dried for 10 min,
188 dissolved in 0.5 or 1 ml of 0.02N NaOH (depending on the considered organ), autoclaved and
189 centrifuged for 2 min at 12600 g. Starch was determined enzymatically on 100 to 400 µl of
190 samples depending on expected concentration [39] (Enzytec Starch Ref. 1002942, R-
191 Biopharm, Darmstadt, Germany)

192 2.6.2 Phenolics and tomatine

193 The extraction procedure was performed on 20 mg of dry powder and followed by separation
194 and quantification of soluble phenolics as described in Royer *et al.* [11].

195 Phenolics contained in the infected tissues were separated on an U-HPLC system (Shimadzu,
196 Japan) equipped with a binary solvent delivery pump and a diode array detector. Three
197 microliters of extract were separated on a C18 Zorbax Eclipse Plus (150 × 2.1 mm, 1.8µm)
198 column (Agilent, USA) by using a gradient elution from 1 to 50% MeOH 0.1% formic acid
199 (FA) in 7.1 min, then 99% MeOH 0.1% FA in 0.8 min with a flow rate of 430 µl min⁻¹. The
200 column was rinsed for 2 min with 99% MeOH 0.1% FA and re-equilibrated to the initial
201 conditions for 2 min prior to the next run. Phenolic quantification was based on the area
202 under peak determined at 320 nm and expressed relative to calibration curves with
203 chlorogenic acid, caffeic acid, ferulic acid, caffeoylputrescine, rutin and kaempferol
204 rutinoside (Sigma, Saint Quentin Fallavier, France).

205 Concentrations of chlorogenic acid, rutin and kaempferol rutinoside in the tissue adjacent to
206 the infection site were measured using HPLC (model LC-20AD, Shimadzu Corp., Kyoto,
207 Japan) on a RP-18 column (4 × 250 mm, 5 µm, Merck, Darmstadt, Germany) fitted with a
208 guard column (Lichrospher RP - 18.5 µm, Merck). The mobile phase consisted of 0.1%
209 formic acid in ultrapure water (A solvent) and 0.1% formic acid in methanol (B solvent).
210 Detection was performed with a diode array detector (200-400 nm) and quantification from
211 peak area was made on the basis of standard curves of rutin, chlorogenic acid and
212 kaempferol-rutinoside (Sigma, Saint Quentin Fallavier, France). The presence of co-eluting
213 peaks made it difficult to quantify caffeoylputrescine. This problem was resolved using an
214 MS detector (LTQ Orbitrap, Thermofisher Scientific, San José, CA, USA) operating in
215 electrospray mode (ESI). Caffeoylputrescine was quantified by following the amount of the

216 [M+H]⁺ peak at m/z 251 and using a range of known concentrations of caffeoylputrescine
217 standard (provided by Dr. Werck-Reichhart from IBMP, Strasbourg).

218 Tomatine from 2 μ l samples previously diluted 50-fold in 70% MeOH with solanine as
219 internal standard (50 μ M, Extrasynthese) was separated and detected on an HPLC-MS (LTQ-
220 MS, Thermo Scientific, San Jose, CA, USA) with the same settings as those in Royer *et al.*
221 [11].

222 **2.7 Data analysis**

223 All data were subjected to mixed two-way analyses of variance ([N] and *Pseudomonas*
224 inoculation as fixed factors and blocks as random) using the lm procedure in R software (R
225 project for Statistical computing, <http://www.r-project.org>). The statistical significance was
226 set at $p < 0.05$ and denoted * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$). Normal Q-Q plots
227 and correlation between variance and means were examined to assess the data distribution
228 and homoscedasticity. When necessary, the data were Log or power transformed.

229 **3. Results**

230 The results described in detail below consider the effects of *Pseudomonas* infection (either *P.*
231 *syringae* or *P. corrugata*) of tomato plants supplied with adequate (1.5 mM, HN) versus low
232 N (0.15 mM, LN) nitrate-N supply. The experiment compares mock inoculated (i.e. control)
233 with bacteria-inoculated plants. The data characterise these effects on stems and leaves
234 sampled outside (adjacent organs) or within the inoculated zone of tomato plants harvested 1
235 or 12 days after inoculation (DAI).

236 The development of necrotic areas induced by both *Pseudomonas* species indicated the
237 development of a reaction by the plant. Twelve DAI, necrotic areas typical of a
238 hypersensitive-like reaction on the leaves inoculated with *P. syringae* (Fig. 1a). On plants

239 inoculated with *P. corrugata* in their stem, necrotic areas expanded internally in the pith
240 around the inoculation point. Mock-inoculated control stems developed small necrotic areas
241 around the point of water inoculation limited to the zone penetrated by the micropipette tip
242 (Fig. 1b).

3.1 Plant growth and CO₂ exchange rates

244 Over the experimental period (1-12 DAI), shoot dry weights increased 4-6 fold (Fig. 2a,b).
245 LN treatment reduced growth rapidly and consistently with time. Shoot dry weight was
246 roughly halved in the LN plants compared to the HN plants (1 DAI: 1.5g for LN vs. 2.5g for
247 HN; 12 DAI: 5g for LN vs. 15g for HN ***). No difference was observed between the two
248 regimes of N nutrition on net photosynthesis (Fig. 2c) or raw photosynthesis (data not
249 shown). Low N fertilisation, however, significantly reduced leaf respiration rate in the dark
250 (Fig. 2d). There was no visual evidence of N deficiency in plants low in N supply during the
251 experiment.

252 Infection with *P. syringae* significantly restricted shoot dry weight (12 DAI *, Fig. 2a), net
253 photosynthesis (***, Fig. 2c) but it stimulated leaf respiration rate in the dark (**, Fig. 2d),
254 and reduced area of infected leaves (Fig. 1e, 1 DAI ** and 12 DAI **). By contrast, *P.*
255 *corrugata* infection produced no significant effect on shoot dry weight (Fig. 2b), net
256 photosynthesis (Fig. 2c) or leaf respiration rate in the dark (Fig. 2d), or adjacent leaf area
257 (Fig. 2f).

258 Infection with *P. syringae* did not affect the leaf area adjacent to the inoculation point (Fig.
259 2g).

260

261 **3.2 Metabolite concentrations in infected organs**

262 **3.2.1 Carbohydrates**

263 Compared to mock inoculation, the presence of *P. syringae* decreased sharply both the
264 glucose and fructose concentrations of inoculated leaves 1 DAI (***, Fig. 3a) and 12 DAI
265 (**, Fig. 3b), the effect being more pronounced 1 DAI (5-13 fold) than 12 DAI (2-5 fold).
266 One DAI, low N nutrition increased fructose concentration in mock inoculated but not
267 infected leaves (*, Fig. 3a). One DAI, *P. syringae* had no significant effect on starch
268 concentration (Fig. 3a) but 12 DAI its value decreased fourfold in response to inoculation,
269 regardless of N treatment (***, Fig. 3b). Sucrose concentration was never affected by *P.*
270 *syringae* (Fig. 3a,b).

271 One DAI, *P. corrugata* increased the stem concentrations of glucose (1.8-2.2 fold, ***),
272 fructose (1.5-2 fold, **) and sucrose (1.1-1.3 fold, *), as compared to mock inoculated stems
273 (Fig. 3c). Twelve DAI these concentration differences were no longer present between
274 control and infected plants (Fig. 3d). Starch concentration in stems was low and not
275 responsive to *P. corrugata* inoculation (Fig. 3c,d).

276 **3.2.2 Defence-related compounds**

277 According to Larbat *et al.*[24], the main phenolics found in healthy tomato leaf and stem
278 extracts are isomers of caffeoyl hexaric acid (CHA1-5), chlorogenic acid (the main form CA
279 and the minor isomers CA_a and b), quercetin derivatives (Rutin, R; Apio-rutin, AR),
280 kaempferol derivatives (Kaempferol-rutinoside, KR; Unknown kaempferol derivative, K
281 Der), caffeoylputrescine (CP; with 2 isoforms, one predominating), feruloylputrescine,
282 coumaroyltyramine and two partially described phenolics assigned as putative phenolamides
283 (Caf Der and Fer Der). Our study confirmed these findings, with 20 phenolic compounds
284 detected (Supplementary Figs. 1, 2). We also observed that LN nutrition increased the

285 concentration of all phenolic compounds in healthy plant leaves and stems, with the
286 exception of CP, Caf Der, Fer Der, and FP in stem, whose concentrations decreased under
287 LN (Fig. 4, Supplementary Tables 1, 2). LN nutrition enhanced also the concentration of the
288 glycoalkaloid tomatine (Fig. 4).

289 The presence of *P. syringae* caused large, rapid and long-lasting accumulation of
290 caffeoylputrescine in inoculated leaves (1 DAI, 20-98 fold increase, ***, Fig. 4a and 12 DAI
291 33-100 fold increase, ***, Fig. 4b) that was amplified by the HN treatment (*). *P. syringae*
292 also increased leaf Caf Der concentration (1 DAI, 2-2.8 fold increase, **; 12 DAI 1.4-2.3
293 fold increase, **; Supplementary Table 1). Inoculation with *P. syringae* immediately
294 decreased (1 DAI) the leaf concentration of chlorogenic acid (1.8-3.1 fold, ***, Fig. 4a).
295 However, this effect disappeared 12 DAI as the concentrations of all phenolic compounds
296 (except caffeoylputrescine, Caf Der and tomatine) in inoculated and mock inoculated leaves
297 were of similar values (Fig. 4a,b; Supplementary Table 1).

298 Inoculation with *P. corrugata* induced a rapid (1 DAI) and slight increase of stem
299 chlorogenic acid concentration (1.2 fold, *; Fig. 4c,d, Supplementary Table 2) but
300 caffeoylputrescine concentration was unchanged. This constituent increased rapidly (1 DAI)
301 but to a small extent. Other constituents increasing in concentrations were: Caf Der (1.4 fold,
302 *), Fer Der (1.4 fold, *), rutin (1.3 fold, *; Fig. 4c,d) and tomatine (1.2 fold, ***, insets of
303 Fig. 4b,d). All these effects disappeared 12 DAI (Fig. 4c,d; Supplementary Fig. 2;
304 Supplementary Table 2).

305 The overall comparison of U-HPLC chromatograms from organs obtained from healthy or
306 inoculated plants with *P. syringae* or *P. corrugata*, revealed that of the two pathogens tested,
307 *P. syringae* induced the most marked changes (Supplementary Figs. 1,2).

308 **3.3 Metabolite concentrations in adjacent organs**

309 **3.3.1 Carbohydrates**

310 Inoculation with *P. syringae* modified the concentrations of several metabolites in stems and
311 leaves distant from the inoculation site. One DAI, a marked decrease in the concentrations of
312 glucose (2.9 fold, *) and fructose (3.7 fold, **) occurred in the adjacent stem of the HN
313 plants only (Fig. 5a). This significant effect eventually extended to both HN and LN regimes
314 12 DAI (glucose: 2-2.2 fold, ***; fructose: 1.8-2 fold, ***; Fig. 5b). Starch concentration in
315 the adjacent stem decreased significantly 12 DAI only in LN inoculated plants (2.1 fold, **;
316 Fig. 5b). In the leaves adjacent to those inoculated with *P. syringae*, sucrose concentration
317 was slightly reduced 1 DAI (1.2 fold, *, Fig. 5c). Twelve DAI, both glucose and fructose
318 concentrations tended to decrease in the adjacent leaves of *P. syringae* inoculated plants but
319 the effect was not statistically significant (Fig. 5d).

320 By contrast, *P. corrugata* infection produced little effect on carbohydrate concentrations in
321 leaves and stems adjacent to the infection site. Only glucose and fructose concentrations
322 increased in adjacent leaves, but solely 1 DAI under HN (1.7 fold ** and 1.6 fold *,
323 respectively; data not shown).

324 **3.3.2 Defensive compounds**

325 Chlorogenic acid, rutin, kaempferol rutinoside and caffeoylputrescine concentrations in
326 adjacent tissues were used as marker molecules to assess the impacts of *P. syringae* and *P.*
327 *corrugata* inoculation on the defensive tomato compounds. One DAI, caffeoylputrescine
328 concentration increased markedly in the adjacent stem (2.2-3.4 fold, ***; Fig. 5a) and leaves
329 (1.6-2.7 fold, **; Fig. 5b). These increases were amplified 12 DAI both in stems (2-9.5 fold,
330 ***; Fig. 5b) and leaves (5.6-48.3 fold, ***; Fig. 5d). *P. syringae* decreased the concentration
331 of chlorogenic acid in the leaves adjacent to the inoculation site 1 DAI (1.1-1.3 fold, *; Fig.

332 5c) and 12 DAI (1.6-1.9 fold, ***; Fig. 5d) but this was not the case in the adjacent stems
333 (data not shown). Twelve DAI but only under LN supply, *P. syringae* inoculation reduced
334 rutin concentration in adjacent leaves (1.5 fold, *; Fig. 5d) and adjacent stems (1.7 fold, ***;
335 Fig. 5b).

336 By contrast, *P. corrugata* infection produced no significant effect on the concentrations of
337 defensive compounds in the adjacent leaves and stems (data not shown).

338 Low N nutrition increased the concentration of all phenolics and tomatine in the adjacent
339 leaves and stems while that of CP was decreased (Fig. 5).

340 4. Discussion

341 The present work reports on the effects of two pathogenic species of *Pseudomonas* on tomato
342 growth and concentrations of primary and secondary metabolites in leaves and stems. It also
343 attempts to assess whether or not N nutrition is able to modulate these responses. We
344 inoculated plants with *P. syringae* or *P. corrugata* and used appropriate mock inoculations
345 with sterile water as controls, then compared plant growth and composition responses one
346 and twelve days after the inoculation. Those two sampling dates integrate the period during
347 which the plant develops short-term metabolic responses and longer-term ones after
348 infection, a common pattern observed with other pathosystems [40-42]. In order to
349 investigate the influence of N in relation to the pathogens, the plants were either supplied
350 adequately with N or with a closely regulated low rate of N supply. By this means, we
351 obtained two groups of plants, one growing at the maximum rate allowed by the climatic
352 conditions of the growth room while growth of the other was limited by plant N uptake.
353 Growth of the low N plants lagged behind that of the high N plants by roughly 50% because
354 of lack of N. Both groups of plants, however, maintained the same photosynthetic rate per
355 unit area. These conditions therefore, fit those described in the framework of the growth-

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356 differentiation balance (GDB) hypothesis [12, 43] since growth was restricted by a lack of N
357 (Fig. 2a,b) without affecting net assimilation rate per unit leaf area (Fig. 2c). N limitation
358 lowered carbon acquisition at plant level as a consequence of restricted leaf area but not of
359 photosynthetic efficiency [26]. Such conditions are favourable to increases in secondary
360 metabolite concentrations in plant tissues [9, 44]. These clear-cut experimental conditions
361 were essential to study the tomato response traits to pathogenic pseudomonas species under
362 conditions of N nutrition limitation.

18 363 ***4.1 Pseudomonas species differed in effect on carbohydrate metabolism*** 19 20 21 364 ***during the early stage of infection***

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24 365 Sugars are a source of energy in plants but are also precursors in the biosynthesis of defence-
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26 366 related compounds [18]. While photosynthesis is clearly the major source of carbon, several
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28 367 sinks compete simultaneously for carbohydrates, such as synthesis of secondary metabolites,
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31 368 plant growth as well as pathogen development [20]. From these viewpoints *P. syringae* and
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34 369 *P. corrugata* behaved very differently.
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37 370 *P. syringae* mediated changes in carbohydrate metabolism. A rapid and sustainable decrease
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39 371 in concentrations of glucose and fructose was observed in the infected leaves, as already
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41 372 described for another strain of *P. syringae* [15] and for the pathogenic fungus *B. cinerea* [20].
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44 373 Starch, which is the main storage form of carbohydrate in tomato [27, 45, 46], was
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46 374 consequently depressed in the long term response to *P. syringae* (Fig. 3b). Contrary to a
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49 375 previous study with *B. cinerea* and tomato [20], the concentration of sucrose, a transport
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51 376 carbohydrate, did not drop after inoculation in our study. The decrease in carbohydrate
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54 377 concentration following *P. syringae* inoculation was likely the consequence of reduction in
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56 378 photosynthesis concomitant with changes in C allocation towards secondary metabolism.
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59 379 Indeed, the gas exchange rates measured in non-necrotic areas of the inoculated leaf revealed
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380 reduced photosynthesis in daylight (Fig. 2c) and enhanced respiration in the dark (Fig. 2d),
381 the latter revealing higher metabolic rates in non-infected zones. This pattern is consistent
382 with the findings of Bonfig *et al.* [47] on *Arabidopsis thaliana* infected with the same strain
383 of *P. syringae*. In tomato, *P. syringae* inoculation causes down-regulation of photosynthetic
384 gene expression [20]. As plant growth was greatly reduced following *P. syringae* inoculation
385 in the present study (Fig. 2a), the higher metabolic activity in non-inoculated zones revealed
386 enhanced plant secondary metabolism. Infection by the tobacco mosaic virus in *Nicotiana*
387 *tabaccum* leaves also mediates an alteration of the metabolic profile in infected leaves, with
388 an increase of compounds belonging to terpenoids and phenylpropanoids such as 5-
389 caffeoylquinic acid and flavonoids [42, 48].

390 Contrary to *P. syringae*, inoculation with *P. corrugata* mediated a rapid transient increase of
391 all soluble carbohydrate concentrations in stems (Fig. 3c,d), which cannot be explained by
392 changes in systemic leaf activities. Leaf necrosis was not observed; neither were net
393 photosynthetic nor dark respiration rates affected by stem inoculation (Fig. 2c,d). *P.*
394 *corrugata* infiltrates host tomatoes at stem or collar levels [49] and in our experiment,
395 necrosis developed markedly in infected stems (Fig. 1). It can thus be postulated that *P.*
396 *corrugata* impaired the performance of the vascular system, causing local sugar
397 accumulation, particularly that of sucrose, the usual form of sugar transport.

398 ***4.2 Pseudomonas species induced different effects on phenolic profiles***

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399 *P. syringae* induced a large disturbance in the phenolic profile of infected leaves, the salient
400 effect being a strong and durable induction of caffeoylputrescine and of two other putative
401 phenolamides. Caffeoylputrescine has previously been reported in healthy leaves and stems
402 of eight tomato cultivars, including Better Bush [24]. Its elicitation by a plant pathogen has
403 only been demonstrated following *P. syringae* and *Phytophthora infestans* infections in

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404 tobacco and potato cell cultures, respectively [50, 51]. Other tomato phenolamides, such as *p*-
405 coumaroyldopamine, feruloyldopamine, *p*-coumaroyloctopamine, *p*-coumaroylnoradrenaline,
406 *p*-coumaroyltyramine, feruloyloctopamine, feruloylnoradrenaline and feruloyltyramine, were
407 previously shown to be induced by inoculation with *P. syringae* [22, 23, 52]. To our
408 knowledge, this is the first evidence of caffeoylputrescine response to *P. syringae* inoculation
409 in tomato and it is reasonable to postulate that discrepancies in phenolamide profiles may
410 reflect cultivar specificity. Phenolamides are considered as antiviral, antibacterial, antifungal
411 and repellent compounds taking part in chemical defence against pest attacks [53]. As for
412 other molecules they can bind with and reinforce cell walls [54]. From a quantitative
413 viewpoint, caffeoylputrescine accumulation in leaves was the major change induced by *P.*
414 *syringae* in the phenolic pathway. Indeed, its 45-fold concentration rise rendered
415 caffeoylputrescine the most concentrated leaf phenolic. The concurrent decline in chlorogenic
416 acid concentration in infected leaves is consistent with a similar finding in Solanaceae and
417 indicative that chlorogenic acid and caffeoylputrescine compete for the same substrate pool
418 of hydroxycinnamoyl coA esters [55, 56]. Thus, it is likely that during our experiment,
419 tomato responded to *Pseudomonas* inoculation by diverting the carbon flux towards
420 caffeoylputrescine synthesis at the expense of chlorogenic acid synthesis. Comparatively, the
421 flavonoid pathway was little affected by *P. syringae*, since 12 DAI only the kaempferol
422 derivative was weakly enhanced.

423 *P. corrugata* induced only slight and transient rises in rutin, tomatine (Fig. 4c,d) and the two
424 nitrogenous phenolic Caf Der and Fer Der stem concentrations. *P. syringae* and *P. corrugata*
425 differed slightly regarding their impact on tomatine concentration, the former brought about
426 no change while the latter produced a small but significant decrease 1 DAI. Interestingly, it
427 has been reported that tomatine is induced by *Ralstonia solanacearum* (formerly
428 *Pseudomonas solanacearum*) in tomato [57].

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429 Thus, the metabolic profile of the infected organs is pathogen-specific. The specificity of the
430 responses is also plant-species dependent, as demonstrated in a recent study in Brassicales;
431 the metabolic profile induced by the attack of *Pieris rapae*, a leaf miner, differed in each of
432 the 3 brassicale host-plant species studied [58].

433 ***4.3 Nitrogen supply influenced concentrations of secondary metabolites***

434 N regime had a major impact on the concentrations of defence-related compounds, both in
435 infected and healthy plants. Higher concentrations were nearly always observed under LN
436 supply, even for the alkaloid tomatine (a N-containing molecule). The only exception to these
437 findings concerned caffeoylputrescine (also a N-containing molecule) whose concentration
438 was lower under LN than HN. These N effects are consistent with earlier observations made
439 on tomato [3, 4, 10, 11].

440 ***4.4 Pseudomonas syringae had a systemic effect on metabolites***

441 For *P. corrugata* chemical response was restricted locally near the point of inoculation for
442 both carbohydrates and secondary metabolites whereas that induced by *P. syringae*
443 inoculation extended to other plant organs. In particular, the elicitation of secondary
444 compounds was clearly systemic for chlorogenic acid (in adjacent leaves) and
445 caffeoylputrescine (in adjacent stems and leaves). There is one report in the literature, that
446 chemical response to *P. syringae* inoculation remains local for *p*-coumaroyldopamine and
447 feruloyldopamine production [22]. Our results (spread of symptoms and no systemic
448 response of tomato plant) show that the interaction between tomato cv. Better Bush and *P.*
449 *corrugata* was compatible. These results are consistent with findings on other tomato
450 cultivars by Gustine *et al.* [59], Bellés *et al.* [60] and Desender *et al.* [61].

451 **5. Conclusions**

452 This study provides a clear illustration that tomato plant response is species-dependent within
453 a same genus of plant pathogens.

454 The inoculation site at which each species develops its pathogenicity may also explain the
455 difference observed. We report that the attack of *P. syringae* induced a large decrease in the
456 hexose concentration of plant tissues while *P. corrugata* produced the opposite effect. *P.*
457 *syringae* elicited the accumulation of caffeoylputrescine, a phenolamide. This result is
458 consistent with previous studies showing that some phenolamides are elicited during *P.*
459 *syringae* inoculation [22]. To our knowledge, our study shows for the first time in tomato, an
460 elicitation of caffeoylputrescine *in planta*. We verify in this study that N limitation increased
461 the concentration of most of the plant-defence related compounds investigated (except the N-
462 containing phenolamides). Our study reveals no clear response pattern in secondary
463 metabolite composition and concentration during the pseudomonas inoculation in response to
464 N supply. During *P. syringae* attack, we demonstrated that organs outside the inoculation
465 zone elicited secondary compounds, suggesting a systemic acquired resistance. Such a
466 hypothesis remains to be tested, for example by following the concentration of salicylic acid
467 in the plant. Our work also provides the first assessment on the impact of *P. corrugata* on
468 carbohydrates and defence-related secondary compounds in tomato.

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669 8. Figures Captions

670 **Figure 1:** a. Leaf pictures shot 12 days after infiltration of water or inoculation with *P.*
671 *syringae* under HN or LN fertilization - b. Pictures of the stem longitudinal section shot 12
672 days after infiltration of water or inoculation with *P. corrugata* under HN or LN fertilization.

673 **Figure 2:** Response of tomato growth and leaf net CO₂ exchanges rates (CER, $\mu\text{mol m}^{-2} \text{s}^{-1}$)
674 responses to N nutrition and infection by *P. syringae* (a, c, e, g) and by *P. corrugata* (b, d, f).
675 Growth measurements were performed one and 12 days after inoculation: dry shoot weights
676 (g per plant, a-b), area of inoculated leaves (cm², e) and of adjacent leaves (cm², f-g). Leaf net
677 CO₂ exchange rates (CER, $\mu\text{mol m}^{-2} \text{s}^{-1}$) at light (190 $\mu\text{mol PPF}$) (c) and in the darkness (d)
678 were performed on non-infected leaf zones, 24 hours after *Pseudomonas* inoculation. LN and
679 HN denote low and high N nutrition offered to tomato plants during growth. Vertical bars
680 denote SE (n=6)

681 **Figure 3:** Carbohydrate concentrations (glucose G, fructose F, sucrose Su and starch S, mg g⁻¹
682 DW) in organs infected by *P. syringae* (leaves: a, b) or *P. corrugata* (stems: c, d). Abscissa
683 and ordinate concern organs from LN and HN tomato plants, respectively. The measurements
684 were performed one (a, c) and twelve days (b, d) after *Pseudomonas* inoculation. Symbols
685 ▲ and ■ denote mock inoculated and infected plants, respectively. The diagonals (straight
686 lines) indicate where LN = HN. It follows that data above the diagonals are depressed by LN,
687 whereas below the line, they are enhanced by LN. Vertical and horizontal bars denote SE
688 (n=6).

689 **Figure 4:** Defence metabolite concentrations (chlorogenic acid CA, rutin R, tomatin T,
690 kaempferol-rutinoside KR and caffeoylputrescine CP, mg g⁻¹ DW) in organs infected by *P.*
691 *syringae* (leaves: a, b) or *P. corrugata* (stems: c, d). Abscissa and ordinate concern organs
692 from LN and HN tomato plants, respectively. The measurements were performed one (a, c)

693 and twelve days (b, d) after *Pseudomonas* inoculation. Kaempferol-rutinoside was detected
694 only in leaves. Symbols ▲ and ■ denote mock inoculated and infected plants, respectively.
695 The diagonals (straight lines) indicate where LN = HN. It follows that data above the
696 diagonals are depressed by LN, whereas below the line, they are enhanced by LN. Vertical
697 and horizontal bars denote SE (n=6).

Figure 5: Primary and secondary metabolite concentrations (chlorogenic acid CA, rutin R,
starch S, sucrose Su, glucose G, fructose F and caffeoylputrescine CP, mg g⁻¹ DW) in organs
adjacent to those infected by *P. syringae* (adjacent stems: a, b ; adjacent leaves: c, d).
Abscissa and ordinate concern organs from LN and HN tomato plants, respectively. The
measurements were performed one (a, c) and twelve days (b, d) after *Pseudomonas*
inoculation. Symbols ▲ and ■ denote mock inoculated and infected plants, respectively. The
diagonals (straight lines) indicate where LN = HN. It follows that data above the diagonals
are depressed by LN, whereas below the line, they are enhanced by LN. Vertical and
horizontal bars denote SE (n=6).

Supplementary Figure 1: U-HPLC chromatograms of soluble phenolics from *P. syringae*
infected (A) and control (B) tomato leaf recorded at 320nm. CHA1-5: Isomers of caffeoyl
hexaric acid; CP: Caffeoyl putrescine; CP_a : minor isomer of caffeoyl putrescine; CA:
Chlorogenic acid; CA_b Chlorogenic acid isomer; Caf Der.: caffeoyl derivative; Put CoHA:
Putative Coumaroyl HydroxyAgmatine; SG: Sinapoyl glucose; FQA: Feruloyl quinic acid;
Fer Der: Feruloyl derivative; AR: Apio-rutin ;T: taxifoline (used as external standard); R:
Rutin; K Der: Kaempferol derivative; KR: Kaempferol rutinoside; CT: Coumaroyl tyramine.

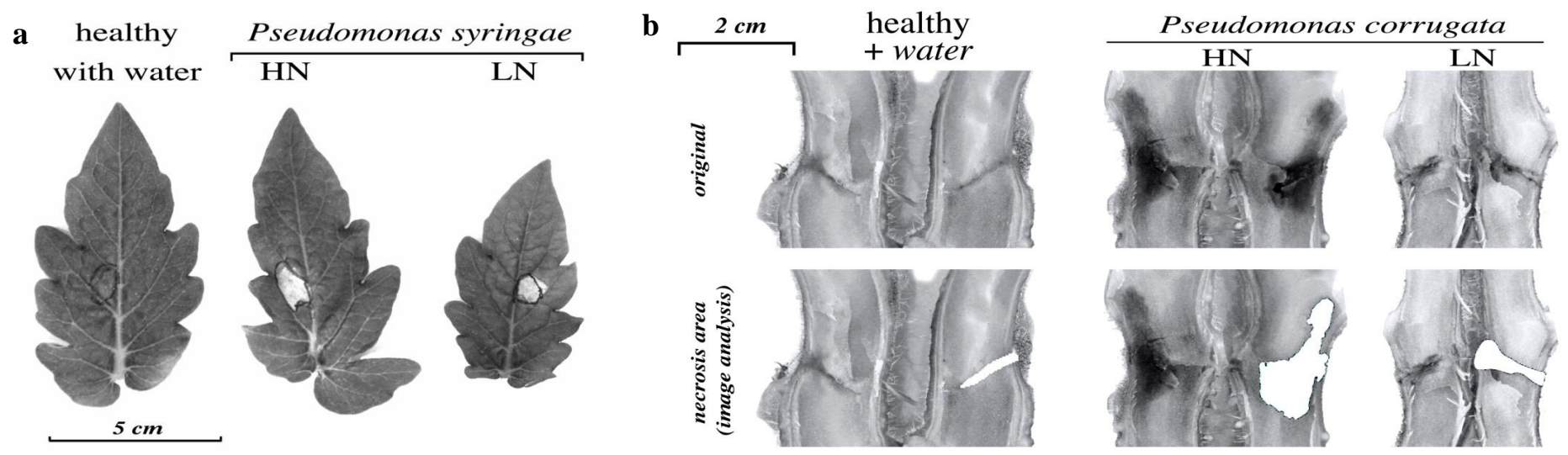
Supplementary Figure 2: U-HPLC chromatograms of soluble phenolics from *P.*
corrugata infected (A) and control (B) tomato stem recorded at 320nm. CHA1-4: Isomers
of caffeoyl hexaric acid; CP: Caffeoyl putrescine; CP_a minor isomer of caffeoyl putrescine ;
CA: Chlorogenic acid; CA_{a-b} Chlorogenic acid isomers; Caf Der.: Caffeoyl derivative; FP:

1 718 Feruloyl putrescine; Put CoHA: Putative Coumaroyl HydroxyAgmatine; SG: Sinapoyl
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3 719 glucose; FQA: Feruloyl quinic acid; T: taxifoline (used as external standard); Fer Der:
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5 720 Feruloyl derivative; AR: Apio-rutin; R: Rutin; CT: Coumaroyl tyramine.
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8 721 **Supplementary Table 1:** Concentration of phenolic compounds in *P. syringae* inoculated
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10 722 and control leaf 1 and 12 DAI. All concentrations are given in $\mu\text{g g}^{-1}$ DW. CHA1-5: Isomers
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12 723 of caffeoyl hexaric acid; CP: Caffeoyl putrescine; CP_a : minor isomer of caffeoyl putrescine;
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14 724 CA: Chlorogenic acid; CA_b Chlorogenic acid isomer; Caf Der.: caffeoyl derivative; FP:
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16 725 Feruloyl putrescine; Put CoHA: Putative Coumaroyl HydroxyAgmatine; SG: Sinapoyl
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18 726 glucose; FQA: Feruloyl quinic acid; Fer Der: Feruloyl derivative; AR: Apio-rutin; T:
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20 727 taxifoline (used as external standard); R: Rutin; K Der: Kaempferol derivative; KR:
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22 728 Kaempferol rutinoside; CT: Coumaroyl tyramine. Values are means of 6 replicates and are
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25 729 given +/- SE.
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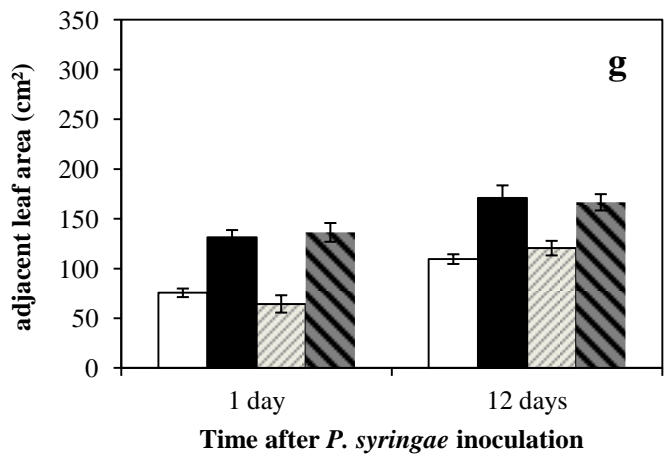
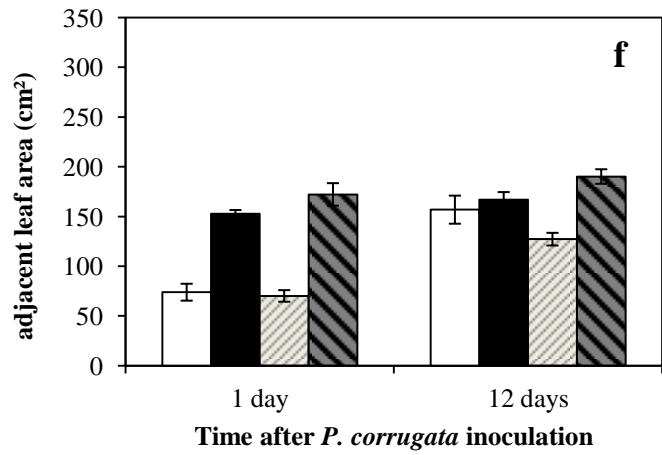
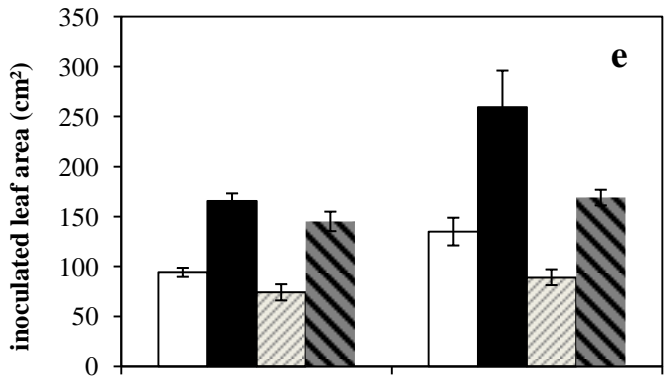
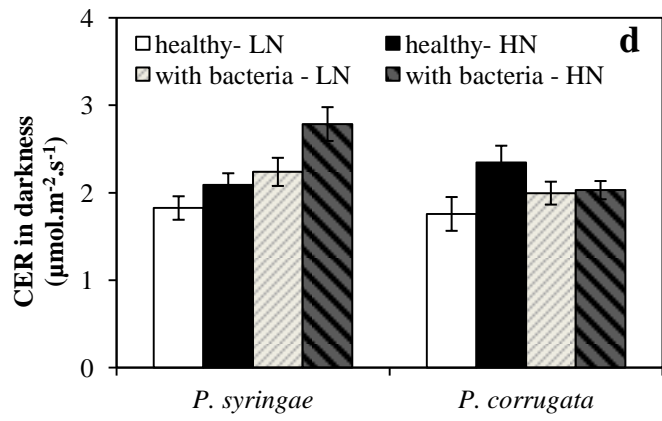
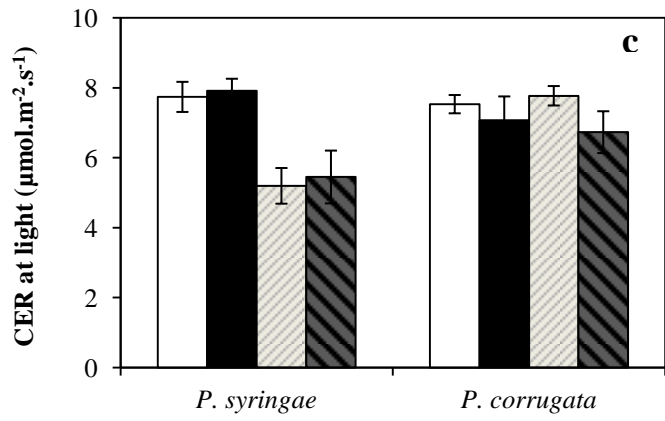
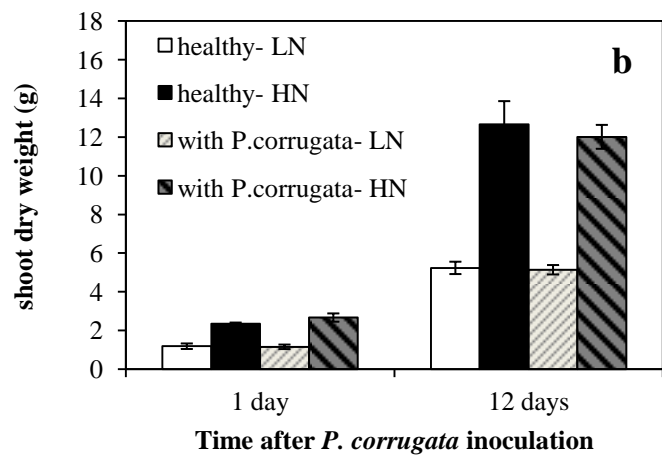
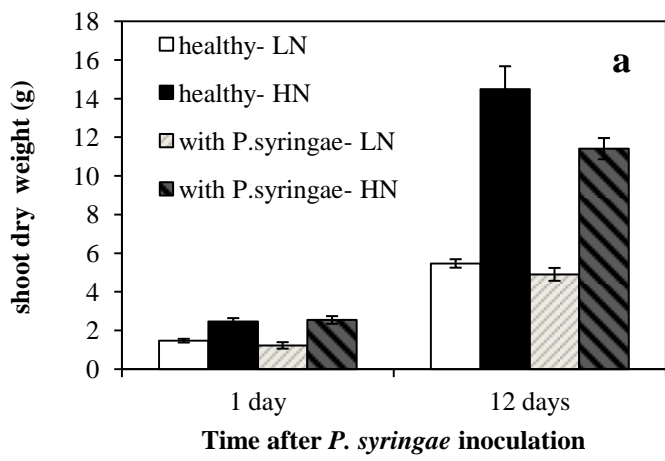
30 730 **Supplementary Table 2:** Concentration of phenolic compounds in *P. corrugata* inoculated
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32 731 and control stem 1 and 12 DAI. All concentrations are given in $\mu\text{g g}^{-1}$ DW. Molecules
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34 732 abbreviations are in supplementary Table 1 legend. Values are means of 6 replicates and are
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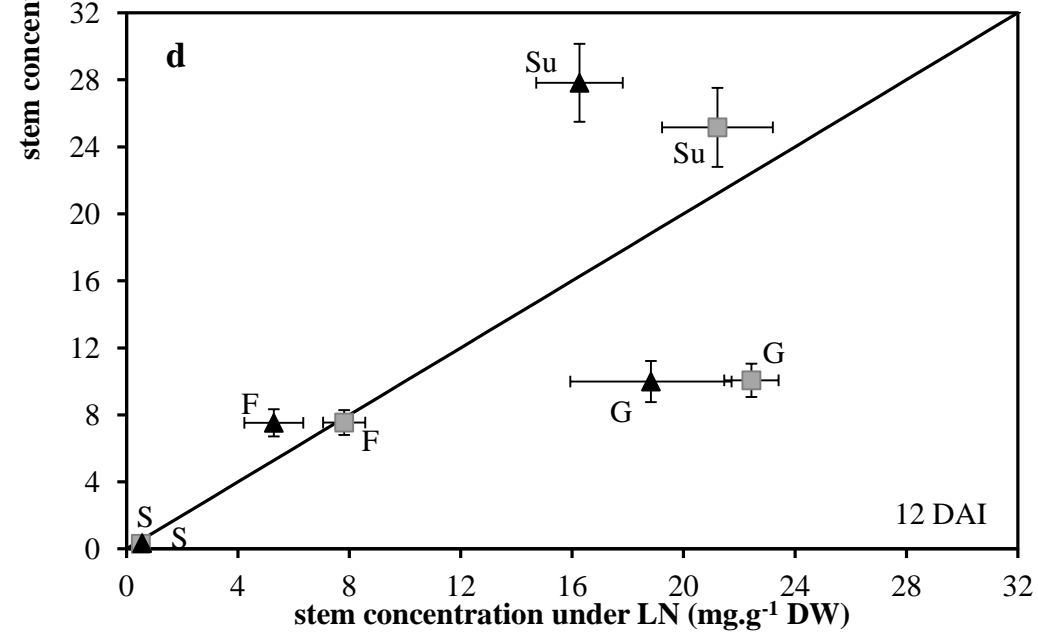
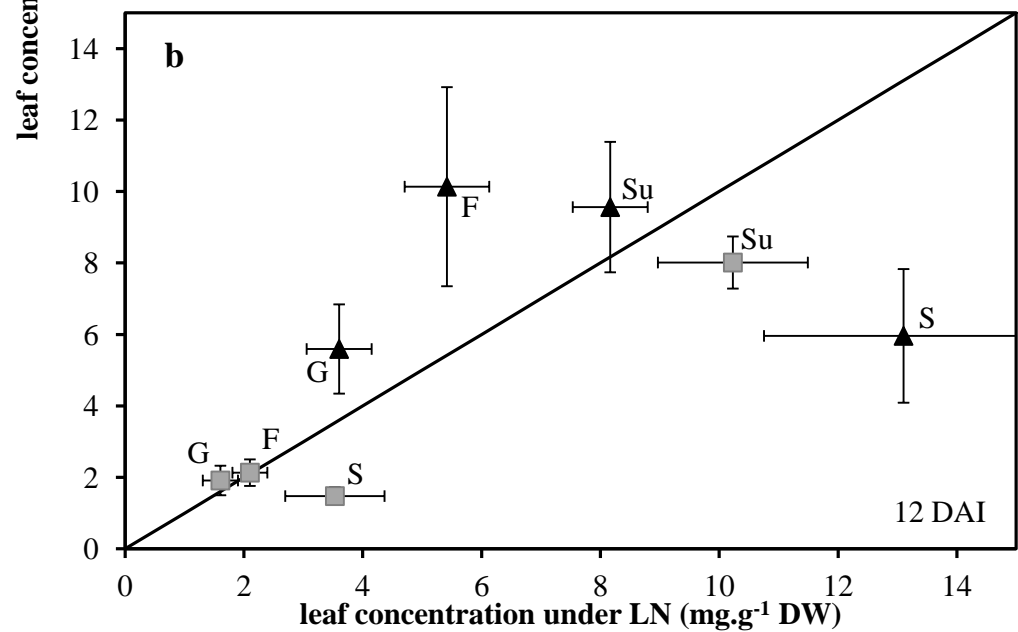
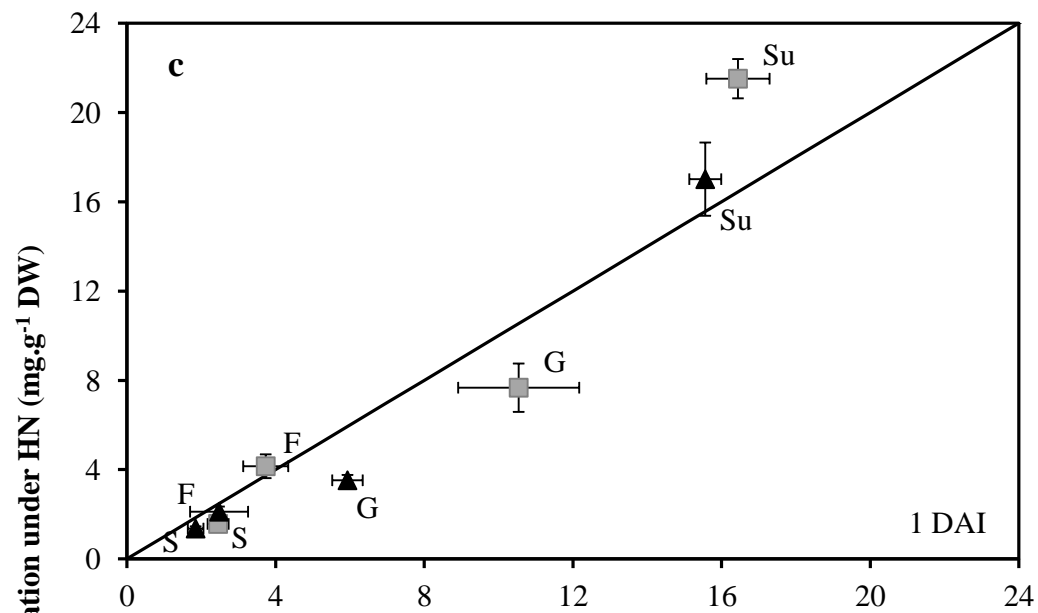
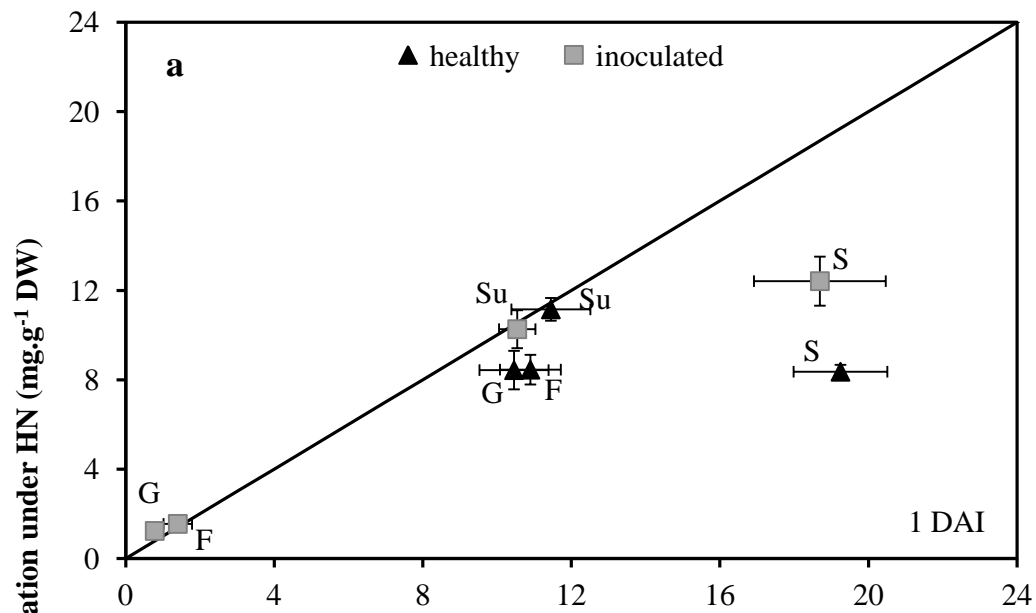


c	Healthy - LN	Healthy - HN	With <i>P. syringae</i> - LN	With <i>P. syringae</i> - HN
	Area of necrosis due to <i>P. syringae</i> on inoculated leaves (cm ²)	0.07 (±0.119)	0.01 (±0.033)	3.01 (±0.453)
	Healthy - LN	Healthy - HN	With <i>P. corrugata</i> - LN	With <i>P. corrugata</i> - HN
	Area of necrosis due to <i>P. corrugata</i> in inoculated stem (cm ²)	0.15 (±0.033)	0.11 (±0.065)	0.29 (±0.144)

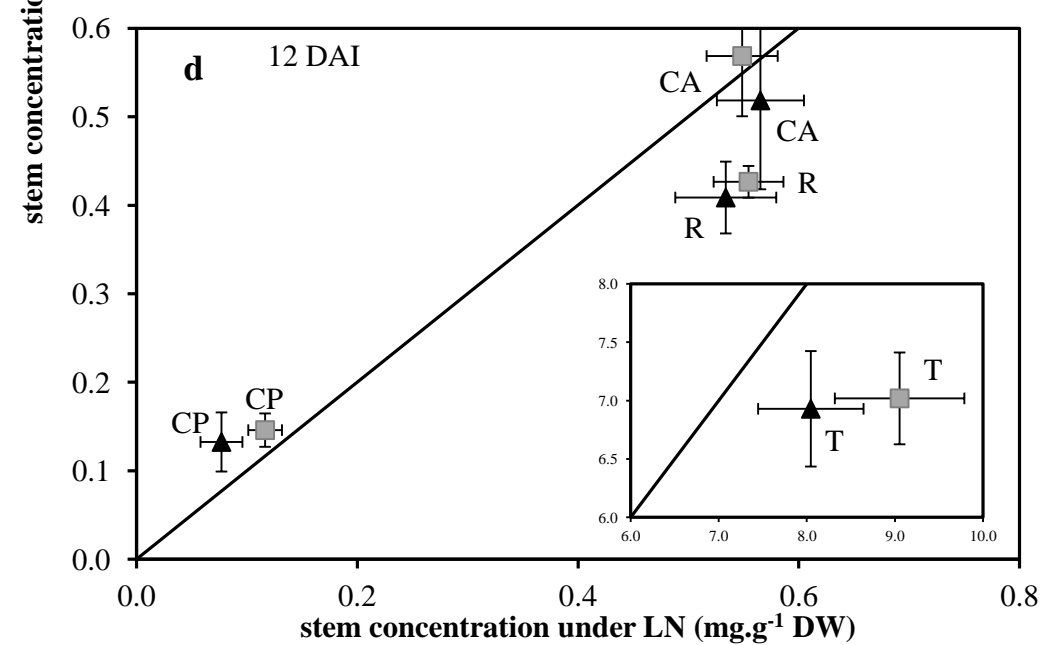
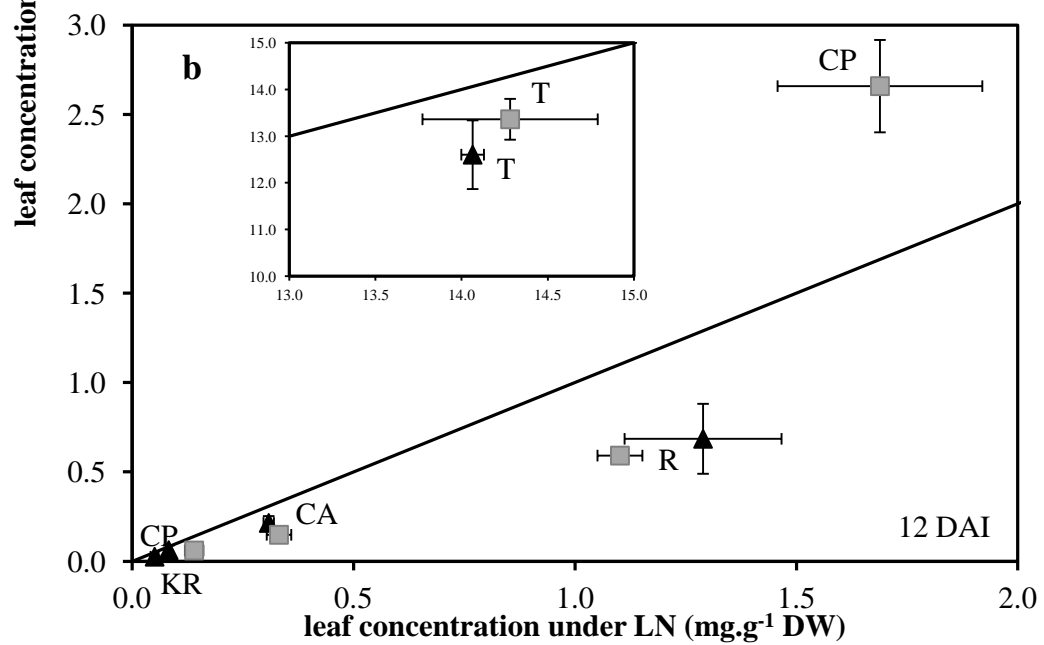
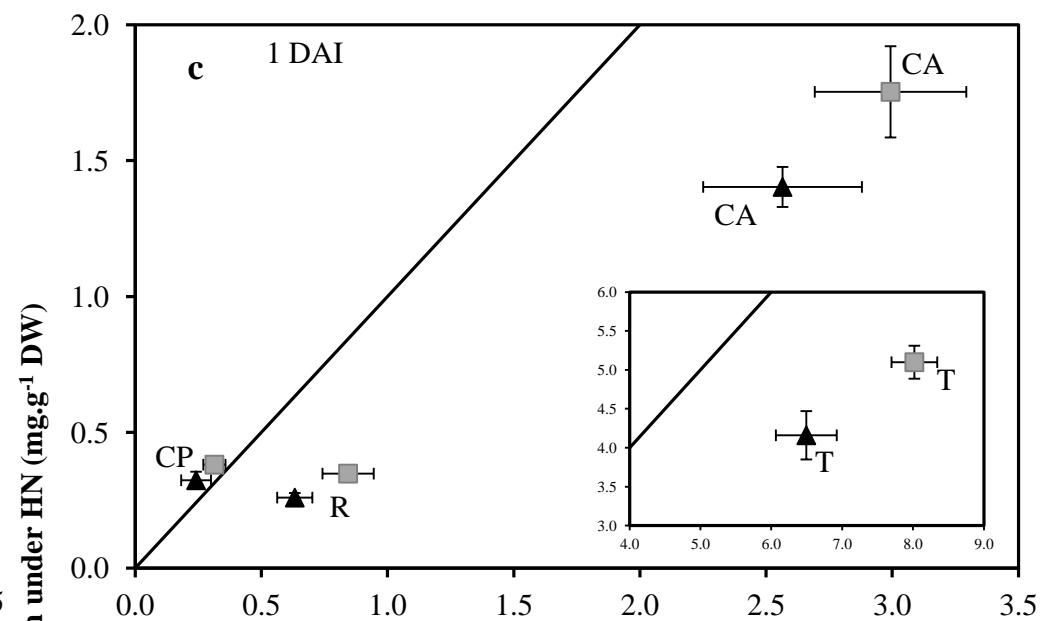
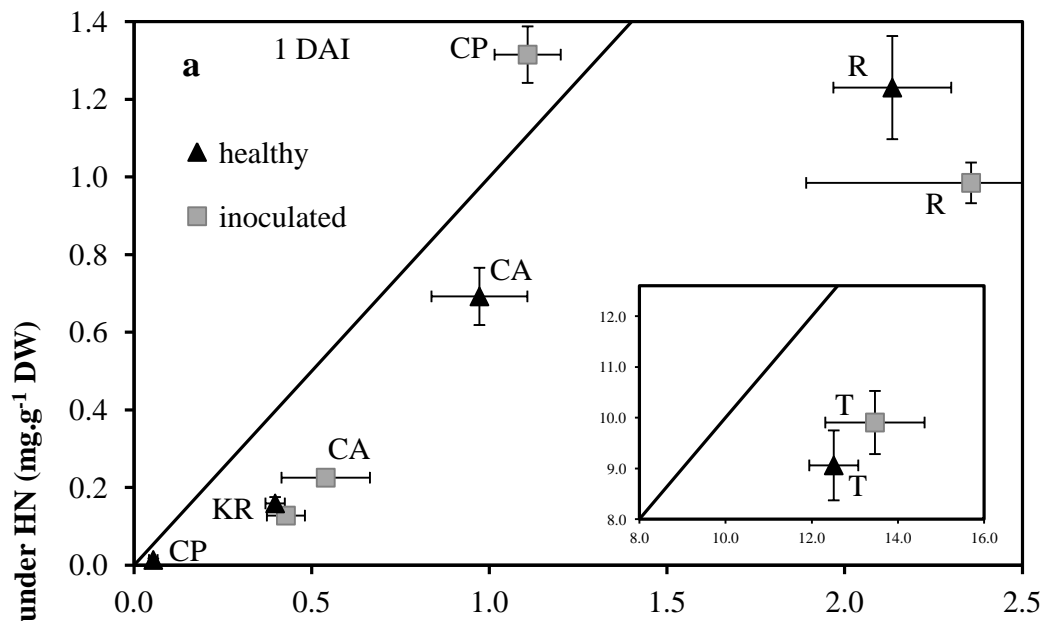
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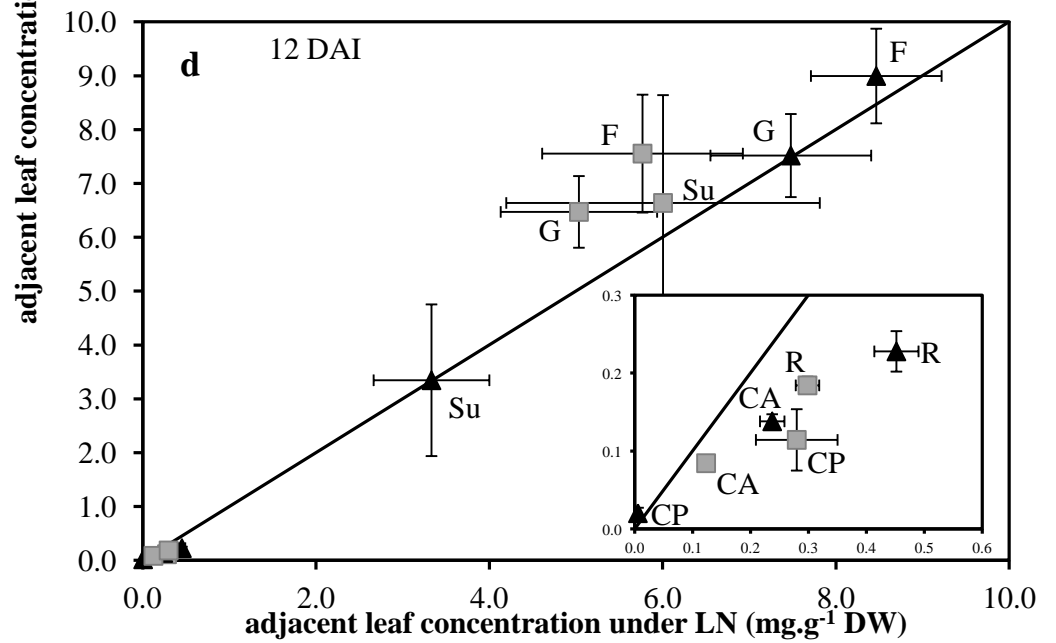
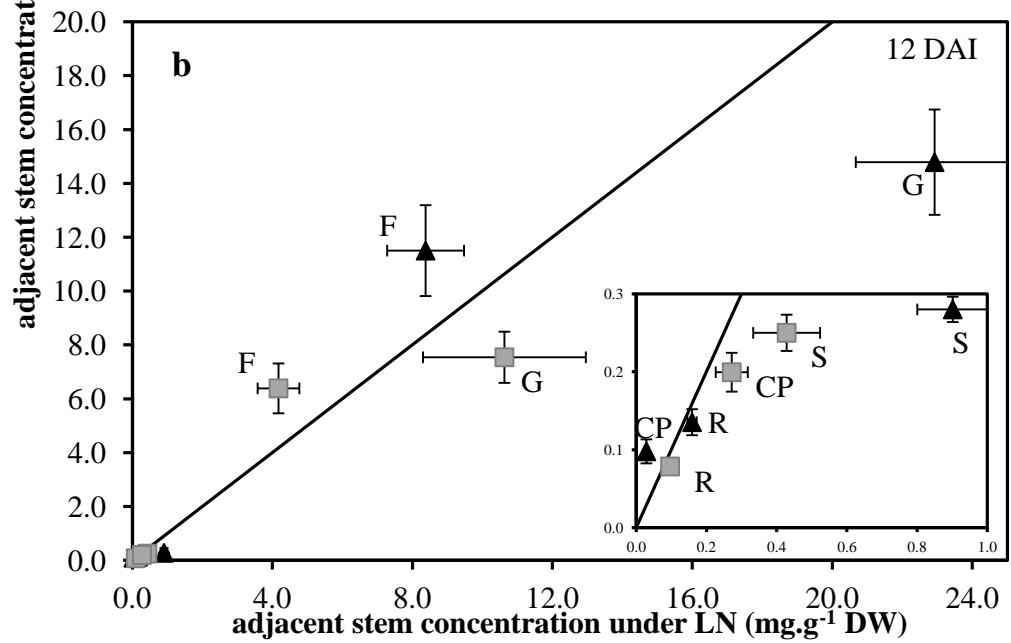
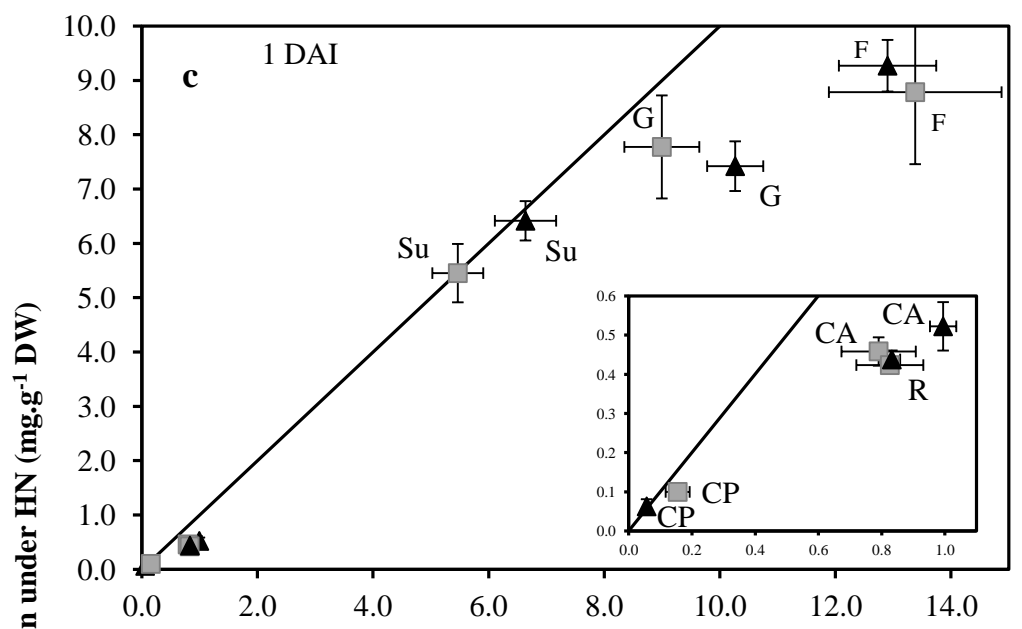
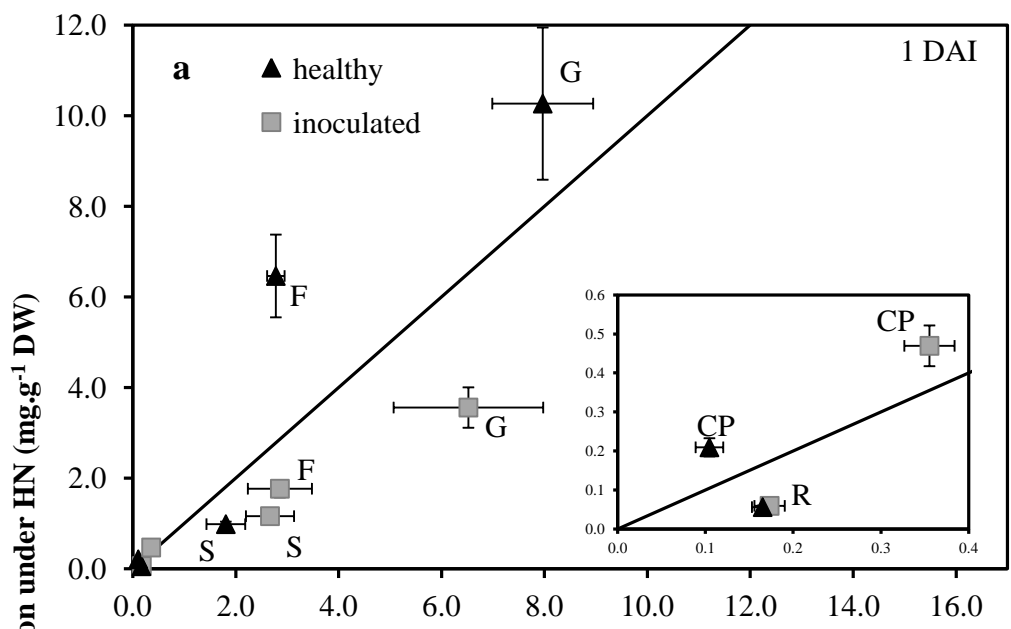
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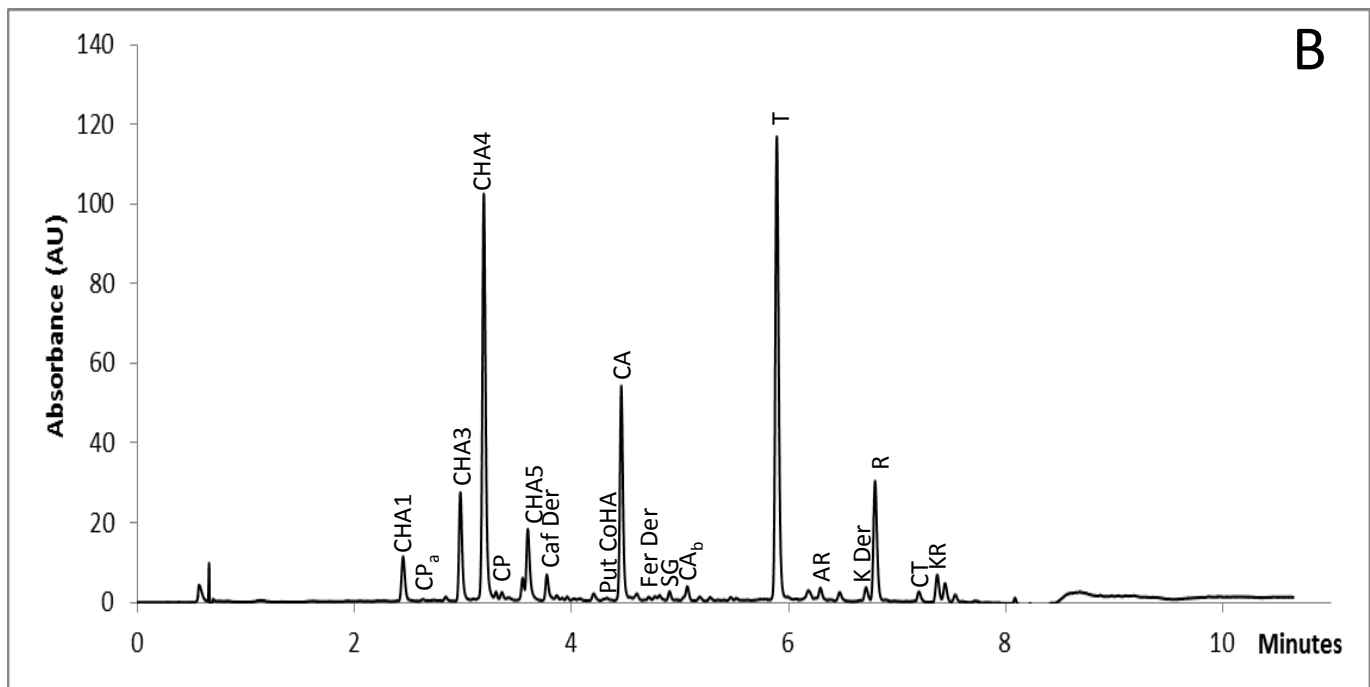
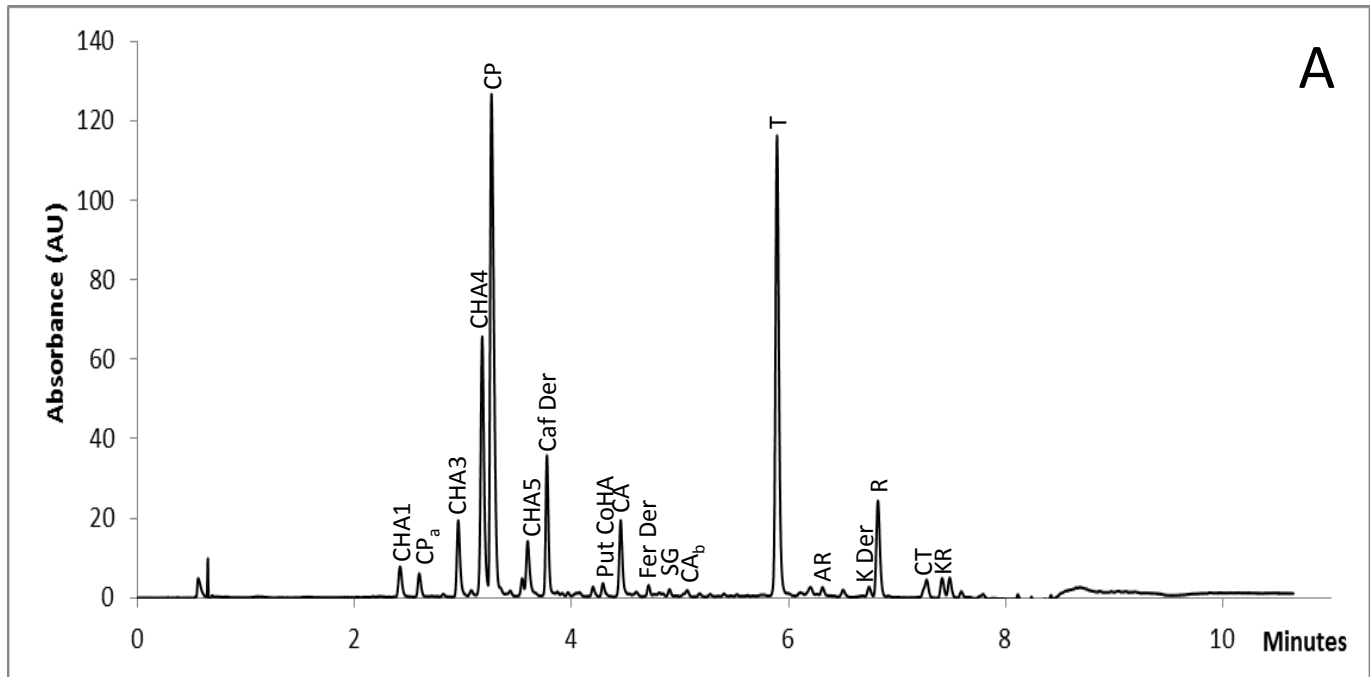
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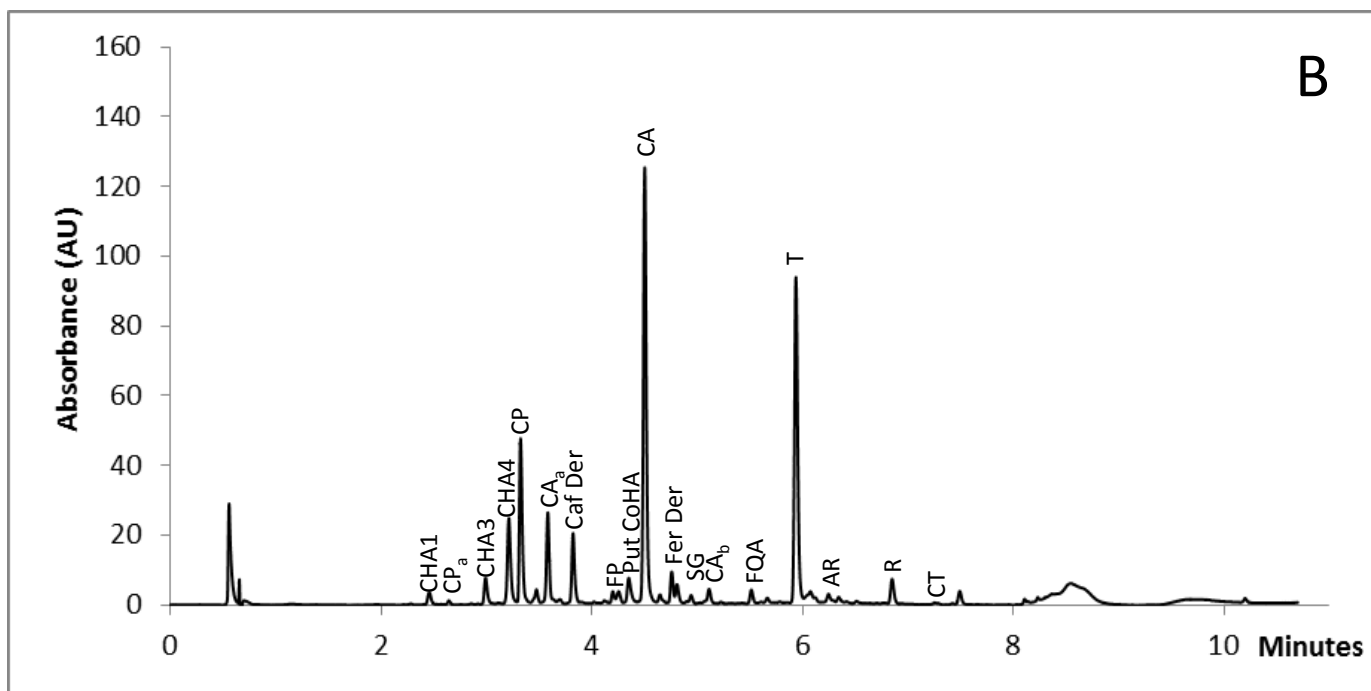
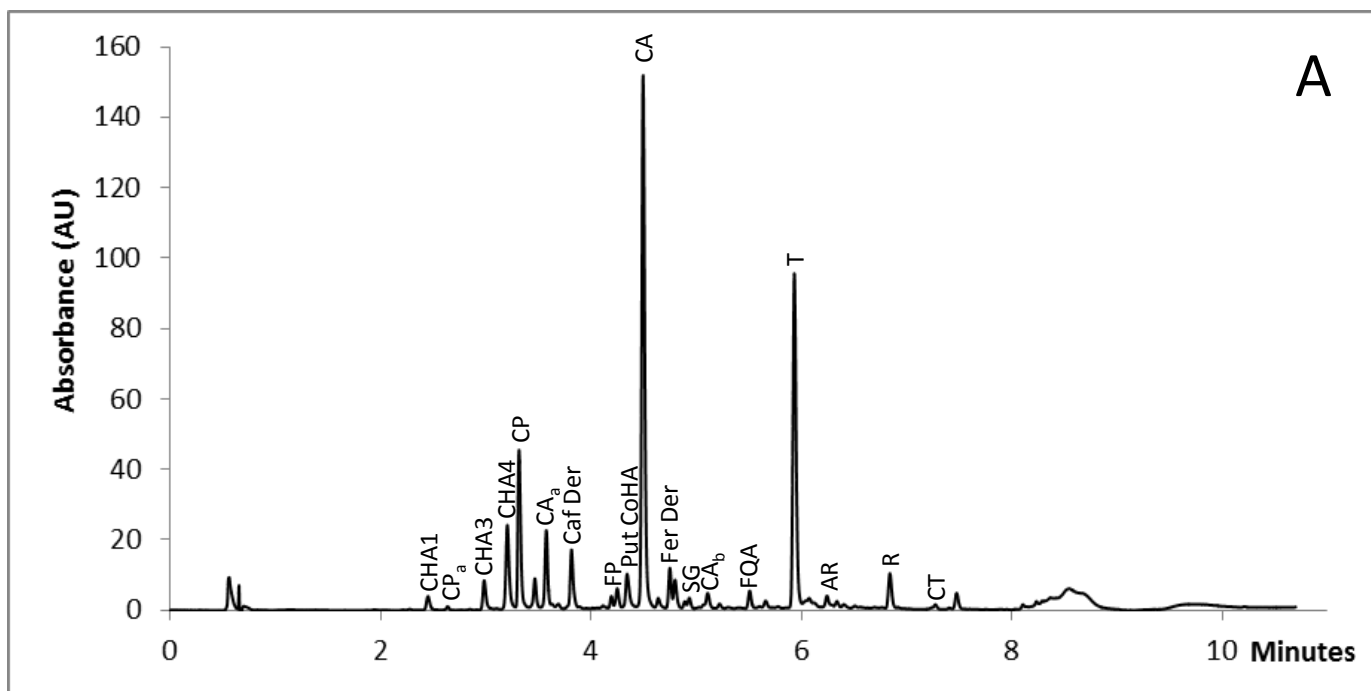
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Table(s)

	1 DAI				12 DAI			
	Healthy		P. syringae inoculated		Healthy		P. syringae inoculated	
	LN	HN	LN	HN	LN	HN	LN	HN
CHA1	772 ± 96	203 ± 29	880 ± 108	135 ± 16	563 ± 21	193 ± 32	574 ± 32	225 ± 12
CP_a	2.4 ± 0.7	3.0 ± 0.1	30 ± 3	61 ± 5	5 ± 1	4 ± 1	213 ± 31	300 ± 43
CHA3	1721 ± 229	234 ± 68	1876 ± 248	307 ± 40	1275 ± 50	400 ± 63	1349 ± 74	505 ± 31
CHA4	5079 ± 535	1486 ± 231	5392 ± 834	916 ± 57	3198 ± 160	1322 ± 233	2916 ± 142	1220 ± 82
CP	54 ± 12	13 ± 3	1107 ± 93	1314 ± 72	51 ± 9	26 ± 7	1687 ± 231	2659 ± 258
CHA5	896 ± 76	332 ± 17	948 ± 134	238 ± 20	1491 ± 73	613 ± 102	1575 ± 40	821 ± 49
Caf Der	69 ± 9	89 ± 23	119 ± 32	252 ± 70	67 ± 3	89 ± 43	97 ± 6	202 ± 47
Put CoHA	14 ± 1	10 ± 1	19 ± 4	27 ± 7	22 ± 2	15 ± 3	21 ± 2	21 ± 5
CA	972 ± 135	692 ± 74	540 ± 124	225 ± 13	320 ± 12	223 ± 40	344 ± 29	154 ± 11
Fer Der	0.9 ± 0.1	1.0 ± 0.2	1.0 ± 0.3	1.6 ± 0.4	4.0 ± 0.1	3.5 ± 0.3	2.9 ± 0.1	1.5 ± 0.1
SG	23 ± 3	25 ± 2	21 ± 4	20 ± 1	24 ± 3	32 ± 3	43 ± 3	29 ± 5
CA_b	46 ± 8	31 ± 5	57 ± 12	26 ± 1	31 ± 2	22 ± 2	32 ± 2	19 ± 1
FQA	13 ± 2	6 ± 1	8 ± 1	3 ± 1	22 ± 4	10 ± 3	17 ± 3	7 ± 2
AR	297 ± 29	132 ± 9	293 ± 31	101 ± 4	278 ± 23	119 ± 37	281 ± 12	166 ± 6
K Der	245 ± 18	82 ± 4	247 ± 24	66 ± 2	192 ± 11	77 ± 30	223 ± 12	90 ± 4
R	2134 ± 165	1230 ± 132	2356 ± 464	984 ± 52	1290 ± 177	685 ± 196	1102 ± 51	591 ± 22
CT	37 ± 3	27 ± 1	47 ± 5	39 ± 3	51 ± 2	29 ± 8	38 ± 4	20 ± 2
KR	397 ± 27	159 ± 17	428 ± 53	127 ± 12	83 ± 2	62 ± 3	140 ± 20	60 ± 4

Table(s)

	1 DAI				12 DAI			
	Healthy		<i>P. corrugata</i> inoculated		Healthy		<i>P. corrugata</i> inoculated	
	LN	HN	LN	HN	LN	HN	LN	HN
CHA1	91 ± 2	42 ± 2	102 ± 4	48 ± 6	45 ± 7	26 ± 3	43 ± 5	24 ± 2
CP_a	8 ± 1	15 ± 4	7 ± 2	14 ± 4	8 ± 3	8 ± 1	8 ± 2	8 ± 1
CHA3	170 ± 7	81 ± 4	169 ± 34	93 ± 12	87 ± 15	48 ± 6	83 ± 10	47 ± 5
CHA4	536 ± 42	307 ± 22	610 ± 38	331 ± 44	262 ± 29	142 ± 13	245 ± 21	144 ± 11
CP	241 ± 59	324 ± 32	314 ± 44	381 ± 25	77 ± 19	133 ± 33	116 ± 15	146 ± 19
CA_a	362 ± 38	177 ± 53	422 ± 47	261 ± 18	408 ± 20	149 ± 20	323 ± 31	161 ± 7
Caf der	96 ± 42	185 ± 31	140 ± 39	272 ± 27	213 ± 39	253 ± 61	216 ± 58	177 ± 20
FP	30 ± 4	28 ± 4	39 ± 3	37 ± 4	55 ± 4	94 ± 10	57 ± 12	80 ± 5
Put CoHA	72 ± 13	69 ± 4	73 ± 10	97 ± 15	80 ± 5	173 ± 15	86 ± 19	153 ± 9
CA	2565 ± 315	1403 ± 74	2993 ± 300	1753 ± 168	565 ± 39	518 ± 100	549 ± 32	569 ± 68
Fer der	60 ± 20	83 ± 12	94 ± 20	168 ± 17	115 ± 25	137 ± 22	124 ± 29	121 ± 12
SG	39 ± 5	40 ± 2	51 ± 9	48 ± 4	37 ± 2	32 ± 5	31 ± 2	33 ± 2
CA_b	99 ± 14	82 ± 16	81 ± 8	84 ± 9	36 ± 3	34 ± 4	36 ± 4	35 ± 5
FQA	95 ± 11	35 ± 2	118 ± 12	48 ± 7	3 ± 1	8 ± 3	2 ± 1	6 ± 1
AR	107 ± 14	55 ± 2	120 ± 15	57 ± 7	52 ± 3	11 ± 1	36 ± 3	11 ± 1
R	632 ± 70	260 ± 17	844 ± 102	348 ± 29	534 ± 46	409 ± 41	554 ± 32	426 ± 18
CT	12 ± 2	14 ± 5	17 ± 2	18 ± 3	35 ± 15	48 ± 15	64 ± 15	32 ± 12