

**The influence of plant species, tissue type and temperature on the capacity of Shigatoxigenic *Escherichia coli* to colonise, grow and internalise into plants.**

**Running title:** STEC growth characteristics in plants

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1 Abstract (247 / 250 words)

2 Contamination of fresh produce with pathogenic *Escherichia coli*, including Shigatoxigenic *E.*  
3 *coli* (STEC), represents a serious risk to human health. Colonisation is governed by multiple  
4 bacterial and plant factors that can impact the probability and suitability of bacterial growth.  
5 Thus, we aimed to determine whether the growth potential of STEC for plants associated with  
6 foodborne outbreaks (two leafy vegetables and two sprouted seed species), is predictive for  
7 colonisation of living plants as assessed from growth kinetics and biofilm formation in plant  
8 extracts. Fitness of STEC was compared to environmental *E. coli*, at temperatures relevant to  
9 plant growth. Growth kinetics in plant extracts varied in a plant-dependent and isolate-  
10 dependent manner for all isolates, with spinach leaf lysates supporting the fastest rates of  
11 growth. Spinach extracts also supported the highest levels of biofilm formation. Saccharides  
12 were identified as the major driver of bacterial growth, although no single metabolite could be  
13 correlated with growth kinetics. The highest level of *in planta* colonisation occurred on alfalfa  
14 sprouts, though internalisation was 10-times more prevalent in the leafy vegetables than in  
15 sprouted seeds. Marked differences in *in planta* growth meant that growth potential could only  
16 be inferred for STEC for sprouted seeds. In contrast, biofilm formation in extracts related to  
17 spinach colonisation. Overall, the capacity of *E. coli* to colonise, grow and internalise within  
18 plants or plant-derived matrices were influenced by the isolate type, plant species, plant tissue  
19 type and temperature, complicating any straight-forward relationship between *in vitro* and *in*  
20 *planta* behaviours.

21 Importance (149 / 150 word)

22 Fresh produce is an important vehicle for STEC transmission and experimental evidence  
23 shows that STEC can colonise plants as secondary hosts, but differences in the capacity to  
24 colonise occur between different plant species and tissues. Therefore, an understanding of the  
25 impact of these plant factors have on the ability of STEC to grow and establish is required for  
26 food safety considerations and risk assessment. Here, we determined whether growth and the  
27 ability of STEC to form biofilms in plants extracts could be related to specific plant metabolites  
28 or could predict the ability of the bacteria to colonise living plants. Growth rates for sprouted  
29 seeds (alfalfa and fenugreek) exhibited a positive relationship between plant extracts and living  
30 plants, but not for leafy vegetables (lettuce and spinach). Therefore, the detailed variations at  
31 the level of the bacterial isolate, plant species and tissue type all need to be considered in risk  
32 assessment.

33

## 34 Introduction

35 Contamination of fresh produce from Shigatoxigenic *Escherichia coli* (STEC) presents a  
36 serious hazard as a cause of food-borne illnesses, diarrhoea and enterohemorrhagic disease.

37 Fresh produce is a major vehicle of transmission of STEC, with foods of plant origin accounting  
38 for the majority of *E. coli* and *Shigella* outbreaks in the USA (47). Fresh produce is often eaten  
39 raw or minimally processed and contamination of the produce can occur at any point along the  
40 food chain from farm to fork, with major outbreaks e.g. from spinach (27) and sprouted seeds  
41 (5). STEC has been shown to interact with plants and can use them as secondary hosts (15,  
42 22), which has implications for pre-harvest contamination, as well as persisting on post-harvest  
43 produce (26, 28, 30).

44 Colonisation of host plants by *E. coli* is governed by a range of environmental, bacterial and  
45 plant factors. Initial contact and attachment of bacteria on plant tissue is defined by motility,  
46 adherence factors and plant cell wall components (58, 59), while establishment is influenced  
47 by a range of plant biotic (40, 61) and abiotic factors (14, 56). The ability of bacteria to grow in  
48 the presence of plant material is a key factor in assessing risk, and although proliferation is  
49 well known to be influenced by physio-chemico factors (4, 53), risk assessments for STEC on  
50 fresh produce tend to consider plants as a homogenous whole (11, 19, 48).

51 STEC preferentially colonise the roots and rhizosphere of fresh produce plants over leafy  
52 tissue and have been shown to internalise into plant tissue, where they can persist in the  
53 apoplastic space as endophytes (12, 66). The apoplast contains metabolites, such as solutes,  
54 sugars, proteins and cell wall components (50) and as such provides a rich environment for  
55 many bacterial species, both commensal bacteria and human pathogens (18, 25). The rate of

56 STEC internalisation is dependent on multiple factors including the plant species and tissue  
57 (67) and how plants are propagated (16, 17). Specificity in the response of STEC to different  
58 plant species and tissue types has been demonstrated at the transcriptional level (8, 34).  
59 Therefore, there is a need to take into account specificity of the STEC-plant interactions that  
60 could impact risk.

61 Determination of the growth potential of a bacterial population takes into account the  
62 probability of growth together with the suitability of the growing population for a particular  
63 environment (20). It is used as a measure in risk assessment, e.g. for growth of STEC in water  
64 (64). In plant hosts, bacterial growth potential is governed by several factors, including  
65 bacterial growth rates, initial adherence and colony establishment, which is often in biofilms, as  
66 well as plant-dependent factors including metabolite availability and plant defence responses  
67 (23). Therefore, the aim here was to determine if *in vitro* growth kinetics and biofilm formation  
68 of STEC in plant extracts, together with plant metabolite analysis, could be related to  
69 colonisation of plants that are associated with food-borne outbreaks, and hence inform on  
70 growth potential of STEC *in planta*. Use of genetically distinct *E. coli* isolates (two STEC, two  
71 environmental and one laboratory isolate) enabled assessment of bacterial phenotypic  
72 variation within plants or plant-derived matrices to be compared. Growth kinetics and biofilm  
73 formation were quantified in different tissue extracts of two leafy vegetables, lettuce and  
74 spinach, and two sprouted seeds, fenugreek and alfalfa sprouts. Growth kinetics was related to  
75 metabolomics of the extracts. Quantification of *in planta* colonisation and internalisation  
76 allowed a correlation analysis for the two STEC isolates.

77

## 78 Results

### 79 *E. coli* growth rates in plant extracts

80 To relate growth potential to colonisation of STEC in fresh produce plants, *in vitro* growth rates  
81 were first measured in plant extracts. Primary modelling of *in vitro* growth data in plant extracts  
82 successfully fitted 86.7 % (117 of 135) growth curves with a non-linear Baranyi model (SM1).  
83 Mis-fits were improved by manually truncating the growth curves to before the observed  
84 decrease in cell density that occurred in stationary phase, resulting in  $R^2_{adj} = 0.996$  (Fig. S1,  
85 Table S1a). Comparison of the maximum growth rates ( $\mu$ ) showed highest growth rates in  
86 spinach extracts, with fastest growth in leaf lysates at 18 °C or apoplast at 25 °C (Fig. 1A),  
87 while in lettuce the fastest growth occurred in apoplastic extract at all temperatures tested (Fig.  
88 1B). All isolates grew consistently faster in fenugreek sprout extracts than in alfalfa, and either  
89 sprout extract supported faster growth than defined medium (RDMG) (Fig. 1C). The *E. coli*  
90 O157:H7 isolates showed differential responses in the different extracts and their growth rates  
91 were as fast or faster than the environmental isolates in almost all extracts. The lowest growth  
92 rates occurred for the laboratory-adapted isolate MG1655. The plant extract tissue-type as well  
93 as the bacterial isolate significantly impacted  $\mu$ , from a two-way ANOVA at 18 °C ( $F(4, 7363) =$   
94  $76.3$ ;  $p < 0.0001$  and  $F(8, 7363) = 436.4$ ;  $p < 0.0001$ , for bacterial isolate and extract type,  
95 respectively) and at 20 °C ( $F(4, 8387) = 160.3$ ;  $p < 0.0001$  and  $F(8, 8387) = 416.1$ ;  $p <$   
96  $0.0001$ , for bacterial isolate and extract type, respectively).

97 Growth was almost always highest at 25 °C, although with exceptions, e.g. for *E. coli* O157:H7  
98 isolate ZAP1589 in lettuce extracts. Growth characteristics were similar at both 18 and 20 °C,  
99 but  $\mu$  were in general lower at 20 °C than at 18 °C. This counterintuitive result was

100 reproducible and occurred in all growth experiments. It meant that secondary modelling for  
101 temperature was not possible. It was possible, however, for temperature-effects of growth in  
102 the defined medium without plant extracts, which produced a linear distribution for temperature  
103 for all five *E. coli* isolates ( $R^2 = 0.996$  to 1) (SM2), indicating the effect was due to the plant  
104 extracts and not a systemic error.

#### 105 Metabolite analysis of fresh produce plant extracts

106 To establish the impacts of different plant components on the growth of the *E. coli* isolates,  
107 metabolite analysis was determined for the extracts. Detection of absolute levels of mono- and  
108 disaccharides (sucrose, fructose, glucose, arabinose) showed the highest abundance in  
109 fenugreek sprout extracts, followed by lettuce apoplast and lettuce leaf lysates (Table 2).  
110 Sucrose was the most abundant sugar in all species and cultivars, except for alfalfa, which had  
111 high levels of fructose and glucose. Arabinose was only detected in the apolastic fluid of  
112 spinach and lettuce, accounting for 0.36 % and 0.23 % of all sugars, respectively. A two-way  
113 ANOVA showed significant differences for tissue types ( $F(7, 60) = 16.5$ ;  $p < 0.0001$ ).

114 The levels of amino acids and other metabolites were determined from identification of 116  
115 polar metabolites, of which 60 were assigned and mapped onto a simplified polar metabolite  
116 pathway for plants to visualise metabolite availability for the bacteria (Fig. S2). The abundance  
117 ratio of each compound against the internal standard ribitol, generated a response ratio (RR)  
118 to allow semi-quantitative comparison (Table S2). Differences occurred between species and  
119 tissue types in a similar pattern to the mono- and disaccharides (Table 2), and for 12  
120 metabolites including fructose, glucose and sucrose, there were significantly different RR (two-  
121 way ANOVA and Tukey multiple comparison,  $F(7, 854) = 37.2$ ,  $p < 0.0001$ ). Small amounts of

122 arabinose could be found in all tissues with no significant differences between host species or  
123 tissue types. Grouping metabolites by structure (Fig. 2A) for monosaccharides,  
124 polysaccharides, amino acids, organic acids and other metabolites, showed that the highest  
125 total saccharides were present in fenugreek sprouts, while alfalfa was higher in  
126 monosaccharides and amino acids. The organic acids in spinach apoplast consisted mainly of  
127 oxalic acid, which was almost double the amount in spinach leaf lysates. The percentage  
128 composition showed that the majority of metabolites in all lettuce extracts are polysaccharides,  
129 compared to mainly of organic acids in all spinach extracts.

130 Significant variation of the metabolite content occurred between plant tissues, as well as for  
131 and individual metabolites (two-way ANOVA assuming a parametric distribution,  $F(420, 854) =$   
132  $43.15$ ;  $p < 0.001$ ). A principal components analysis (PCA) showed that the first five  
133 components accounted for ~ 85 % of variance, and 50 % of the variance for all detectable  
134 polar metabolites ( $n=116$ ) was attributed to PC1 and 2 (Fig. 2B). This was supported by  
135 significant positive correlation for leaf lysates and apoplast extracts of lettuce and spinach ( $R^2$   
136  $> 0.97$ ), a weak correlation for the root lysates based on species ( $R^2$  0.542 – 0.757), with no  
137 significant correlation between any species for the tissues.

#### 138 The influence of plant extract metabolites on *E. coli* growth

139 To relate any specific plant metabolites to bacterial growth, a correlation analysis was carried  
140 out between the plant extracts growth rates for two *E. coli* O157:H7 isolates (Sakai and  
141 ZAP1589) and the assigned metabolites. Several organic acids positively associated with  
142 maximal growth rates ( $\mu$ ), although there was a temperature-dependent effect. Metabolites  
143 associate with growth at 18 °C for isolate Sakai were galactosyl glycerol, threonic acid, and



144 oxoproline ( $p \sim 0.04$ ); at 20 °C, malic acid, fumaric acid and quinic acid ( $p = 0.014 - 0.048$ );  
145 and at 25 °C oxalic acid ( $p = 0.009$ ), aspartic acid ( $p = 0.038$ ), glutamic acid ( $p = 0.046$ ),  
146 coumaric acid ( $p = 0.011$ ) and uridine ( $p = 0.011$ ). Chlorogenic acid (*trans*-5-O-caffeoyl-D-  
147 quinate) was consistently associated with growth for all temperatures ( $p = 0.04$  at 18 °C,  $p = 0.004$   
148 at 20 °C, and  $p = 0.04$  at 25 °C). *E. coli* isolate ZAP1589 gave similar results, although there was  
149 also a bacterial isolate effect as there were no significant associations at 20 °C. Therefore, no  
150 single metabolite was identified as the major factor influencing *E. coli* growth rate, with a  
151 significant impact from growth temperature.

152 The main metabolite groups were then investigated as groups that could influence bacterial  
153 growth, by generating defined 'artificial' growth media comprising the main plant extract  
154 metabolites. The six most abundant metabolites were selected from lettuce apoplast or sprout  
155 extracts to represent contrasting metabolite profiles (Table 3). Each of the major groups of  
156 saccharides (SA), organic acids (OA) or amino acids (AA) were assessed independently by  
157 dilution, to restrict their effect, and at temperatures relevant to lettuce (18 °C) and sprouts (25  
158 °C). Maximal growth rates were similar in the sprout and lettuce extract artificial medium (Fig.  
159 3), although reduced compared to the 'complete', natural extracts (Fig. 1). Growth rates were  
160 significantly reduced when the concentration of the saccharide group (SA) was reduced for  
161 both artificial media (all  $p < 0.0049$ ), while restriction of the amino acids (AA) or organic acids  
162 (OA) had no impact (Fig. 3). The SA-dependent effect occurred for all *E. coli* isolates, although  
163 there were also significant isolate dependencies (two-way ANOVA,  $F(16, 28637) = 39.5$ ;  $p <$   
164  $0.0001$  at 25 °C; two-way ANOVA,  $F(4, 9544) = 401.3$ ;  $p < 0.0001$  at 18 °C).

165 The influence of plant extracts on *E. coli* biofilm formation

166 On host tissue *in planta*, bacterial colonies are more likely to be present in biofilms rather than  
167 as single cells. Therefore, the influence of the plant extracts of the leafy vegetables was tested  
168 for *E. coli* biofilm ability in isolation, i.e. on polystyrene surfaces. Spinach leaf lysates and root  
169 lysates were the only extracts that induced biofilm for all isolates, albeit minimal for isolate  
170 MG1655 ( $p < 0.0011$ , compared to isolate MG1655) (Table 4). The remaining extracts were  
171 not as conducive for biofilm formation, with the exception of one of the environmental isolates  
172 (JHI5025). This was not explained by different growth rates since this isolate did not exhibit the  
173 fastest growth rates in the extracts compared to the others (Fig. 1) and presumably therefore  
174 reflect increased adherence in the presence of the plant extracts. A qualitative risk ranking was  
175 determined for implementation of biofilm formation as a risk factor for the *E. coli* O157:H7  
176 isolates (Sakai and ZAP1589) that identified spinach roots as the highest risk (from highest to  
177 lowest): spinach roots > spinach leaves > lettuce roots > lettuce leaves > spinach apoplast >  
178 lettuce apoplast.

179 *E. coli* O157:H7 colonisation and internalisation *in planta*

180 *E. coli* O157:H7 colonisation of leafy vegetables and sprouts was quantified to determine  
181 whether growth kinetics and biofilm formation in the extracts were predictive of *in planta*  
182 colonisation. Colonisation of the *E. coli* O157:H7 isolate (ZAP1589) was quantified on spinach  
183 and lettuce, and for both isolates (ZAP1589 and Sakai) on sprouted seeds. Our previous *in*  
184 *planta* data for lettuce and spinach plants showed that the highest levels of *E. coli* isolate Sakai  
185 occurred on spinach roots (67). Inoculation of spinach and lettuce with the high dose ( $10^7$  cfu  
186  $\text{ml}^{-1}$ ) of *E. coli* isolate ZAP1589 also resulted in higher levels of bacteria on the roots compared

187 to leaves, with similar levels on spinach and lettuce roots, e.g.  $2.53 \pm 0.97$  and  $2.69 \pm 0.88$  log  
188 (cfu g<sup>-1</sup>) at day 14, respectively (Fig. 4A, B). *In planta* colonisation of sprouted seeds by the  
189 two *E. coli* O157:H7 reference isolates was quantified for plants grown under conditions that  
190 mimic industry settings (hydroponics at 25 °C, three days) (Fig. 4C-F). A low inoculation dose  
191 of 10<sup>3</sup> cfu ml<sup>-1</sup> was used and total viable counts on day 0 were estimated by MPN since they  
192 fell below the direct plating detection threshold. Total counts of isolate Sakai increased by 4.5  
193 log (cfu g<sup>-1</sup>) on alfalfa sprouts and 3 log (cfu g<sup>-1</sup>) on fenugreek sprouts, between 0 and 2 dpi.  
194 Viable counts for isolate ZAP1589 were generally lower on both sprouted seeds compared to  
195 isolate Sakai, but still reached  $6.00 \pm 0.253$  log (cfu g<sup>-1</sup>) on alfalfa 2 dpi.

196 Internalisation was also assessed since endophytic behaviour is a feature of *E. coli* O157:H7  
197 colonisation of fresh produce plants and growth potential could be reflected by growth in the  
198 apoplast washings. Internalisation of isolate ZAP1589 occurred to higher levels in spinach  
199 roots compared to lettuce roots (Fig. 4A, B), although the prevalence was similar in both plant  
200 species (60 % and 58.3 % of plants contained endophytic bacteria). In contrast, internalisation  
201 in sprouts only occurred on three occasions in all the experiments: isolate Sakai in alfalfa (1.07  
202 log (cfu g<sup>-1</sup>)) and fenugreek (1.53 log (cfu g<sup>-1</sup>)) on day 1, and isolate ZAP1589 in alfalfa (1.87  
203 log (cfu g<sup>-1</sup>)) on day 2. The prevalence was 7.1 % (1/14 samples positive), although the viable  
204 counts were close to the limit of detection by direct plating. Therefore, internalisation of *E. coli*  
205 O157:H7 isolates Sakai and ZAP1589 appeared to be a rare event on sprouted seeds,  
206 although they colonised the external sprout tissue to higher levels than on lettuce or spinach.

207 Correlating *in planta* colonisation with plant extract growth rate kinetics

208 To relate growth kinetics in extracts with *in planta* growth, growth rates were estimated for *in*  
209 *planta* growth. This was possible for sprouted seeds since colonisation levels increased over  
210 time (Fig. 4). Alfalfa plants supported significantly faster growth rates for both *E. coli* O157:H7  
211 isolates compared to fenugreek, at  $2.23 \pm 0.213 \log \text{ cfu g}^{-1}$  per day ( $R^2 = 0.720$ ) and  $1.50 \pm$   
212  $0.0913 \log \text{ cfu g}^{-1}$  ( $R^2 = 0.863$ ) for Sakai on alfalfa and fenugreek sprouts, respectively, and for  
213 isolate ZAP1589, rates of  $2.24 \pm 0.159 \log \text{ cfu g}^{-1}$  ( $R^2 = 0.822$ ) and  $0.710 \pm 0.116 \log \text{ cfu g}^{-1}$  ( $R^2$   
214  $= 0.464$ ) per day on alfalfa and fenugreek sprouts, respectively. The difference in growth rate  
215 between the isolates on fenugreek sprouts was significant ( $p < 0.0001$ ). Although *in planta*  
216 growth rates for *E. coli* isolates Sakai were estimated on spinach tissues (leaves, roots or  
217 internalised in leaf apoplast) or lettuce (leaves, roots) from low inoculation dose ( $10^3 \text{ cfu ml}^{-1}$ )  
218 (67) these were non-significant since growth over the 10 day period was minimal or completely  
219 constrained, with a high degree of plant-to-plant variation. Growth rate estimates were not  
220 made when a high starting inoculum was used since the colonisation levels decreased over  
221 time (Fig. 4).

222 Comparison of the *in planta* and extract growth rate estimates were made for both *E. coli*  
223 O157:H7 isolates on sprouted seeds (at 25 °C) or in spinach and lettuce (at 18 °C) (Fig. 5). A  
224 positive correlation occurred for growth rate estimates in the sprouted seeds ( $R^2 = 0.516$ ),  
225 although this was not significant. Since *in planta* growth in spinach or lettuce tissues was  
226 minimal, there was no correlation with growth rates in corresponding extracts. Therefore, the  
227 restrictions in bacterial growth that occurred with living plants meant that growth rates in

228 extracts could not be extrapolated to *in planta* growth potential for leafy vegetables, but did  
229 bear a positive relationship for sprouted seeds.

230

## 231 Discussion

232 The potential for food-borne bacteria to grow in fresh produce food commodities is a key  
233 consideration in quantitative risk assessment. Factors that influence bacterial growth are the  
234 plant species and tissue, the bacterial species or isolate, and the surrounding environment.  
235 The growth potential of a bacterial population consists of proportion of the growing sub-  
236 population and the suitability of the environment for growth, and it provides a quantitative  
237 description of probability of growth (20). Therefore, the factors that influence growth potential  
238 of STEC in edible plants include plant-dependent and physio-chemico factors, as well as  
239 bacterial isolate-specific responses. Metabolically active components of plants can be  
240 extrapolated from plant extracts for bacterial growth dynamic measurements coupled with  
241 metabolite analysis. They also represent a bacterial growth substrate in their own right that  
242 could arise during the post-harvest production process e.g. from cut surfaces. A number of  
243 studies show growth of food-borne bacteria on plant extracts during the production process  
244 (31, 51, 52) and growth potential for *E. coli* O157:H7 has been evaluated in water (64). Here,  
245 maximum growth rates in plant extracts were strongly influenced by the plant tissue type and  
246 species, as well as the *E. coli* isolate tested and overlaid by temperature-dependent effects. *In*  
247 *planta* growth rates, however, was markedly different between the sprouted seeds and leafy  
248 vegetables, with a growth restriction evident in the leafy vegetables. The plant-dependent  
249 factors that could account for this difference include plant age, defence response, growth  
250 conditions and associated microbiomes. As such growth rates in the extracts could not be  
251 used to infer *in planta* growth potential for spinach or lettuce. In contrast, proliferation on  
252 sprouted seeds did bear a positive relationship to growth rates in extracts, although it was also  
253 dependent on the plant species and on bacterial isolate tested.

254 Saccharides were shown to be the major driving force for *E. coli* growth, which is unsurprising  
255 given their role in central metabolism (37). Although the levels of the most abundant sugars,  
256 glucose, fructose and sucrose (the disaccharide of glucose and fructose) could explain the  
257 high growth rates in sprout extracts, similarly rapid growth did not occur in lettuce leaf lysate  
258 extract, despite an abundance of sugars, indicating that plant species-specific inhibitory  
259 compounds exist. This is supported by the occurrence of more rapid growth rates in spinach  
260 leaf extracts compared to lettuce. Plant-dependent factors that could influence bacterial growth  
261 potential include the innate defence response (29) and antimicrobial activity of plant secondary  
262 metabolites (65). Plant development stage is an important factor since sprouted seeds, which  
263 were abundant in glucose and fructose, are at a distinct developmental stage to mature plants,  
264 and young plants of a variety of species can serve as preferential secondary plant hosts for  
265 STEC (68).

266 Bacterial growth rates were not significantly impacted by manipulation of the major amino or  
267 organic acids from the extracts, although the phenolic acid, chlorogenate (*trans*-5-O-caffeoyl-  
268 D-quinic acid) was positively associated with growth. This contrasts to reports of its ability to  
269 inhibit fatty acid synthesis in *E. coli* isolate MG1655 (33) and prevent *E. coli* growth (69), but  
270 may be explained by differences in concentration between the extracts and exogenous  
271 application. Oxalate levels were relatively high in spinach, in keeping with previous reports that  
272 show an average as high as ~ 1000 mg / 100 g fresh weight (44) and correlated with growth for  
273 isolate Sakai at 25 °C. Amino acids levels were substantially higher in sprouted seed extracts  
274 compared to the leafy vegetables, which is likely a reflection of different developmental stages  
275 of the plants (3). It was notable that the artificial media did not support equivalent growth rates  
276 to the 'complete', natural extract media, indicating that other, minor nutrients in the extracts

277 were utilised for maximal bacterial growth and also need to be accounted for in growth  
278 dynamics.

279 Bacteria including STEC, tend to form biofilms in association with plant tissue (10, 67, 68).  
280 Here, a risk ranking could be inferred from biofilm formation in the extracts, with spinach roots  
281 ranked highest. Curli is an important biofilm component for STEC associated with plants (6),  
282 but other biofilm components are likely to be responsible for the biofilm formation in extracts,  
283 since isolate Sakai did not form biofilms in spinach apoplast extract *in vitro* although does  
284 produce curli during endophytic colonisation and biofilm formation in leaves (67). This indicates  
285 that specific *in planta* cues induce different biofilm components. Alternative biofilm components  
286 that may be involved include Type 1 fimbriae, which was shown to be expressed by the  
287 environmental isolates JH15025 and JH5039 at 20 °C and promoted binding to spinach roots  
288 (36).

289 Internalisation of STEC into apoplastic spaces in plants presents a hazard as pathogens  
290 cannot be removed by conventional sanitation methods. However, growth potential for  
291 internalised *E. coli* O157:H7 could not be inferred from growth in apoplast extracts since  
292 endophytic proliferation was prevented or reduced in the apoplast (67). As the apoplast is a  
293 habitat for plant-associated endophytes (60) and phytopathogens (57), it appears that for *E.*  
294 *coli* additional factors such as the plant defence response need to be considered. The  
295 increased likelihood of internalisation into tissues of leafy vegetables compared to sprouted  
296 seeds for the *E. coli* O157:H7 isolates could be due to multiple factors including plant age, the  
297 competing microbiota and access to nutrients. Plant dependent factors have also been shown  
298 to impact colonisation of lettuce cultivars by STEC (54).



299 *In planta* colonisation of *E. coli* O157:H7 isolate Sakai was significantly higher than isolate  
300 ZAP1589, in both leafy tissue types and on both sprouted seed species (67). In contrast,  
301 growth rates in the plant extracts and in artificial media overlapped, albeit with specific extract-  
302 specific differences. Since isolate ZAP1589 was found to be flagellate but non-motile, this may  
303 reflect a role for flagella in plant colonisation (59). ZAP1589 growth rates on sprouted seeds  
304 were similar to the rates reported for other *E. coli* O157:H7 isolates on 2-day old alfalfa sprouts  
305 (7). Growth rates of both *E. coli* O157:H7 isolates in the extracts was, in general, as high as  
306 the environmental isolates, indicating similarities in fitness levels for STEC and environmental  
307 *E. coli* in the plant environment. As anticipated, almost all growth rates were lowest for the  
308 laboratory adapted K-12 isolate, and biofilm formation was essentially absent.

309 The ability of *E. coli* isolates to metabolise different carbon sources varies and could contribute  
310 to the isolate-dependent variations in growth rates. Although less than 50 % of *E. coli* isolates  
311 can metabolise sucrose (37), *E. coli* O157:H7 isolate Sakai encodes the sucrose transport  
312 genes (1) and sucrose degradation genes were expressed by this isolate on exposure to  
313 spinach extracts (8). The sucrose translocator from *S. enterica* serovar Typhimurium was  
314 expressed by a related epiphyte *in planta* (43). In contrast, fructose and glucose are sufficient  
315 sole carbon source-metabolites for *E. coli* and their role in bacterial metabolism is well  
316 characterised (37). An *E. coli* fructose metabolism gene has also been expressed in a related  
317 epiphyte *in planta* (32).

318 Growth rates normally positively correlate with temperature (55), as was observed for growth  
319 rates in the defined medium without plant extracts, which exhibited a linear distribution from 18  
320 °C to 25 °C. However, maximal growth rates in the extracts were influenced in a non-linear  
321 manner by temperature. Similarly, a non-linear effect was reported in a meta-study on growth

322 of STEC on lettuce (38). Since *E. coli* Sakai exhibits distinct metabolic responses to different  
323 plant tissues (8), it is possible that a temperature-dependent effect on metabolite content  
324 similarly impacted bacterial metabolism and resultant growth. This may explain the different  
325 organic acid-growth correlations that occurred at 20 °C 'vs' 18 °C. The implications are that a  
326 linear approximation, e.g. such as a Ratkowsky model, is not sufficient to describe *E. coli*  
327 growth in plant extracts, although it has been used to model growth on plants (39, 55).

328 In conclusion, growth potential *in planta* was described in part, by growth rates in plant  
329 extracts, but only for sprouted seeds. On the other hand, biofilm formation in plant extracts  
330 showed some relation to *in planta* colonisation in leafy vegetables. Plant species- and tissue-  
331 type dependent differences in metabolites meant that no single metabolite could be correlated  
332 with growth, and the only positive association was with the combined group of saccharides.  
333 The marked differences in *in planta* colonisation between the sprouted seeds and leafy  
334 vegetables reinforces the higher risk associated with very young plants, grown under  
335 conditions conducive for bacterial growth (68). Therefore, although this data can inform hazard  
336 identification and risk analyses, it is evident that important specificities within each plant-  
337 microbe system need to be considered, and it is not possible to take a generalised view of  
338 STEC-plant colonisation.

339

## 340 Materials and Methods

### 341 Bacteria and media

342 The bacterial isolates panel comprised five isolates: two *E. coli* O157:H7 isolates, two  
343 environmental *E. coli* isolates and an *E. coli* K-12 isolate (Table 1). *E. coli* ZAP1589 is a Stx  
344 negative derivative, generated from isolate H110320350. Regions flanking *stx* genes were  
345 amplified using specific primers: No-stx1 (5'-ttgctggctcggtagccggg  
346 AGTGCTGTGACGATGATGCGATG), Ni-stx1 (5'-cgctcttgcggccgcttgaacgg  
347 ATTACACAATACTCCTTGAGCAC), Co-stx1 (5'-tcccattgccaccggtcgac  
348 GCGGGTCCGGACGGTCATATGTC), Ci-stx1 (5'-ccgtccaagcggccgaagagcg  
349 CAGAATAGCTCAGTGAAAATAGC), and No-stx2 (5'-ttgctggctcggtagccggg  
350 CCAAGCACGCCATTGCATCTTAC), Ni-stx2 (cgctcttgcggccgcttgaacgg  
351 ATACAAGGTGTTCCCTTTGGCTG), Co-stx2 (5'-tcccattgccaccggtcgac  
352 AACCTCTCCTGCCGCCAGCAAAG), Ci-stx2 (5'-ccgtccaagcggccgaagagcg  
353 GGCATAACCTGATTCGTGGTATG) for *stx1* and *stx2*, respectively. The PCR fragments were cloned  
354 into pTOF25 and verified by sequencing. The kanamycin resistant gene from pTOF2 (41) was cloned  
355 into the *stx1*-deletion construct and tetracycline resistance gene from pTOF1-TcR (63) was cloned into  
356 the *stx2*-deletion construct. The plasmids were transformed into isolate H110320350 for allelic  
357 exchange to delete *stx1* and *stx2* sequentially, these were confirmed absent by PCR using primers:  
358 *stx1* (5'-ATAAATCGCCATTTCGTTGACTAC and 5'-AGAACGCCCACTGAGATCATC) and *stx2*  
359 (5'-GGCACTGTCTGAAACTGCTCC and 5'-TCGCCAGTTATCTGACATTCTG). Motility of isolate  
360 ZAP1589 and isolate H110320350 was tested on motility agar (0.7 %), and presence of the H7  
361 flagella was confirmed by agglutination with the monoclonal H7 antibody.

362 Bacteria were cultured overnight in Lysogeny-broth medium (LB) at 37 °C (2), with shaking at  
363 200 rpm. Prior to experimentation an aliquot of the overnight culture was inoculated 1:100 in  
364 rich defined 3-(N-morpholino)propanesulfonic acid (MOPS) medium (45) with 0.2 % glycerol  
365 and essential and non-essential amino acids, termed 'rich defined MOPS glycerol' (RDMG), for  
366 24 h at 18 °C and 200 rpm. Bacteria were collected by centrifugation, washed in phosphate  
367 buffered saline (PBS) and adjusted to the required starting optical density (OD) 600 nm. Media  
368 was supplemented with 30 µg ml<sup>-1</sup> kanamycin, if required. Defined artificial 'lettuce apoplast' or  
369 'sprout extract' media was generated by adding each group of constituents (Table 3) to a base  
370 minimal MOPs medium (MMM) lacking a carbon source and amino acids. Each component  
371 group was added at the defined concentration to represent the concentrations and composition  
372 present in lettuce apoplast or sprout extracts and by dilution of one major group at a time at:  
373 1:50 saccharides (SA), 1:10 amino acids (AA) or 1:20 organic acids (OA), while the other  
374 groups were at 1:1. The pH of the sprout defined medium was 7.2 and lettuce apoplast defined  
375 medium 7.05. Viable counts were determined from 10-fold dilutions plated on MacConkey  
376 (MAC) agar, incubated overnight at 37 °C and counted manually the next day. All experiments  
377 were conducted in triplicate. Viable counts and OD<sub>600</sub> nm were plotted in Excel 2010.

### 378 Plant extracts and metabolite analysis

379 Lettuce (*Lactuca sativa*) var. All Year Round and spinach (*Spinacia oleracea*) var. Amazon  
380 were grown individually in 9 cm<sup>3</sup> pots in compost for microbiological assays, or in vermiculite  
381 for metabolite analysis, in a glasshouse for three weeks. Fenugreek (*Trigonella foenum-*  
382 *graecum*) and alfalfa (*Medicago sativa*) seeds were soaked in sterile distilled water (SDW) for  
383 3 h at room temperature (RT), surface sterilized with 3 % calcium hypochlorite (20,000 ppm ml<sup>-1</sup>  
384 <sup>1</sup> active chlorite) for 15 min, washed five times with SDW and soaked for 2 h in SDW at RT.

385 Sprouts were transferred aseptically on distilled water agar (DWA) (0.5 % agar) and sprouted  
386 for two (alfalfa) or five (fenugreek) days at 25 °C in darkness. Leaf apoplastic washings were  
387 collected as described previously (Methods SM3), optimised for spinach and lettuce to  
388 minimize cytoplasmic contamination (35). All tissue extracts were made as described  
389 previously (8). In brief, vermiculite was gently washed off the roots with tap water and rinsed  
390 with SDW. Leaves and roots were separated with a sterile scalpel, macerated in liquid nitrogen  
391 with a pestle in a mortar and stored at -20 °C until use and pre-processed for sample  
392 clarification by mixing 1 g with 20 ml SDW, soaked on a shaker for 4 h, centrifuged at 5000 rcf  
393 for 15 min, and the supernatant heated to 50 °C for 30 min. The extract was centrifuged at  
394 5000 rcf for 15 min and filter sterilised through a 0.45 µm filter for root tissue or 0.1 µm filter for  
395 leaf tissue. Sprouts were macerated in liquid nitrogen, processed as described above without a  
396 washing step to remove vermiculite, and filter sterilised through a 0.22 µm filter. Apoplast  
397 extracts were filtered sterilised through a 0.1 µm filter (Durapore, Merck, Germany). Extracts  
398 were made from ~ 5 plants per sample for leaves and roots and up to 24 plants for apolastic  
399 washings or for sprouts. 10 ml plant extract samples were used for GC-MS analysis as  
400 described in Methods SM4. Lysates were prepared for HPLC described previously by (62).

#### 401 Growth rate parameterisation

402 Representative edible species associated with food-borne outbreaks were used: two leafy  
403 greens (lettuce, spinach) and two sprouted seeds (fenugreek, alfalfa). Plant tissues used  
404 represented edible, non-edible and internalised tissues of the leafy greens from total lysates of  
405 leaves or roots, and apoplastic washing recovered from leaves, respectively, while total sprout  
406 lysates were used to represent edible sprouts. A panel of five *E. coli* was assessed (Table 1) to

407 compare relative fitness of two STEC O157:H7 Stx- isolates to two environmental isolates from  
408 plant roots and soil. A K-12 faecal-derived and laboratory-adapted isolate was included for  
409 reference. Growth was assessed at three temperatures (18, 20 and 25 °C) to represent  
410 relevant growth temperatures of field-grown leafy greens in northern temperate zones and  
411 sprouted seeds grown under controlled conditions. Growth kinetics were measured from  
412 optical densities derived from a plate reader (as described by others (20)).

#### 413 Bacterial growth rates

414 Bacterial growth rates were determined using a pre-warmed plate reader Bioscreen C plate  
415 reader (Oy Growth Curves Ab Ltd, Finland), set to different temperatures. The *E. coli* isolates  
416 were grown as described above, adjusted to an OD<sub>600</sub> of 0.05 in PBS (~ 2.1 x 10<sup>7</sup> cfu ml<sup>-1</sup>) and  
417 inoculated at a 1:10 dilution in plant extracts (at 1:20 w/v in dH<sub>2</sub>O) or defined media (Table 3),  
418 in 200 µl total volume, in multi-well plates. Growth for the *E. coli* isolates was measured at 18,  
419 20 and 25 °C in 100-microwell plates (Honeycomb, Thermo Fisher, USA). Wells were  
420 randomised in duplicate on the plate with negatives included. All growth curves in extracts  
421 were repeated three times with four replicates on plates. Measurements were recorded every  
422 15 min for 48 hours and multi-well plates were shaken for 60 seconds pre- and post-  
423 measurement. Results were exported from plate reader proprietary software as tab-delimited  
424 files. For model fitting, 12 replicates of each isolate and medium type were averaged and  
425 converted to viable counts log (cfu h<sup>-1</sup>) (Methods SM5). A conversion factor of 4.2 x 10<sup>8</sup> cfu ml<sup>-1</sup>  
426 was applied so that all growth curves could be modelled using DM-Fit (Methods SM1).  
427 Secondary modelling was applied for different temperature as described (Methods SM2). A 2-

428 way ANOVA was carried out for multiple comparisons (isolate / extract type) in Prism v6  
429 (GraphPad Software Inc., USA).

#### 430 Biofilms

431 Bacterial biofilms were measured as described previously (42). Bacteria were grown  
432 aerobically in LB at 37 °C for 12 h, sub-cultured (1:1000 v/v) in RDMG for 18 h at 18 °C, diluted  
433 in PBS to OD<sub>600</sub> of 0.05 and inoculated into plant extracts as per the growth rates  
434 determination in a 96 well polystyrene plate and incubated statically for 48 h at 18 °C. The  
435 washed wells were stained with 0.1 % crystal violet solution and solubilised with 95 % ethanol.  
436 The solution was transferred into a fresh plate and absorbance measured at 590 nm with a  
437 plate reader (Multiskan Go, Thermo Scientific, USA). Results were exported with the software  
438 SkanIt™ (Thermo Scientific, USA) to Microsoft Excel 2010 for analysis. A 2-way ANOVA was  
439 carried out for multiple comparisons (isolate / extract type) in Prism v6 (GraphPad Software  
440 Inc., USA).

#### 441 Plant colonisation assay

442 Lettuce and spinach plants (~ 3 weeks old) were transferred to a growth chamber (Snijders) at  
443 21 °C; 75 % humidity and 16 h light – 8 h dark cycle (400 µE/m<sup>2</sup>.s (30.000 lux)) three days  
444 prior to inoculation and were not watered for ~ 18 h prior to inoculation. Roots were inoculated  
445 by placing pots in a plastic box containing a 1 litre suspension of *E. coli* Sakai or ZAP1589,  
446 diluted to OD<sub>600</sub> of 0.02 (equivalent to 10<sup>7</sup> cfu ml<sup>-1</sup>) in SDW, which partially submerged pots.  
447 After 1 h inoculation, the pots were transferred to the growth chamber until sampling. Sprouts  
448 were inoculated with 10<sup>3</sup> cfu ml<sup>-1</sup> bacteria in 0.5 l SDW for 1 h, rinsed with 0.5 x Murashige and  
449 Skoog (MS) basal medium (no sucrose), and transferred to petri dishes containing distilled

450 water agar (DWA) (0.8 % agar) and incubated for up to three days at 25 °C. Negative controls  
451 were incubated with SDW without bacteria.

452 Lettuce and spinach roots were sampled at 0, 5, 10 and 14 days post infection (dpi),  
453 aseptically removed from aerial tissue with a sterile scalpel, the compost removed by washing  
454 with SDW, and the roots were transferred into 50 ml tubes, washed with PBS and the fresh  
455 weight determined. Sprouts were sampled at 0, 1, 2 dpi, where half were used to enumerate  
456 the total viable counts of *E. coli* and stored in PBS until further use (~ 30 min), and surface-  
457 associated bacteria were removed from the other half of the samples by surface sterilization  
458 with 200 ppm Ca(ClO)<sub>2</sub> for lettuce/spinach roots or 20,000 ppm Ca(ClO)<sub>2</sub> for sprouts, for 15  
459 min. Surface decontamination of sprout tissue required at least 15,000 ppm of Ca(ClO)<sub>2</sub> to  
460 eradicate external *E. coli*, but endophytes appeared to be protected from the active chlorite  
461 since endemic internalised bacteria occurred on recovery media after surface decontamination  
462 with 20,000 ppm Ca(ClO)<sub>2</sub>. The root/sprouts were washed five times with PBS to ensure  
463 removal of all loosely adherent bacterial cells and residual chlorine. Surface sterilisation was  
464 validated as described (67). Any samples containing surface-associated bacterial colonies  
465 were removed from subsequent analysis. Roots/sprouts were macerated using mortar and  
466 pestle in 2 ml PBS and ~ 50 mg sterile sand. The supernatant was diluted once for spinach  
467 and lettuce (1:1), three times for fenugreek (1:3) or four times for alfalfa (1:4) with PBS and  
468 100 µl plated on MAC plates using a spiral plater (WASP, Don Whitley Scientific, UK) and  
469 incubated for 24 h at 37 °C. Plates were counted using a counting grid (WASP, Don Whitley  
470 Scientific, UK), multiplied by the dilution factor and converted to cfu ml<sup>-1</sup>. The experiment was  
471 repeated three times with five replicate samples per time point, and sprout samples comprised  
472 multiple (> 15) sprouts. The limit of detection from direct plating was 20 cfu ml<sup>-1</sup>, below which



473 values were manually levelled to  $< 1 \log$  (cfu ml<sup>-1</sup>) for lettuce and spinach root data. Since the  
474 level of inoculation of sprouts for day 0 was below the detection limit, the numbers were semi-  
475 quantified by most probable number (MPN) method for 3 tube assay as described by Oblinger  
476 and Koburger (46). Samples were diluted 6-fold in buffered peptone water (BPW) and  
477 incubated overnight at 37 °C, and positive samples confirmed by plating triplicate 100  $\mu$ l  
478 samples on MAC agar and incubating overnight at 37 °C.

479

480

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487 **Conflict of interest disclosure**

488 The authors declare no conflicts of interest.

489

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## 682 Tables and Figures

## 683 Tables

684 **Table 1** Bacterial isolates used in this study

685 ST = sequence type, Stx = Shiga toxin presence, nd = not determined, n/a = not applicable.  
 686 Isolate Sakai used here is the *stx*-inactivated derivative (9). \* Isolate ZAP1589, derived from  
 687 H110320350 (Perry et al., 2013) has both *stx*-encoding regions removed, and is H7 positive  
 688 but non-motile. \$ GenBank, ENA or BioProject accession numbers are provided for whole  
 689 genomes.

Isolate Name	Serotype	ST	Stx	Source	Reference	Genome <sup>\$</sup>
MG1655	OR:H48	98	n/a	faecal/lab	(21)	NC_000913.1
JHI5025	nd	2055	n/a	soil	(24)	ERS1939526
JHI5039	nd	2303	n/a	root	(24)	ERS1939531
Sakai	O157:H7	11	negative	sprout / clinical	(9)	NC_002695.2
ZAP1589	O157:H7	11	negative	leek /	(49)*	PRJNA248042

690

691 **Table 2** Quantification of saccharides from plant extracts

692 Concentrations of mono- and disaccharides determined by HPLC ( $\mu\text{g mg}^{-1}$ ). ND – not  
 693 detected.

	glucose	fructose	sucrose	arabinose
fenugreek	24.5 ± 3.1	24.9 ± 3.7	75.6 ± 6.3	ND
alfalfa	35.4 ± 0.8	35.8 ± 18.6	3.5 ± 0.3	ND
lettuce apoplast	19.4 ± 1.8	23.4 ± 2.8	53.4 ± 20.7	0.226 ± 0.001
lettuce leaf lysates	10.7 ± 0.3	14.6 ± 0.4	50.1 ± 3.1	ND
lettuce root lysates	9.9 ± 0.1	20.0 ± 0.9	22.5 ± 0.4	ND
spinach apoplast	11.8 ± 2.0	8.0 ± 1.7	38.3 ± 7.0	0.211 ± 0.049
spinach leaf	21.9 ± 2.9	6.1 ± 0.8	32.8 ± 2.6	ND
spinach root	17.4 ± 1.2	9.00 ± 0.9	29.4 ± 1.5	ND

694

695 **Table 3** Composition of defined artificial media supplements

696 Concentration ( $\mu\text{g ml}^{-1}$ ) as determined by HPLC and GC-MS for the major six components in  
 697 sprout extracts (alfalfa and fenugreek combined), lettuce apoplast, used to generate defined  
 698 'artificial' media.

<b>Metabolite</b>	<b>Sprouts</b>	<b>Lettuce apoplast</b>
<b><i>Saccharides (SA)</i></b>		
Sucrose	3021.4	2116.2

32



Fructose	1443.4	926.5
Glucose	1425.0	769.8
<b>Amino acids (AA)</b>		
Asparagine	814.3	n/a
Alanine	766.1	n/a
Serine	327.4	n/a
Oxoproline	n/a	63.4
<b>Organic acids (OA)</b>		
Malic acid	n/a	194.0
2,3-dihydroxy-propanoic acid	n/a	143.5

699

700 **Table 4** Biofilm formation for reference *E. coli* isolates in plant tissue extracts. Biofilms  
 701 were formed on polystyrene multiwall plates following incubation in spinach (Sp.) and lettuce  
 702 (Lt.) extracts (apoplast; leaf; root) and rich defined MOPS medium with glycerol (RDMG) at 18  
 703 °C, for 48 hrs in static conditions. The average ( $\pm$  variance) density of crystal violet at OD<sub>590 nm</sub>  
 704 is presented. P value summaries are provided per isolate for each extract type vs' RDMG (ns p  
 705 > 0.05; \* p  $\leq$  0.05; \*\* p  $\leq$  0.01; \*\*\* p  $\leq$  0.001; \*\*\*\* p  $\leq$  0.0001).

Treatment /	Sakai	ZAP1589	JHI5025	JHI5039	MG1655

33

Isolate					
Sp. apoplast	0.002 ± 0.001 (ns)	0.011 ± 0.001 (ns)	0.372 ± 0.007 (****)	0.013 ± 0.000 (ns)	0.001 ± 0.002 (ns)
Sp. leaf	0.071 ± 0.000 (***)	0.128 ± 0.001 (****)	0.218 ± 0.034 (****)	0.113 ± 0.001 (****)	0.000 ± 0.000 (ns)
Sp. root	0.173 ± 0.000 (****)	0.148 ± 0.017 (****)	0.179 ± 0.015 (****)	0.126 ± 0.000 (****)	0.013 ± 0.000 (ns)
Lt. apoplast	0.000 ± 0.002 (ns)	0.005 ± 0.000 (ns)	0.125 ± 0.005 (****)	0.001 ± 0.000 (ns)	0.000 ± 0.000 (ns)
Lt. leaf	0.000 ± 0.000 (ns)	0.018 ± 0.001 (ns)	0.151 ± 0.002 (****)	0.007 ± 0.000 (ns)	0.001 ± 0.000 (ns)
Lt. root	0.008 ± 0.000 (ns)	0.029 ± 0.001 (ns)	0.066 ± 0.001 (ns)	0.025 ± 0.000 (ns)	0.000 ± 0.000 (ns)
RDMG	0.000 ± 0.000	0.000 ± 0.000	0.013 ± 0.000	0.000 ± 0.000	0.000 ± 0.000

706

707

708 **Figure Legends**

709 **Figure 1** Maximum growth rates ( $\mu$ ) of reference *E. coli* isolates in plant extracts.

710 Maximum growth rates ( $\mu$ ) were calculated using the Baranyi model for the reference *E. coli*  
711 isolates in spinach **(A)** or lettuce **(B)** aploplast (circles), leaf lysates (triangles) and root lysates  
712 (diamonds) extracts, or in alfalfa (circles) or fenugreek (triangles) sprouts lysate extracts **(C)**  
713 with RDMG (diamonds) as no-plant extract control, at 18, 20 or 25 °C. Each point is the  
714 average rate (n = 12), with standard errors indicated by bars. P value summaries from multiple  
715 comparison analysis by isolate 'vs' MG1655 or by extract type 'vs' RDMG are provided in  
716 Table S1b and Table S1c, respectively.

717 **Figure 2** Plant extract metabolomics and grouping

718 The 60 assigned metabolites from all species and tissues are separated into amino acids,  
719 organic acids, mono- and polysaccharides and others **(A)** by their mean total response ratio  
720 (with SD indicated by bars). **(B)** Score plot of principal component 1 (31 % variance) and  
721 component 2 (19 %) for all 116 polar metabolites, for alfalfa (ALF) in red, fenugreek (FEN) in  
722 blue, spinach (SAP, SLL, SRL) green and lettuce (LAP, LLL, LRL) black.

723 **Figure 3** Maximum growth rates ( $\mu$ ) in artificial media mimicking plant extracts.

724 Maximum growths rates ( $\mu$ ) calculated using the Baranyi model for the *E. coli* isolates at 18 °C  
725 and 25 °C in media mimicking **(A)** lettuce apoplast or **(B)** sprout lysates (a mixture of alfalfa  
726 and fenugreek sprout metabolites) with specified dilutions. The base minimal MOPS medium  
727 (MMM) was supplemented with saccharides (SA), organic acids (OA) or amino acids (AA) at  
728 the dilution specified. Each point is the average rate with standard errors indicated by bars.

729 **Figure 4** Total and internalised counts for *E. coli* O157:H7 *in planta*.

730 The number of *E. coli* isolate ZAP1589 recovered from inoculation ( $10^7$  cfu ml<sup>-1</sup>) of **(A)** spinach  
731 (var. Amazon) or **(B)** lettuce (var. All Year Round) roots at 0, 5, 10 and 14 dpi.. The number of  
732 *E. coli* isolate ZAP1589 recovered from alfalfa **(C)** or fenugreek **(D)**, and *E. coli* isolate Sakai  
733 recovered from alfalfa **(E)** or fenugreek sprouts **(F)**, from inoculation at  $10^3$  cfu ml<sup>-1</sup>, sampled at  
734 0, 1 and 2 dpi.. Averages (lines) and individual samples counts are shown for the total (black)  
735 or internalised population (red) (n = 15: ~ 1.5 g per sample for sprouts, individual plants for  
736 spinach & lettuce). Sprout d0 data was assessed by MPN (level of detection = 0), otherwise  
737 minimum counts were manually levelled to the direct plating detection limit of 10 cfu g<sup>-1</sup> on d1.

738 **Figure 5** Comparison of *in planta* and extract growth rates for *E. coli* isolates Sakai and  
739 ZAP1589

740 Growth rates for *in planta* estimates were plotted against estimates for plant extract extracts,  
741 on a Log<sub>10</sub> cfu day<sup>-1</sup> basis for *E. coli* isolates Sakai and ZAP1589, normalised per g fresh  
742 weight for plant tissues or per ml for plant extracts. Estimates for sprouted seeds (alfalfa – Alf;  
743 fenugreek – Fen) were obtained for growth at 25 °C, and at 18 °C for spinach (Sp.) or lettuce  
744 (Lt.) tissues (apoplast – A; leaves – L; roots – R).

745 **Supplemental Figure 1** Manual correction of growth rate misfits in DMFIT.

746 Example of a correction with *E. coli* isolate JHI5039 grown in lettuce leaf lysate, 18 °C. **A)**  
747 DMFIT could not fit a non-linear curve on data (n = 193) with a decrease in the stationary  
748 phase ( $R^2_{adj} = 0.001$ ). **B)** Data was cut off manually (n = 49) to achieve better fits ( $R^2_{adj} =$   
749 0.996). A complete list of fits including data points are in Supplemental Table 3.

750 **Supplemental Figure 2** Simplified polar metabolic pathways in plants

751 Interaction between major polar pathways (colour coded) in green leafy plants. Metabolism of  
752 carbohydrates degradation (green) is linked to amino acid degradation (dark blue and purple),  
753 which feed into the TCA cycle (red). The arrows pointing outside are entries into the non-polar  
754 fatty acid pathway. The glutamate group (orange) leads into the urea cycle. The light blue  
755 cycle described the acyl chain synthesis. Modified from the metabolomic pathway in *Solanum*,  
756 based on Dobson, et al. (13).

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