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Vacuolar localisation of anthocyanin pigmentation in microgreen cotyledons of basil, cabbage and mustard greens does not impact on colonisation by Shiga-toxigenic Escherichia coli O157:H7

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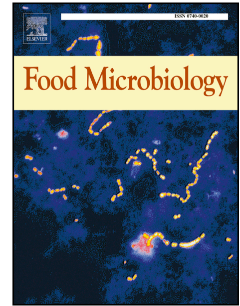

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Vacuolar localisation of anthocyanin pigmentation in microgreen cotyledons of basil, cabbage and mustard greens does not impact on colonisation by Shiga-toxigenic *Escherichia coli* O157:H7.

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Running title

No impact of anthocyanin on *E. coli* O157:H7 colonisation of microgreens

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Abstract

Microgreens, the immature plants harvested after a few weeks of growth, are perceived as a heathy, nutritious food ingredient but may be susceptible to colonisation by human pathogens including Shiga-toxigenic *Escherichia coli* (STEC). Some microgreen cultivars accumulate anthocyanins or secrete essential oils which, when extracted or purified, have been reported to inhibit bacterial growth. Therefore, the impact of anthocyanins on bacterial colonisation by STEC (Sakai) was compared for three species that have pigmented cultivars: basil (*Ocimum basilicum* L.), cabbage (*Brassica oleracea* L.) and mustard greens (*Brassica juncea* L.). Inoculation with low concentrations of STEC (Sakai) (3 log_{10} colony forming units/ml (CFU/ml)) during seed germination resulted in extensive colonisation at the point of harvest, accumulating to ~ 8 log_{10} CFU/g FW in all cultivars. Bacterial colonies frequently aligned with anticlinal walls on the surface of epidermal cells of the cotyledons and, in basil, associated with peltate and capitate gland cells. Crude lysates of pigmented and non-pigmented basil

cultivars had no impact on STEC (Sakai) growth rates, viability status or biofilm formation. Anthocyanins are located within plant vacuoles of these microgreen cultivars and did not affect colonisation by STEC (Sakai) and pigmentation therefore cannot be considered as a controlling factor in bacterial interactions.

KEYWORDS

Microherb, fresh produce, foodborne pathogen, vegetable

Wordcount:

Abstract – 197

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1 # 1 INTRODUCTION

2 'Microgreens', 'microleaf' and 'microherbs' are marketing terms for immature plants, grown in
3 light and harvested less than a month after germination (Di Gioia et al., 2015). Whilst often
4 consumed in Asian cultures, they are increasingly popular as ingredients in Western countries,
5 providing colour and intense flavour, and may have enhanced nutritional and potentially
6 nutraceutical value (Kyriacou et al., 2016). The method of growth of microgreens can be
7 favourable for proliferation of food-borne pathogens including Shiga-toxigenic *Escherichia coli*
8 (STEC) (Riggio et al., 2019; Wright and Holden, 2018) similar to the situation for sprouted
9 seeds. Several STEC outbreaks have been associated with sprouted seeds (Buchholz et al.,
10 2011; CDC, 2016) and STEC can interact with plants as alternative hosts (Holden et al., 2009;
11 Méric et al., 2013). STEC has the potential to colonise a range of microgreen species to high
12 levels under commercially relevant growth conditions (Wright and Holden, 2018). In addition
13 to the public health drivers, foodborne illness incurs large financial costs, whether at the retail
14 or national levels (Bartsch et al., 2018; Hussain and Dawson, 2013).

15 The red colouration of some cultivars of plant species including basil (*Ocimum basilicum* L.),
16 cabbage (*Brassica oleracea* L.) and mustard greens (*Brassica juncea* L.) is due to the
17 presence of anthocyanins. These and other flavonoids have a role in protecting plants from
18 abiotic stresses like high light intensities and UV-B radiation and in protection against
19 phytopathogens (Harborne and Williams, 2000; Tattini et al., 2017). The synthesis of
20 anthocyanins is understood to be localised on the cytosolic side of the endoplasmic reticulum,
21 but they are primarily located in the vacuole, where the pH influences the resulting colour.
22 Anthocyanins have been the subject of much recent interest into their pharmacological
23 potential as antioxidant, anti-inflammatory and antimicrobial agents (Liu et al., 2021). In
24 particular, they are under investigation by the food industry as natural colourants, flavouring,
25 antioxidant or antibacterial agents to replace synthetic chemical additives or preservatives
26 (Demirdöven et al., 2015). Here, we tested whether pigmentation from anthocyanins impacted
27 the colonisation of microgreens during propagation, by the foodborne pathogen STEC
28 O157:H7. Different green, or red, anthocyanin-producing, cultivars of three species, basil
29 ('Genovese' and 'Dark Opal'), cabbage ('Golden acre' and 'Red Drumhead') and mustard
30 greens ('Wasabini' and 'Red Carpet'), were inoculated with an STEC isolate (Sakai),
31 previously associated with large-scale disease outbreaks from contaminated white radish
32 sprouts (Michino et al., 1999). The experimental set-up was designed to mimic commercial
33 growth hydroponic conditions, and the plants harvested at a suitable size for microgreen
34 consumption.

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37 # 2 MATERIAL and METHODS

38 ## 2.1 Bacterial strain and growth conditions

39 *Escherichia coli* O157:H7 Stx-negative strain Sakai was used for biosafety reasons, which
40 contains an insertional inactivation of *stx2* with a kanamycin cassette and partial deletion of
41 the *stx1a* coding sequence and 5' region (Dahan et al., 2004). It was transformed with the
42 plasmid-borne GFP fluorescent reporter *pgyrA-gfp* (termed STEC (Sakai)) was used for
43 visualisation and for quantification (for details see Wright and Holden, 2018). Bacteria were
44 routinely cultured for ~18 h in lysogeny broth (LB) at 37°C with aeration, and prior to use sub-
45 cultured 1:100 in rich defined MOPS (RDM) glucose and grown at 18°C for ~20 h, in the
46 presence of chloramphenicol (25 µg/ml). Long-term stocks were stored at -80°C in 20 %
47 glycerol.

48 ## 2.2 Plant material

49 Seed of the following red or green plant cultivars (cvs.) were used: basil (*Ocimum basilicum*)
50 cvs. Micro Leaf Basil 'Dark Opal' (M) and 'Genovese' (C); cabbage (*Brassica oleracea*) cvs.
51 'Red Drumhead' (C) and 'Golden Acre' (D), mustard greens (*Brassica juncea*) cvs. 'Red
52 Carpet' (C) and 'Wasabini' (S); purchased from Marshalls seeds, Huntingdon (M), Chiltern
53 Seeds, Wallingford (C), Sarah Raven Seeds, Wiltshire (S), or Dobies, Paignton, UK (D).

54 ## 2.3 Seed sterilisation, plant growth and inoculation with bacteria

55 Seeds were treated with 5 % Domestos (Unilever: includes 10 % sodium hypochlorite, 0.1-1
56 % sodium hydroxide and surfactant) diluted in sterile distilled water (SDW) for 5 min followed
57 by 6 rinses in SDW. Under sterile conditions, seeds were transferred to round (7.5 cm diameter
58 x 8 cm high) polypropylene containers lined with dry matting (GrowFelt Purple, CN Seeds Ltd.,
59 Ely, UK). Seed surface sterilisation reduces endemic microbes and helps to standardise
60 conditions. STEC (Sakai) were diluted to OD_{600} of 0.2 (~8.0 log_{10} CFU/ml) in 0.5 x Murashige
61 and Skoog medium (Murashige and Skoog, 1962) including vitamins adjusted to pH 5.8 with
62 NaOH (Duchefa, M0222); the same medium required to maintain plant growth and reduce the
63 variable factors to 'bacterial inoculant' only. They were further diluted to 3.0 log_{10} CFU/ml in
64 0.5 x MS prior to seed inoculation. The matting was moistened with 15 ml of bacterial
65 suspension, and the containers fitted with lids incorporating a sun cap closure (Sigma-Aldrich
66 S5939) to allow gas exchange. They were then transferred to a growth cabinet (16 h light,
67 22.8 ± 0.02°C, humidity 96.5 ± 0.05%; 8 h dark, 20.6 ± 0.01°C, humidity 99.2 ± 0.04%) until
68 the time of harvest. All containers, matting or plant growth media were sterilised by autoclaving
69 prior to use. For quantification, plants were cut just above the crown to harvest the edible
70 portion of tissue, weighed and macerated with a pestle and mortar in 1 ml phosphate buffered
71 saline (PBS). These extracts were serially diluted 10-fold in PBS, with 4 replicate 10 µl aliquots

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72 plated as micro-drops on to 24.5 cm square bioassay plates containing MacConkey agar with
73 chloramphenicol (Pious et al 2015), and incubated overnight at 37°C. Resultant colonies were
74 expressed as CFU/g of fresh weight (FW) with a detection limit of 403 CFU/g FW (=2.6 log_{10}
75 CFU/g FW or 7 CFU/g $FW^{1/3}$), based on presence of at least 1 bacterial colony in one of four
76 samples and converted by the maximum plant weight. For each species, five containers were
77 sown with either the red or green cultivar, with one plant being sampled from each container
78 at each harvest date, and the experiment was repeated twice, independently. Due to the
79 different growth rates, basil was harvested between 6 and 9 days, and cabbage and mustard
80 between 5 and 8 days from sowing.

81 **2.4 Leaf lysate extraction and STEC (Sakai) inoculation**

82 Basil cultivars 'Dark Opal' and 'Genovese' were germinated in standard compost and grown
83 at ~ 21 °C in a glasshouse. Two weeks after germination, the leaves were aseptically
84 harvested using sterilised scissors and macerated to a fine powder in liquid nitrogen. 10 g leaf
85 powder was added to 40 ml SDW, vortexed for 1 min and harvested by centrifugation (4,000
86 g for 20 min). The supernatant was filter sterilised and stored at -20 °C. Leaf extracts were
87 used to supplement RDM medium with or without 0.2 % (v/v) glycerol, at a concentration of
88 40 % (v/v), as previously (Crozier et al., 2016). To determine growth rates, 2 ml of STEC
89 (Sakai) were harvested by centrifugation (4,000 g for 5 min) and re-suspended in 2 ml RDM,
90 or in LB as a control. The cell density was measured and adjusted to a final OD_{600} of 0.05 and
91 200 µl was added per well to a multiwell plate (Honeycomb; Thermo Fisher, USA), in sample
92 reps of 4. The cultures were incubated at 21 °C for 60 hours and cell density read in a
93 prewarmed plate reader (Bioscreen C; Oy Growth Curves Ab Ltd., Finland), with
94 measurements taken every 15 min, and the multiwell plates shaken for 60 s pre- and post-
95 measurement. The results were exported from the plate reader proprietary software as tab-
96 delimited files. To determine the cell viability status, bacteria were grown as above, incubated
97 at 21 °C for 60 hours, serially diluted 10-fold in PBS and plated onto MacConkey plates, half
98 of which contained 0.1 % Na-pyruvate to resuscitate stressed, non-culturable cells (Mizunoe
99 et al., 1999), and incubated at 37 °C for 24 hours. The colony number were converted to log_{10}
100 CFU/ml. Biofilm formation was assessed from cells grown as above, with 200 µl per well added
101 at a density of OD_{600} 0.02 and incubated at 21 °C for 60 hours. Biofilms were assessed by
102 crystal violet staining, as described previously (Merget et al., 2019).

103 **2.5 Confocal microscopy**

104 Cotyledons were harvested and mounted in SDW on microscope slides under a coverslip held
105 in place using double-sided tape and observed using a Nikon A1R confocal laser scanning
106 microscope mounted on an NiE upright microscope fitted with an NIR Apo 40x 0.8W water

107 dipping lens and GaAsP detectors. GFP (green) and chlorophyll (blue) were excited at 488
108 nm with emissions gathered at 500-530 nm and 663-738 nm respectively Anthocyanin was
109 excited sequentially at 561 nm with emissions gathered at 570-620 nm (magenta). Images are
110 false colour single confocal fluorescence sections or maximum intensity projections, produced
111 using NIS-elements AR software. Colour plates were prepared using Photoshop CS5 software
112 using enhancement of levels where appropriate.

113 **2.6 Statistical analysis**

114 Differences between green and red cultivars for the three species were analysed using linear
115 mixed effects (LME) models. In order to normalise the response variable, CFU/g FW plant
116 tissue, a cube root transformation was used (Shapiro-Wilk Normality test, $W = 0.997$, $p =$
117 0.951) as the usual $\log_{10}$ transformation failed to meet the normality assumption ($W = 0.831$,
118 $p < 0.0001$). The fixed effects were cultivar as a factor and age, defined as day from sowing,
119 as a continuous variable. The addition of a fixed effect for experiment had no significant effect
120 for any species ($p < 0.6$). As replicate experiments were undertaken for each species, and
121 plants were taken from the same five pots at each sample age, pot nested within experiment
122 was used as a random effect. Based on lower Akaike Information Criterion (AIC) score,
123 inclusion of this random effect structure was justified compared to a general linear model. The
124 LME models were implemented using LMER in the package LME4, R4.21.

125 Bacterial growth rates were determined by fitting to the Gompertz model using DMFit
126 (ComBase) add-in for Excel to obtain the maximum growth rate (μmax) and the maximum cell
127 density (y_max). ANOVA was run to determine differences in viability status and biofilm
128 formation.

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131 **3 RESULTS**

132 **3.1 Localisation and colonisation dynamics of STEC (Sakai) on microgreens**

133 To first determine whether red-pigmented cultivars exhibited altered localisation of STEC,
134 seeds of different green or red cultivars of three species; basil, cabbage and mustard greens,
135 were inoculated with relatively low concentration of STEC (Sakai) (3 log_{10} CFU/ml) during
136 germination and examined at the 'microgreen' stage. There were no observable differences
137 between the cultivars in that the seedlings became extensively colonised, with the majority of
138 bacteria being located on the epidermal cell surfaces (Fig. 1). Bacteria were observed in close
139 proximity to the gland cells of basil ('Genovese' Fig. 1a and b) and ('Dark Opal' Fig. 1c and d)
140 and aligned with the anticlinal walls of cabbage ('Golden Acre' Fig. 1e, 'Red Drumhead' Fig.
141 1f) or mustard greens ('Wasabini' Fig. 1g or 'Red Carpet' Fig. 1h).

142 The number of STEC (Sakai) colonising each cultivar was quantified over four days, starting
143 five days (cabbage and mustard greens) or six days (basil) from sowing. Germination of seeds
144 on matting watered with relatively low concentrations of bacteria resulted in extensive
145 colonisation of the cotyledons to around 8.0 log_{10} CFU/g FW (464 CFU/g $FW^{1/3}$ Fig. 2). There
146 was no significant difference in CFU/g FW between basil and cabbage (*p*=0.575), in a model
147 where cultivar was nested in species, but mustard differed with respect to intercept and slope
148 with days from sowing (Table 1). In general, the level of colonisation decreased with time from
149 the start of sampling at day 5 post-sowing (day 6 for basil) until the end of the experiment (Fig.
150 3), although this trend was only significant in cabbage when considered in a single species
151 model (p=0.041). There was no significant difference between the green and red cultivars for
152 any species either when considering the full model or for species models in which the effect
153 of days from sowing and cultivar were compared ($p > 0.27$).

154 **3.2 Leaf lysate extracts from purple basil do not inhibit STEC growth, viability or**
155 **biofilm formation**

156 As there was no apparent antimicrobial impact for intact, growing plants, the bacterial growth
157 response was tested in crude extracts, using basil as a representative species. The impact of
158 both basil cultivars was tested on STEC growth and viability, using freeze-dried leaf lysate
159 extracts that were used to supplement defined media. Extracts from either basil cultivar
160 supported growth of STEC (Sakai) at 21 °C (Fig. 4a), with no obvious difference between the
161 rates for either cultivar in the base RDM medium. Although growth rates were faster in RDM
162 glycerol medium supplemented with 'Dark Opal' extract compared to 'Genovese' extract, the
163 difference was marginal (0.020 'vs' 0.018, respectively). Growth rates and maximum cell
164 densities were similar to that for LB medium, but cultures in media that lacked glycerol reached
165 only 35 – 38 % of the cell density of cultures grown in media containing glycerol. The viability

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166 status of the cells (as determined from resuscitation medium) was not significantly affected
167 during growth in the extracts compared to viable plate counts, as measured at 24 hours
168 (p=0.6177) or 60 hours (p=0.445) at 21 °C, or after incubation of 25 days at 21 °C in either of
169 the neat (100 %) extracts (p=0.496). Neither of the basil cultivar extracts particularly enhanced
170 or suppressed biofilm formation, as measured after 60 hours at 21 °C, in media with or without
171 glycerol (Fig. 4b: p=0.057).

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174 ## 4 DISCUSSION

175 Although concentrated, extracted anthocyanins possess antibacterial properties, we show that
176 such activity did not occur *in planta* since pigmented cultivars of three microgreen species
177 were colonised equally well by the foodborne pathogen, STEC O157:H7 (isolate Sakai) as the
178 green, non-pigmented cultivars of the same species. Extracted anthocyanins have been
179 reported to have beneficial health effects including antimicrobial activities (Khoo et al., 2017).
180 For example, proanthocyanidins isolated from cranberry show bacterial anti-adhesion activity
181 against strains of uropathogenic P-fimbriated *E. coli* (Howell, 2007) whilst anthocyanins,
182 incorporated into dental copolymer, are being tested as natural antibacterial agents against
183 *Streptococcus mutans* (Hrynash et al., 2014). *In planta* anthocyanins are compartmented
184 primarily in the vacuoles of the epidermal cells (Zhao and Dixon, 2010) so that bacteria are
185 unlikely to encounter them unless the plant cells are damaged. This lack of exposure of STEC
186 bacteria to anthocyanins, together with the concentrated levels in extracts, may therefore
187 explain the lack of any impact on *in planta* colonisation for the pigmented compared to the
188 non-pigmented varieties. Furthermore, the effect was consistent across different pigmented
189 plant species. Since red or green cultivars tested are genetically distinct, albeit in the same
190 species, there are additional factors that could impact colonisation.

191

192 Multiple other plant-derived secondary metabolites may also show antimicrobial activity,
193 including essential oils from basil (Sakkas and Papadopoulou, 2017). Therefore, crude
194 aqueous soluble extracts of basil were assessed, which from the 'Dark Opal' cultivar
195 potentially include oils secreted by the gland cells as well as anthocyanins released from the
196 vacuoles. However, neither extract of either purple pigmented or green non-pigmented basil
197 impacted STEC (Sakai) growth, viability status or biofilm formation. Similarly, a clinical isolate
198 of *Salmonella enterica* serovar Senftenberg was equally unaffected by growth on (green) basil,
199 exhibiting resistance to basil oil and its components (Kisluk et al., 2013). The extracts used
200 here were not concentrated or purified in contrast to forms investigated by others for
201 antimicrobial activity (Burt, 2004; Suppakul et al., 2003), which may in part at least, explain
202 the effect. The structure of the gland cells on the cotyledons of both green and red basil
203 appeared essentially similar to those observed on young and more mature basil leaves, and
204 the aroma released during extract preparation was indicative of some oil production. STEC
205 (Sakai) were observed growing in close proximity to both the 2-celled capitate and 4-celled
206 peltate gland cells involved in the synthesis and storage of phenylpropenes that are
207 components of the essential oils secreted by basil (Gang et al., 2001; Werker et al., 1993).
208 This suggests that oils potentially secreted do not influence bacterial colonisation and
209 distribution. Instead, the recessed positioning of the gland cells may offer a protected
210 microclimate for growth of the STEC bacteria, similar to the stomatal pores and channels

211 formed above anticlinal walls of the epidermal cells (Monier and Lindow, 2004), as shown here
212 by colonisation of cabbage or mustard greens by STEC. This distribution on the surface of,
213 and occasionally within, cotyledons is similar to previous observations of basil and other
214 species including amaranth and broccoli (Wright and Holden, 2018). The decrease in numbers
215 of colonising bacteria with age, also observed previously in broccoli (Wright and Holden,
216 2018), may be the result of decreased nutrient availability on the cotyledons. However, for
217 microgreens where the cotyledons are consumed, this is unlikely to reduce the potential risk
218 at the point of consumption.
219

220 **4.1 Conclusion**

221 The cotyledons of numerous species consumed as microgreens, including basil, cabbage and
222 mustard greens, have the potential for extensive colonisation by STEC (Sakai) and, *in planta*,
223 intracellular anthocyanins do not play any antimicrobial role reducing their incidence. This
224 raises the need for microbial risk management for consumption of microgreens as raw
225 products, pigmented or not, in line with that for sprouted seeds. Risk management also needs
226 to take account of innovative technologies used for plant propagation, which lend themselves
227 to this type of product. The information can be incorporated into existing microbial risk
228 guidance for growing fresh produce.
229

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230 **5 ACKNOWLEDGMENTS**

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232 Analytical Services Division, Strategic Research Programme, in Food Safety (B6 and
233 previously in RD3.1.3)

234 **6 CONFLICT OF INTEREST**

235 The authors declare that they have no conflict of interest.

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237 ## 7 FIGURES AND TABLES

238 **FIGURE 1 Colonisation of microgreens by STEC (Sakai)**

239 STEC (Sakai) expressing GFP close to peltate (P), capitate (C) gland cells or stomata (S) on
240 the surface of cotyledons of 'Genovese' (**a** and **b**) and 'Dark Opal' (**c** and **d**) basil seen in
241 maximum intensity projections (**a** and **c**) or single sections (**b** and **d**). STEC overlying the
242 anticlinal epidermal cell walls of 'Golden Acre' (**e**) 'Red Drumhead' (**f**) cabbage, 'Wasabini' (**g**)
243 or 'Red Carpet' (**h**) mustard greens. Scale bars represent 50 μm (**a**, **c**), 10 μm (**b**, **d-h**).
244

245 **FIGURE 2. Observed colonisation rates**

246 Differences in colonisation for STEC (Sakai) plotted in relation to days from sowing for
247 anthocyanin-pigmented (magenta) and green-pigmented (green) cultivars of three microgreen
248 species;- basil, cabbage and mustard greens. Results from two experiments are indicated by
249 symbols (circle and triangle).
250

251 **FIGURE 3. Predicted colonisation rates.**

252 Fitted relationship for colonisation of STEC (Sakai) in relation to days from sowing based on
253 the full interaction linear mixed model together with lower and upper pointwise standard
254 errors for each cultivar. The colonisation data was cube root transformed to conform to
255 normality and homogeneity of variance assumptions.
256

257 **FIGURE 4 Growth and biofilm formation of STEC (Sakai) in basil leaf lysate extracts.**

258 **(a)** Maximum growth rates (Log_{10} CFU/h - X) and maximum cell densities (Y-max (OD_{600nm} -
259 n) of STEC (Sakai) obtained from data fitted to growth models (in DMFit). Growth was
260 quantified in a multi-well reader over 60 hours at 15-minute intervals at 21 °C. The fitted rates
261 with standard errors of the rates are provided from three experimental replicates (n=12).
262 Growth rates in LB were included for reference: a maximum growth rate of 0.021 (LB) equates
263 to a generation time of 5.52 hours. **(b)** Biofilm formation was quantified from crystal violet
264 retained measured at OD_{590nm} after 60 hours at 21 °C. The averages and variance are provided
265 from two experimental reps (n=8). RDM and RDMg refer to rich defined MOPS medium without
266 and with glycerol respectively; DO and G refer to leaf lysate extracts (40 %) of 'Dark Opal' and
267 'Genovese' cultivars respectively.
268
269

270 **TABLE 1.** Linear Mixed Model of colonisation ($CFU.gFW^{1/3}$) in relation to two factors;
271 species and cultivar (red or green) and the continuous variable, days from sowing.
272 Estimates, confidence interval and significance values given for the fixed effects are
273 presented, together with R^2 of model fit.

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Predictors	*Estimates*	*Confidence Intervals*	*p*
(Intercept)	594.70	387.50 – 801.89	**<0.001**
Cabbage	80.92	-201.78 – 363.62	0.575
Mustard	327.94	45.24 – 610.64	**0.023**
Days from sowing	-42.87	-63.47 – -22.28	**<0.001**
Basil * Red	34.95	-11.1 – 81.00	0.137
Cabbage * Red	38.92	-7.13 – 84.98	0.098
Mustard * Red	-44.52	-90.57 – 1.53	0.058
Cabbage * Days from sowing	4.94	-24.19 – 34.06	0.740
Mustard *Days from sowing	-34.90	-64.03 – -5.78	**0.019**
Observations	240		
Marginal R^2 / Conditional R^2	0.345 / 0.558		

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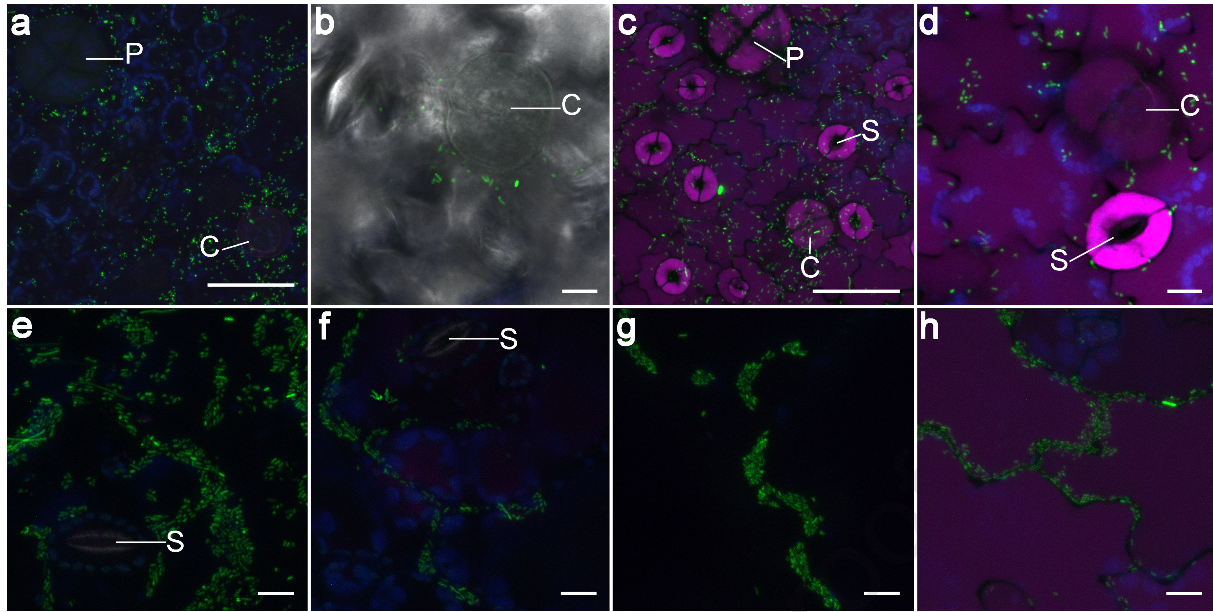

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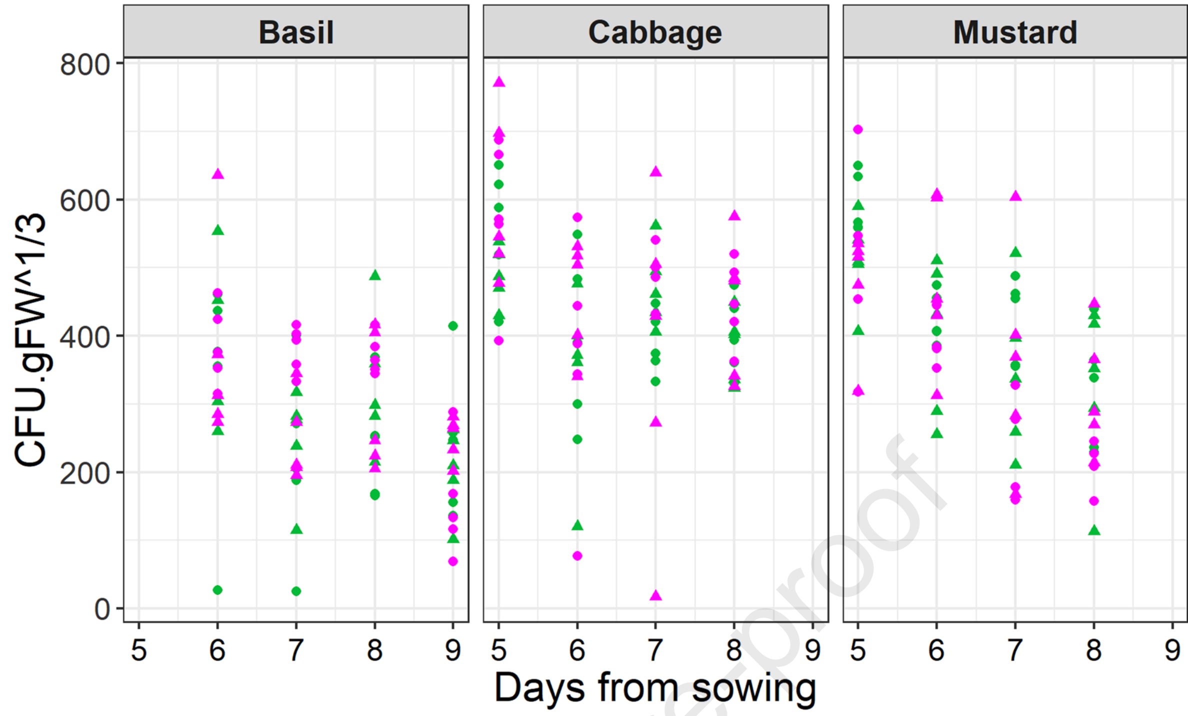

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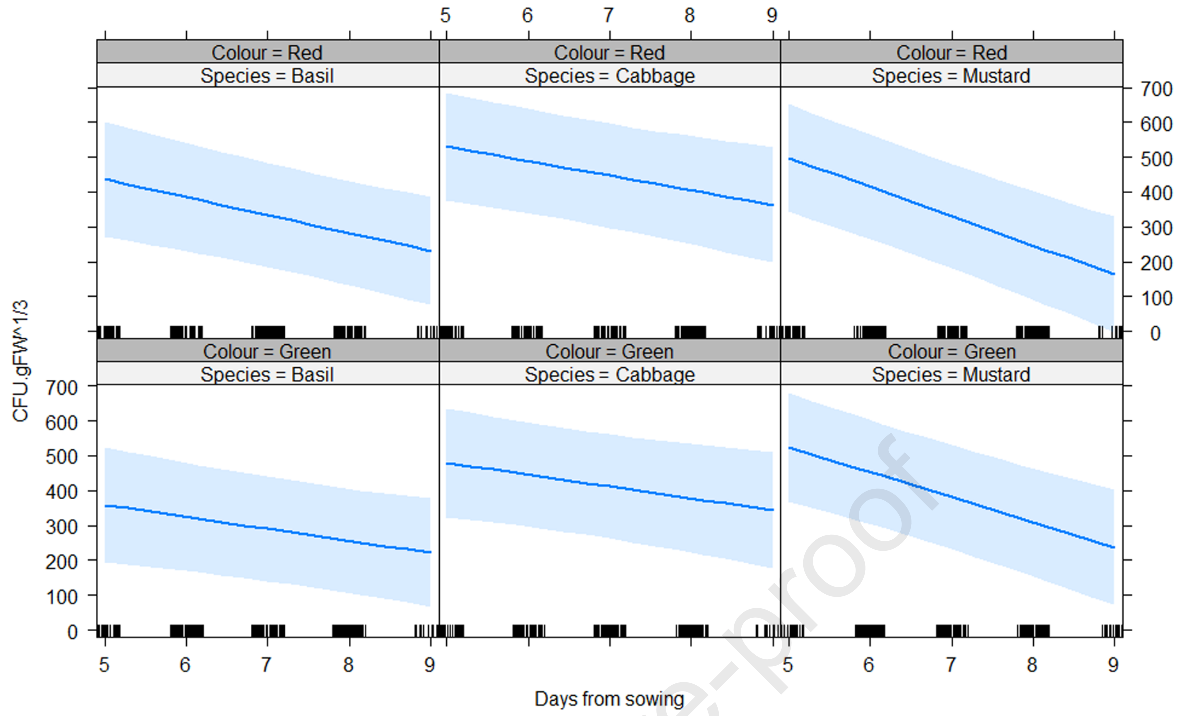

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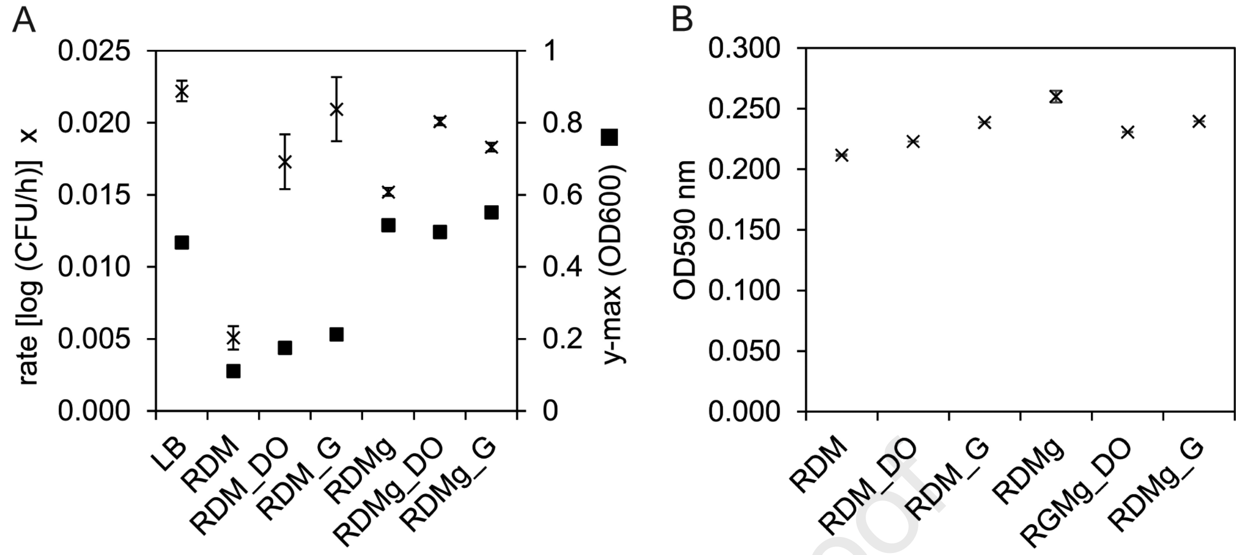

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Vacuolar localisation of anthocyanin pigmentation in microgreen cotyledons of basil, cabbage and mustard greens does not impact on colonisation by Shiga-toxigenic *Escherichia coli* O157:H7.

Kathryn M. Wright [a], Jacqueline Marshall [a], Peter J. Wright [b], Nicola J. Holden [a c*]

Each Highlight can be no more than 85 characters, including spaces

- Microgreens are susceptible to contamination by foodborne bacterial pathogens
- Many microgreens or microherbs are naturally coloured, or pigmented
- *E. coli* O157 grew equally well on microgreens with or without pigments
- *E. coli* O157 was also unaffected by crude extracts of basil microgreens
- Pigmentation in microgreens does not imply a reduced risk of foodborne pathogens

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Anthocyanin pigmentation in microgreen cotyledons does not impact on colonisation by Shigatoxigenic *Escherichia coli* O157:H7.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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