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Silicon-induced root nodulation and synthesis of essential amino acids in a legume is associated with higher herbivore abundance

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Manuscripts

Silicon-induced root nodulation and synthesis of essential amino acids in a legume is associated with higher herbivore abundance

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Running headline: Silicon indirectly promotes aphid abundance

1 Summary

- 2 1. Ecologists have become increasingly aware that silicon uptake by plants, especially the
3 Poaceae, can have beneficial effects on both plant growth and herbivore defence. The
4 effects of silicon on other plant functional groups, such as nitrogen-fixing legumes, have
5 been less well studied. Silicon could, however, indirectly promote herbivore performance in
6 this group if reported increases in N₂-fixation caused improvements in host plant quality for
7 herbivores.
- 8 2. We tested how silicon supplementation in the legume *Medicago sativa* affected plant growth
9 rates, root nodulation and foliage quality (silicon content and amino acid profiles) for an
10 insect herbivore (*Acyrtosiphon pisum*).
- 11 3. Plants supplemented with silicon (Si⁺) grew three times as quickly as those without
12 supplementation (Si⁻), almost entirely in shoot mass. While root growth was unaffected by
13 silicon uptake, root nodules containing nitrogen-fixing bacteria were 44% more abundant
14 on Si⁺ plants. Aphid abundance was twice as high on Si⁺ plants compared to Si⁻ plants and
15 was positively correlated with silicon-stimulated plant growth.
- 16 4. Si⁺ plants accumulated more than twice as much silicon as Si⁻ plants, but did not have
17 higher silicon *concentrations* because of dilution effects linked to the rapid growth of Si⁺
18 plants. Si⁺ plants showed a 65% increase in synthesis of essential foliar amino acids,
19 probably due to increased levels of root nodulation.
- 20 5. These results suggest that increased silicon supply makes *M. sativa* more susceptible to *A.*
21 *pisum*, mainly because of increased plant growth and resource availability (i.e. essential
22 amino acids). While silicon augmentation of the Poaceae frequently improves herbivore
23 defence, the current study illustrates that this cannot be assumed for other plant families

24 where the beneficial effects of silicon on plant growth and nutrition may promote herbivore
25 performance in some instances.

26 **Key-words:** amino acids, aphids, legume, nodulation, nitrogen fixation, plant defence, silica, silicon

27

28 **Introduction**

29 Plant silicon has multiple functional roles in plants and it is unusual in that it has been suggested to
30 provide a particularly broad range of benefits to plants, including resistance to both abiotic and
31 biotic stresses (Cooke, DeGabriel & Hartley 2016; Cooke & Leishman 2016). These include drought
32 and salinity (Liang et al. 2007; Ma & Yamaji 2008), herbivores (Reynolds, Keeping & Meyer 2009;
33 Hartley & DeGabriel 2016) and diseases (Fauteux et al. 2006). Silicon is the second most abundant
34 element in the Earth's crust, which plants acquire from the soil via uptake of soluble silicic acid (Ma
35 & Yamaji 2008). The Poaceae, in particular, often accumulate large amounts of silicon, sometimes in
36 excess of 10% of dry mass, more than any other inorganic constituent (Epstein 1999). The process
37 of silicon uptake and deposition is still incompletely characterised, but both active and passive
38 transport mechanisms have been identified in a range of crop species (Ma et al. 2006; Hartley 2015;
39 Deshmukh & Belanger 2016). Silicon can be deposited within or between cells, in the cell wall or as
40 discrete opaline phytoliths (Cooke & Leishman 2011). Deposition patterns can be altered markedly
41 by changes in silicon supply (Hartley et al. 2015).

42

43 Agronomists have recognised since the 1960s that applying silicon to soils can dramatically
44 increase rates of plant growth with benefits for crop yield in a range of systems (Epstein 1999;
45 Guntzer, Keller & Meunier 2012). However, most of these studies demonstrated these benefits in
46 the Poaceae, where silicon can be used as a structural material to support more erect growth as
47 well as increased rigidity and resistance to lodging (Schoelynck et al. 2010; Stromberg, Di Stilio &

48 Song 2016). It is also metabolically 'cheaper' than other structural biochemicals (e.g. lignin), so this
49 silicon-supported upright growth potentially allows plants to increase photosynthetic efficacy and
50 compete more effectively for light and space (Schoelynck et al. 2010; Stromberg et al. 2016).
51 However, the existence of these benefits is less-well characterised in other plant functional groups
52 such as nitrogen-fixing legumes, though there have been some studies which demonstrate growth
53 increases in silicon supplemented legumes (Horst & Marschner 1978; Miyake & Takahashi 1985;
54 Guo et al. 2006). In addition to improving plant structure, silicon supplementation may have
55 another potential benefit for legume growth via increased rates of root nodulation and symbiosis
56 with nitrogen fixing bacteria (Nelwamondo & Dakora 1999; Mali & Aery 2008). Increased
57 nodulation and biological nitrogen fixation, however, frequently makes legumes more susceptible
58 to herbivory via increased plant growth and nitrogen availability in the plant (e.g. Gerard 2001;
59 Johnson & McNicol 2010; Guo et al. 2013). This seems especially true for fluid-feeding aphids,
60 which benefit from fluxes in amino acid concentrations in the phloem sap (e.g. Johnson, Ryalls &
61 Karley 2014; Ryalls et al. 2016). Silicon supplantation may therefore indirectly promote the
62 performance of some herbivores if it causes such changes in the host plant.

63

64 In addition to promoting plant growth, silicon deposition in plant tissues has been shown to be an
65 effective defence against both vertebrate (Massey & Hartley 2006; Wieczorek et al. 2015) and
66 invertebrate herbivores (Keeping & Meyer 2006; Massey, Ennos & Hartley 2006; Frew et al. 2016).
67 Silicon negatively affects herbivores via abrasive effects on herbivore mouthparts (Massey &
68 Hartley 2009; but see Kvedaras et al. 2009), reduced digestive efficiency (Massey et al. 2006; Massey
69 & Hartley 2006; Wieczorek et al. 2015) and induction of secondary metabolites (Reynolds et al.
70 2009), which can include metabolites involved in indirect defences (e.g. recruitment of herbivore
71 natural enemies) (Kvedaras et al. 2010).

72

73 The range of effects of silicon supplementation on legumes presents a dichotomy whereby
74 increasing silicon availability could either make legumes better defended against herbivores via
75 increased silicon defences in the foliage (Reynolds et al. 2009) or make them more nutritious for
76 herbivores via increased nodulation increasing nitrogen availability (Mattson 1980). The relative
77 balance of these two effects, operating either directly (e.g. defence) or indirectly (e.g. nutritional
78 quality), has not yet been tested experimentally and is the rationale for this study.

79

80 The objective of this study was to determine whether increasing silicon availability for lucerne
81 (*Medicago sativa* L.) affected an insect herbivore (the aphid *Acyrtosiphon pisum*) either via
82 increased silicon uptake into the foliage and/or altered concentrations of amino acids in the
83 foliage. Based on observations that silicon can increase root nodulation in legumes (Nelwamondo
84 & Dakora 1999; Mali & Aery 2008), we predicted that silicon supplementation would increase plant
85 growth rates and root nodulation, which would increase foliar amino acid concentrations and
86 consequently herbivore performance.

87

88 **Materials and Methods**

89 *Experimental Procedure*

90 Lucerne (*M. sativa* L., cv. Sequel) plants were grown from seed at 26/18°C (day:night) in a
91 glasshouse receiving supplemental light (15:9 light:dark) and humidity controlled at 55% (see Ryalls
92 et al. 2013 for further technical details). Eighty eight plants were grown in 70 mm diameter pots
93 that were 135 mm deep (allowing unrestricted root growth for the duration of the experiment).
94 Each contained c. 700g of soil excavated from the Hawkesbury campus of Western Sydney
95 University (latitude -33.608847, longitude 150.747016). The soil is typified as low-fertility sandy
96 loam in the Clarendon Formation (Chromosol), which has low bioavailable Si content of 17 mg kg⁻¹
97 (see Barton et al. 2010 for full details). Sandy loam soils typically contain c. 35 mg kg⁻¹ of

98 bioavailable Si (Environmental Analysis Laboratory, Southern Cross University personal
99 communication). Plants were irrigated with c. 70 ml of tap water (3ppm Si) three times a week.
100 After two weeks of growth, half of the plants continued to receive tap water at the same intervals
101 while the other half received 70 ml of 500 mg l⁻¹ soluble Si in the form of NaSiO₃.9H₂O (Sigma-
102 Aldrich, Castle Hill, NSW, Australia) three times a week for six weeks. NaSiO₃.9H₂O has been used in
103 numerous studies to increase silicification of leaves and deter herbivores (reviewed by Reynolds et
104 al. 2009). When plants were six weeks old, 22 of the plants receiving the silicon supplementation
105 and 22 of the plants receiving tap water were each inoculated with two teneral female *A. pisum*.
106 Plants were configured in a randomised fashion in the glasshouse. *Acyrtosiphon pisum* used in
107 the experiment were taken from an established culture originating from an individual
108 parthenogenetic adult female collected from a lucerne field on campus (Ryalls et al. 2017). Cultures
109 were maintained on spare lucerne plants (cv. Sequel) for at least six generations prior to the
110 inoculation period.

111

112 White mesh (organza) bags (125 x 170 mm) were applied tightly around the rim of all pots
113 confining aphids to their allocated plants. After two weeks, bags were removed from all plants,
114 aphid colonisation assessed (% plants with > 1 aphid present) and individuals removed with a fine
115 paintbrush to be counted. Plants were separated and cleaned free of soil with water before
116 measuring shoot height, maximum rooting depth and counting the number of root nodules
117 (housing nitrogen-fixing bacteria). Plants were freeze dried for 48 hr and weighed. Leaves were
118 separated from the stems and ball-milled to a fine power prior to chemical analysis.

119

120 *Chemical analysis*

121 Chemical analysis was conducted on insect-free plants (44 of the 88 plants). To provide enough
122 dried leaf material to perform both amino acid and silicon analysis to be conducted on the same

123 plants it was necessary to pool foliar samples (2-3 plants per sample). This resulted in nine
124 replicates of Si⁺ and Si⁻ plants. Foliar silicon concentrations were analysed with X-ray fluorescence
125 spectrometry according to the methodology set out in Reidinger, Ramsey & Hartley (2012). In brief,
126 plant material was ground to a fine powder and pressed into 13mm-diameter pellets. Following the
127 methods of Reidinger et al. (2012), foliar silicon concentration was determined using a Niton XL3t
128 XRF analyzer (Thermo Fisher Scientific, Inc., MA, USA), for a measurement time of 30 seconds.
129 Results were expressed as foliar silicon concentration (as % of dry mass), calibrated against plant-
130 certified reference material of known silicon content (Garbuzov, Reidinger & Hartley 2011).

131

132 Soluble amino acids were extracted and analysed from milled foliar samples (10-15 mg) following
133 the protocol set out by Ryalls et al. (2015). Foliar amino acid composition is tightly correlated with
134 phloem amino acids in *M. sativa*, so analysing foliar material is a reliable proxy for phloem quality
135 (Ryalls et al. 2017). Amino acid standards within the AAS-18 (Fluka, Sigma-Aldrich) reference amino
136 acid mixture were supplemented with asparagine and glutamine (A0884 and G3126, respectively,
137 from Sigma, Sigma-Aldrich). Nine essential amino acids (i.e. those that cannot be synthesised by
138 insects *de novo*), including arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine,
139 threonine and valine (Morris 1991) and 10 non-essential amino acids (alanine, asparagine, aspartic
140 acid, cysteine, glutamic acid, glutamine, glycine, proline, serine and tyrosine) were detected using
141 this method.

142

143 *Statistical analysis*

144 The effects of silicon supplementation and aphid inoculation and the interaction between these
145 terms on plant dry mass, shoot height, rooting depth, root nodule abundance and root nodules per
146 cm of roots were analysed with two way analysis of variance (ANOVA) tests. Differences in silicon
147 content (log transformed) and concentrations between treatment groups were analysed with a

148 one-way ANOVA (silicon supplementation included as a fixed factor). The effects of Si addition on
149 aphid colonisation success and aphid abundance were analysed with generalised linear models
150 with binomial error structure and a logit link function and Poisson error structure with a log link
151 function, respectively. Silicon supplementation was included as a fixed effect and the dispersion
152 parameter was estimated. Pearson's correlation tests were used to explore relationships between
153 plant parameters and aphid abundance. Where appropriate, data transformations (see Figure and
154 Table legends for details) were chosen to meet model assumptions and give residual diagnostic
155 plots which fitted a normal distribution and showed least heteroscedasticity. Permutational
156 multivariate analysis of variance (PERMANOVA) was used to explore the impacts of silicon
157 supplementation on amino acid composition and concentrations for total, essential and non-
158 essential amino acids. ANOVA and generalised linear models were performed with Genstat (version
159 17, VSN International, UK), whereas the PERMANOVA was conducted in R v3.3.1 using the using
160 the R-package vegan (Oksanen et al. 2017).

161

162 **Results**

163 Silicon supplementation (Si+ plants) increased plant biomass almost three-fold compared to those
164 grown without silicon supplementation (Si- plants), whereas the presence of aphids had no
165 significant impact on plant biomass (Fig. 1 A). This increase in biomass in Si+ plants was attributed
166 to increased growth in the shoots rather than the roots, the latter being similar in Si+ and Si- plants
167 (Table 1). Plants inoculated with aphids grew slightly less tall than those without (Table 1). Silicon
168 supplementation also resulted in c. 44% increase in root nodulation (Fig. 1B), which was reflected in
169 higher nodule density on the roots (Table 1). Nodulation was unaffected by aphids (Fig. 1B, Table
170 1).

171

172 Aphid colonisation success was similar on Si- and Si+ plants (73% and 64%, respectively; $F_{1,42} =$
173 0.42 , $P = 0.517$). Aphids were substantially more abundant (+112%) on Si+ plants than Si- plants
174 (Fig. 2). Moreover, aphid abundance was positively correlated with plant biomass (Fig. 3A) and
175 plant height (Fig. 3B).

176

177 Si+ plants accumulated over twice as much silicon as Si- plants (mean \pm standard error: $262 \pm$
178 48.14 mg and 124.35 ± 14.05 , respectively) ($F_{1,16} = 12.05$, $P = 0.003$). However, because Si+ plants
179 grew so much bigger than Si- plants, this diluted foliar silicon concentrations by 26% in Si+ plants
180 (Fig. 4A). PERMANOVA analysis showed that Si+ plants had 65% higher concentrations of essential
181 amino acids than Si- plants (Fig. 4B). Overall there was no difference in total and non-essential
182 amino acid concentrations between the two groups, but there was a significant increase in the
183 concentrations of 11 of the 18 amino acids tested in the silicon treated plants compared to controls
184 (Fig. S1; Table S1).

185

186 Discussion

187 This study has shown that silicon supplementation caused a significant increase in root nodulation
188 in a legume, despite no increase in root length. This contributed to silicon-induced increases in
189 shoot biomass and enhanced foliar resources in the form of essential amino acids. While silicon
190 accumulation increased substantially in silicon supplemented plants, foliar silicon concentrations
191 were diluted due to this rapid increase in plant growth. These silicon-induced changes to plant
192 physiology and chemistry were associated with significant increases in herbivore abundance.

193

194 Enhanced growth of the meristems and increased synthesis of essential amino acids were the most
195 likely drivers of increased aphid abundance on Si+ plants. Aphids often feed on actively growing
196 stems because nutrients, specifically amino acids, are being translocated to these tissues for growth

197 (Raven 1983; Dixon 1998; Johnson, Elston & Hartley 2003). We found a positive correlation
198 between aphid abundance and plant growth (both dry mass and plant height), which supports the
199 conclusion that aphids performed better on the faster growing plants. Silicon supplementation also
200 increased concentrations of essential amino acids in the foliage by 65% relative to plants without
201 silicon supplementation. The concentration of essential amino acids in the phloem sap of plants is
202 comparatively low (10-30% of total protein amino acids; Douglas 2003), so silicon-induced
203 increases could promote aphid performance. In particular, >90% of total amino acid content of
204 aphid tissues are essential amino acids (Douglas 2003) so this group is very important for aphid
205 nutrition. We did not attempt to directly relate aphid abundance to amino acid concentrations of
206 specific plants on which aphids were feeding because aphids themselves have large qualitative and
207 quantitative effects on phloem amino acids (Douglas 2003), particularly in *Medicago* spp. (Guo et al.
208 2013). This confounds interpretation of how silicon supplementation was affecting amino acid
209 concentrations on aphid-infested plants, not least because aphid populations were much larger on
210 Si+ plants (and possibly having larger impacts on amino acids than on Si- plants). Nonetheless, it
211 seems likely that silicon-induced increases in foliar essential amino acids were at least partly
212 responsible for increased aphid abundance.

213

214 While silicon has been shown to increase root nodulation and nitrogen fixation in legumes
215 (Nelwamondo & Dakora 1999; Dakora & Nelwamondo 2003; Mali & Aery 2008), the mechanisms
216 for this have yet to be demonstrated. The simplest explanation is that increased root growth
217 increases potential invasion sites for rhizobial bacteria (Mali & Aery 2008), but we found that silicon
218 increased nodule density and had little impact on root growth *per se*. Using comparable application
219 rates (480 mg l⁻¹) as the present study (500 mg l⁻¹), Nelwamondo & Dakora (1999) similarly found
220 that nodule density increased on sand-grown cowpea (*Vigna unguiculata*) plants.

221 In a follow up study, Nelwamondo et al. (2001) showed that silicon supplementation increased the
222 abundance of bacteroids and symbiosomes (the plant-derived membrane that encases the
223 bacteroids) in root nodules, which they suggested could explain enhanced N₂ fixation. They also
224 found that silicon increased cell wall thickness of root nodules which appeared to decrease
225 intercellular spaces which should, in theory, reduce N₂ fixation because of lower gaseous diffusion
226 (Nelwamondo et al. 2001). The fact that N₂-fixation actually increased led Nelwamondo et al. (2001)
227 to speculate that silicification reduced the need for lignin, which is costly in terms of carbon; carbon
228 could then be channelled into bacteroid respiration to increase N₂-fixation. It is also possible that
229 carbon could be channelled towards enhanced nodule organogenesis. Nelwamondo et al. (2001)
230 also proposed that silicon supplementation had parallels with phosphorus fertilisation in terms of
231 stimulating root nodulation, including increased production of compounds that upregulate
232 nodulation genes (Dakora & Nelwamondo 2003). This has yet to be tested.

233

234 The efficacy of silicon defence against sap-feeders, such as aphids, appears less clear cut than on
235 chewing herbivores and has been the subject of debate (Massey et al. 2006; Keeping & Kvedaras
236 2008; Kvedaras et al. 2009). Published studies have shown silicon has either no (e.g. Hogendorp,
237 Cloyd & Swiader 2009; Cherry et al. 2012; Keeping, Miles & Sewpersad 2014) or negative (e.g.
238 Gomes et al. 2008; Costa, Moraes & DaCosta 2011; Dias et al. 2014) effects on this feeding guild.
239 The variation in these published findings probably reflects the fact that whilst silicon can negatively
240 affect aphids via epidermal resistance to stylet penetration, aphids can circumvent some of the
241 consistent negative impacts reported for chewing herbivores (e.g. mandible wear and impaired
242 food breakdown) simply by virtue of their feeding behaviour (i.e. fluid acquisition via a stylet).
243 Further experimental work and meta-analysis of existing studies would help to address the extent
244 to which different herbivore guilds are affected by silicon defences. In the present study, silicon

245 application did not affect aphids adversely because Si⁺ plants did not have higher concentrations
246 of silicon in the foliage.

247

248 In addition to promoting physical defences at the leaf surface, silicon can also stimulate
249 phytohormonal pathways in the plant, such as the jasmonic acid (JA) pathway (Ye et al. 2013),
250 which underpins synthesis of secondary metabolites with defensive functions (Wu & Baldwin 2010).
251 Stimulation of the JA pathway is known to suppress the salicylic acid (SA) pathway, which is often
252 triggered by phloem-feeding herbivores and can result in plants mounting a defensive response
253 against these herbivores (Ode, Johnson & Moore 2014). We still know very little about how silicon
254 affects other plant defences, but if silicon application were to stimulate the JA pathway (and
255 suppress the SA pathway), this may have also contributed to the success of aphids on silicon
256 supplemented plants in this study. We can conclude, however, that if defences were activated by
257 silicon application, they had minimal impacts on aphids in this system.

258

259 This study has shown that lucerne plants benefitted from silicon supplementation in terms of both
260 increased growth and resource acquisition via root nodulation, whereas the concentrations of
261 silicon in the foliage tended to decrease in silicon supplemented plants. Aphid abundance
262 increased substantially on these faster-growing plants. There a number of hypotheses considering
263 the relationships between resources (nitrogen, water), plant growth and herbivory (reviewed by
264 Hartley & Jones 1997). Our results are consistent with the resource availability hypothesis (Coley,
265 Bryant & Chapin 1985), which predicts that faster growing plants, with increased access to
266 resources (e.g. either through silicon fertilisation or biologically fixed nitrogen) will be less well
267 defended against herbivores. Increasing the rate of silicon supplementation may increase foliar
268 silicon concentrations, but increasing the concentrations of silicon application much beyond those
269 used in this study would most likely inhibit nodulation and N₂-fixation, as has been reported for

270 cowpea plants (Mali & Aery 2008). In that study, high doses of silicon application reduced calcium
271 concentrations in the plant; calcium increases biosynthesis of isoflavonoid nodulation signals in
272 legumes (Dakora & Phillips 1996) so it was suggested this was the reason for high silicon
273 application diminishing nodulation (Mali & Aery 2008).

274

275 To our knowledge, this is the first study to report beneficial effects of silicon addition on herbivore
276 performance which arose indirectly via promotion of root nodulation and amino acid biosynthesis.
277 Further work on non-grass species is needed, but our results suggest that in some functional
278 groups of plants, such as legumes, the positive effects of silicon on plant growth and nitrogen
279 acquisition may outweigh any additional defensive function of silicon supplementation. Our
280 findings suggest that silicon supplementation of legumes may not provide the benefits that such
281 applications provide to grasses such as cereals (Guntzer et al. 2012), at least in part because the
282 positive effects on nodulation, nutrient content and yield may make these crops more susceptible
283 to aphids pests and the diseases they vector. There is an increasing need to extend the use of
284 legumes in agriculture because of their benefits to soil fertility, so understanding how silicon
285 promotes nodulation and N₂-fixation and its indirect impact on plant susceptibility to herbivores
286 could be a promising area of research.

287

288 **Author contributions**

289 SNJ, JMWR, AF and ANG conceived the experimental design. SNJ, JMWR, AF, JDG, MD and ANG
290 acquired and processed data with JMWR and SEH specifically undertaking chemical analysis. SNJ
291 and JMWR analysed the data and all authors contributed to the interpretation and drafting of the
292 manuscript.

293

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299

300 **Data accessibility**

301 Data from this paper are available at the Dryad digital repository doi:

302

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462

463

465 **Table 1.** Plant (shoot) height, maximum rooting depth and number of root nodules per cm of root
 466 length in response to silicon supplementation (Si+) and without silicon supplementation (Si-) and
 467 aphid inoculation.

Silicon supplementation	Aphid inoculation	Plant (shoot)¹ height (cm)	Maximum rooting depth (cm)	Root nodules per cm of root²
Si-	Control	7.52 ± 0.76	10.06 ± 1.01	0.68 ± 0.10
	Aphids	6.70 ± 0.60	11.47 ± 0.79	0.35 ± 0.05
Si+	Control	13.84 ± 1.32	11.40 ± 1.17	0.78 ± 0.19
	Aphids	10.22 ± 0.93	9.92 ± 1.11	0.86 ± 0.20
<i>Statistical analysis</i>				
	Silicon	$F_{1,84} = 27.11,$ $P < 0.001$	$F_{1,84} = 0.01,$ $P = 0.918$	$F_{1,84} = 4.19,$ $P = 0.044$
	Aphids	$F_{1,84} = 3.99,$ $P = 0.049$	$F_{1,84} = 0.01,$ $P = 0.970$	$F_{1,84} = 0.71,$ $P = 0.401$
	Silicon x Aphids	$F_{1,84} = 1.12,$ $P = 0.293$	$F_{1,84} = 1.12,$ $P = 0.164$	$F_{1,84} = 1.82,$ $P = 0.181$

468 ¹ log transformed

469 ² log+1 transformed

470

471

472 **Figure Legends**

473 **Figure 1.** Impacts of silicon supplementation and aphid inoculation on the (A) biomass (log
474 transformed) and (B) abundance of root nodules on *M. sativa*. Mean values \pm standard error shown.

475 N = 22.

476 **Figure 2.** Impact of silicon supplementation on aphid (*A. pisum*) abundance on colonised plants.

477 Mean values per plant \pm standard error shown. N = 14 (Si-) and N = 16 (Si+).

478 **Figure 3.** Correlations between aphid abundance and (A) plant biomass and (B) plant (shoot)

479 height. N= 30. Solid lines represent linear regression through all the points. Dashed lines represent

480 95 % confidence intervals

481 **Figure 4.** Impacts of silicon supplementation on foliar (A) silicon concentrations (logit transformed)

482 and (B) essential amino acids in *M. sativa*. Mean values \pm standard error shown. N = 9.

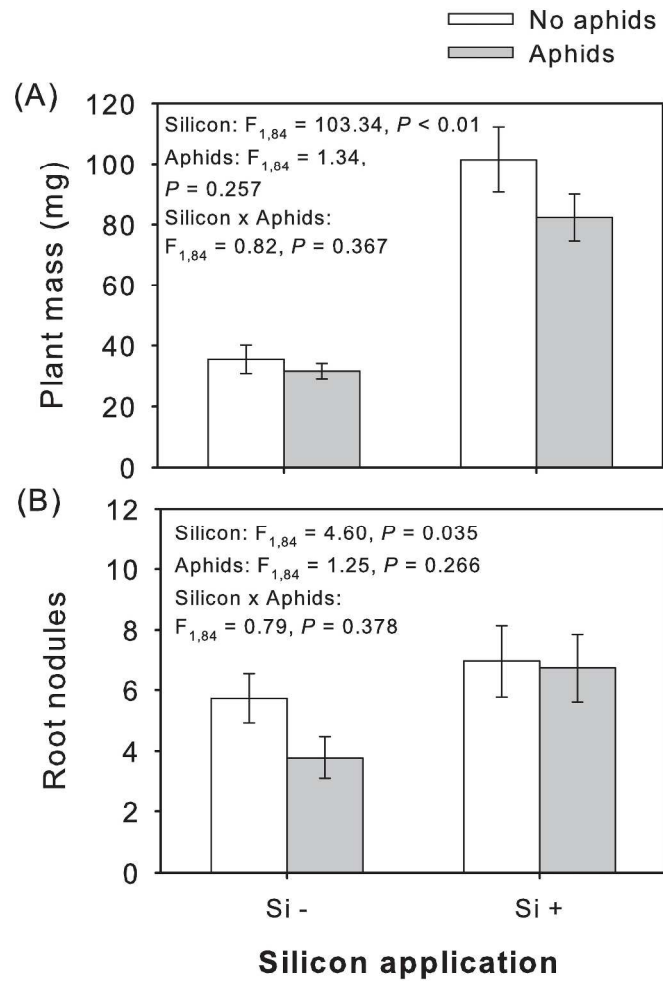


Figure 1

296x420mm (300 x 300 DPI)

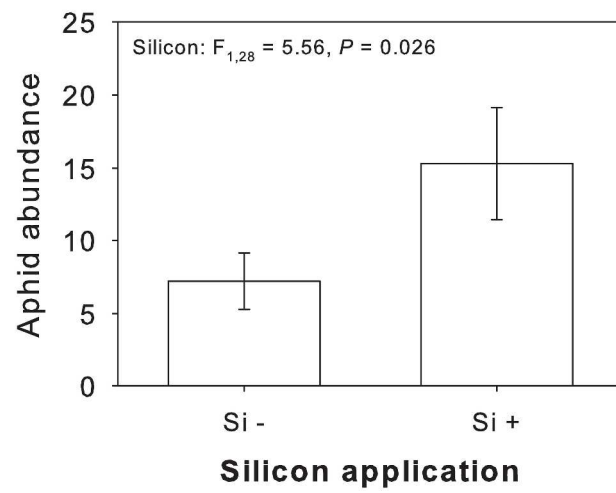


Figure 2

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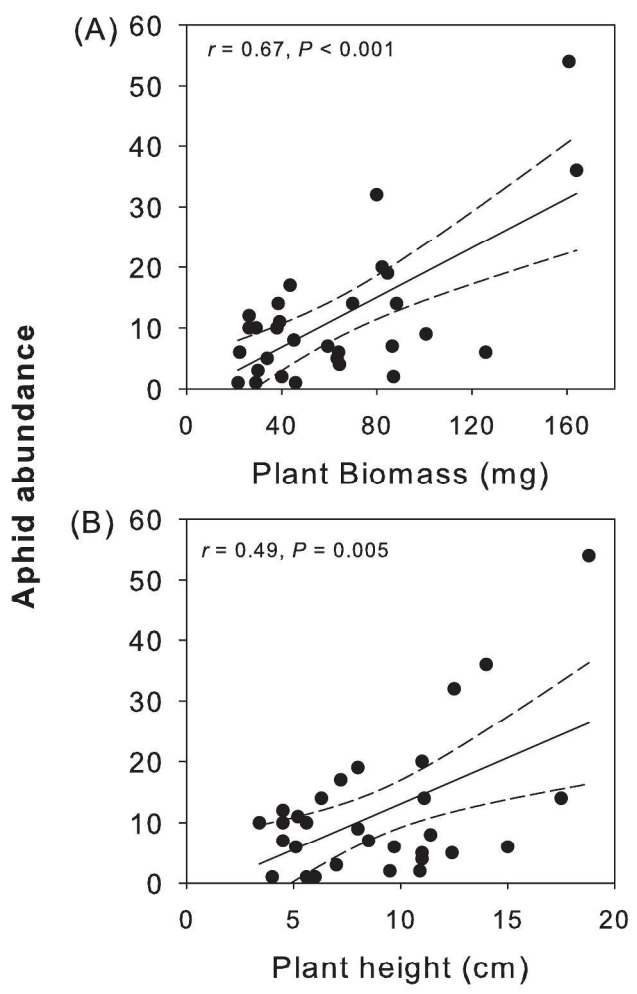


Figure 3

296x420mm (300 x 300 DPI)

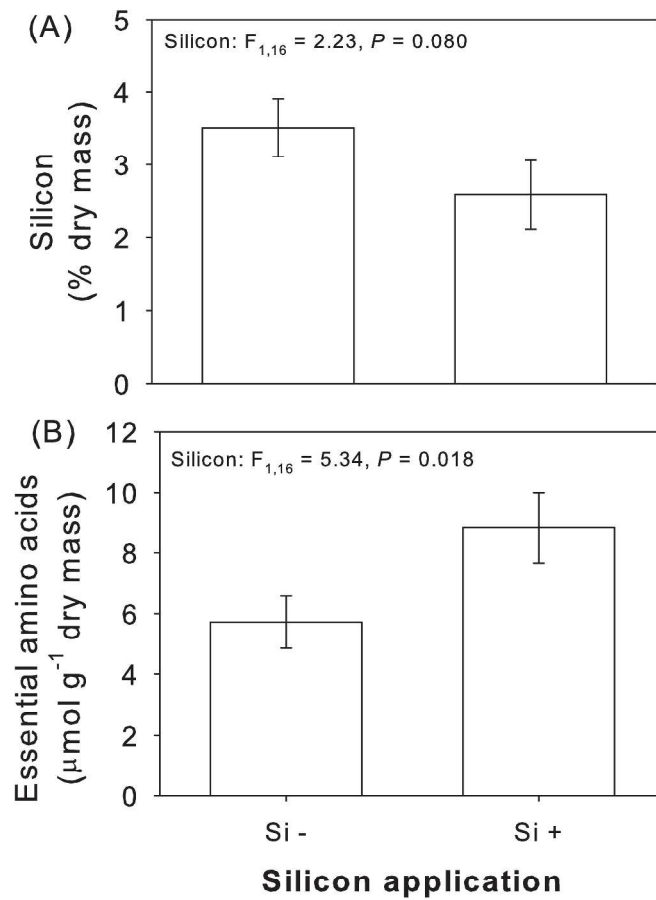


Figure 4

296x420mm (300 x 300 DPI)

Supplemental Material

Table S1. Statistical analysis for amino acid concentrations shown in Figure S1. Statistically significant differences between silicon supplemented and non-supplemented plants shown in bold.

Amino acids	F_{1,16}	P
Total amino acids	1.82	0.163
Non-essential amino acids	1.44	0.244
Aspartic acid	7.01	0.018
Glutamic acid	3.02	0.102
Serine	8.79	0.009
Glycine	9.63	0.007
Histidine	0.05	0.829
Arginine	2.20	0.157
Threonine	4.83	0.043
Tyrosine	29.99	< 0.001
Valine	9.69	0.007
Cysteine	8.15	0.01
Isoleucine	11.50	0.004
Leucine	4.54	0.04
Phenylalanine	1.49	0.24
Lysine	0.62	0.81
Methionine	5.69	0.03
Alanine	2.11	0.17
Proline	10.48	0.005
Asparagine	0.19	0.67

Supplemental Figure

Figure S1. Impacts of silicon supplementation (filled bars represent Si+ plants, open bars are Si- plants) on foliar amino acids grouped by total amount, non-essential and individual amino acids. Mean values \pm standard error shown. N = 9. Statistically significant difference between plants (see Table S1) indicated * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

