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1 Targeted plant defence: silicon conserves hormonal defence 2 signalling impacting chewing but not fluid-feeding herbivores

3
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11
12 *Running Title: Silicon integrates into defence signalling*

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16 17 **Abstract**

18 Plants deploy an arsenal of chemical and physical defences against arthropod herbivores, but it is
19 most cost efficient to produce these only when they are attacked. Herbivory activates complex
20 signalling pathways involving several phytohormones, including jasmonic acid (JA), which
21 regulate production of defensive compounds. The Poaceae also have the capacity to take up large
22 amounts of silicon (Si) which accumulates in plant tissues. Si accumulation has anti-herbivore
23 properties, but it is poorly understood how Si defences relate to defence hormone signalling.
24 Here we show that Si enrichment causes the model grass *Brachypodium distachyon* to show
25 lower levels of JA induction when attacked by chewing herbivores. Triggering this hormone
26 even at lower concentrations, however, prompts Si uptake and physical defences (leaf hairs)
27 which negatively impact chewing herbivores. Removal of leaf hairs restored performance.
28 Crucially, activation of such Si-based defence is herbivore-specific and occurred only in
29 response to chewing and not fluid-feeding (aphid) herbivores. This aligns with our meta-analysis
30 of 88 studies that showed Si defences were more effective against chewing herbivores than fluid-

31 feeders. Our results suggest integration between herbivore defences in a model Si-accumulating
32 plant, which potentially allows it to avoid unnecessary activation of other costly defences.

33 **Key-words:** Herbivory, insects, jasmonic acid, plant defence, physical defence, silica, silicon
34

35 **Introduction**

36 Grasses obtain more Si than any other inorganic constituent (Raven 1983, Cooke and
37 Leishman 2011) and there is consensus that Si has an important functional role in plants for
38 alleviating diverse biotic and abiotic environmental stresses (Coskun et al. 2019). Amongst
39 these, Si has been widely reported to have anti-herbivore properties (McNaughton et al. 1985,
40 Reynolds et al. 2016). Si deposition between and within cell walls most likely confers physical
41 resistance to herbivory (Clissold 2008, Hunt et al. 2008). Moreover, Si may augment leaf
42 trichomes / hairs and form discrete structures (e.g. opaline phytoliths) on the leaf surface
43 (Hartley et al. 2015). These can interfere with feeding, wear down mouthparts and reduce
44 nutrient acquisition by herbivores once ingested (Massey et al. 2006, Massey and Hartley 2009).

45 In addition to physical defences, plants have the capacity to synthesise a diverse range of
46 defensive secondary metabolites with anti-herbivore properties. These defences may exist
47 constitutively, but often they are produced following herbivore attack (Karban and Baldwin
48 1997). Herbivory activates complex signalling pathways involving several phytohormones,
49 including jasmonic acid (JA), salicylic acid (SA) and ethylene, which regulate expression of
50 defence genes and downstream production of defensive compounds (Howe and Jander 2008, Erb
51 et al. 2012). The JA signalling pathway is regarded as the master regulator of plant resistance to
52 arthropod herbivores and pathogens (Erb et al. 2012). In general, JA regulates defences against
53 tissue-chewing herbivores, whereas defences against fluid-feeding (i.e. piercing/sucking)
54 herbivores are regulated by both SA and JA pathways (Züst and Agrawal 2016).

55 Despite the emergence of numerous ecological theories to explain the evolution and
56 maintenance of anti-herbivore plant defences (Stamp 2003), Si-based defences and secondary
57 metabolite-based defences have largely been studied separately. In particular, attempts to
58 characterise how Si interacts with the JA pathway when challenged by herbivory (real or
59 simulated) have received limited attention (four studies to our knowledge; Ye et al. 2013, Kim et
60 al. 2014, Hall et al. 2019, 2020). Moreover, these studies found conflicting patterns and only
61 considered chewing-type damage despite plant defences operating very differently depending on
62 how herbivores feed (i.e. chewing or fluid-feeding herbivory) (Schweiger et al. 2014).

63 Accumulation of Si in higher concentrations (> 1% dry mass) relies on active uptake
64 which has a metabolic cost to the plant (Ma and Yamaji 2015). Furthermore, Si accumulation is
65 known to be an inducible defence across a range of plant species experiencing herbivory
66 (Massey et al. 2007, Johnson et al. 2019). This induction is also an active process, so potentially
67 costly to the plant (Ma and Yamaji 2015, McLarnon et al. 2017). If herbivores induce active Si
68 uptake, plants may therefore downregulate production of secondary metabolite-based defences,
69 which are potentially even more costly than Si-based defences (Hall et al. 2019). For example, Si
70 accumulation imposed lower penalties on plant growth than synthesis of phenolic compounds in
71 several grass species (Johnson and Hartley 2018). Such “trade-offs” between the allocation of
72 plant resources to Si-based versus secondary metabolite defences is likely to be a tightly
73 controlled process regulated by phytohormones.

74 We investigated the defensive role of Si in the model grass *Brachypodium distachyon*
75 challenged by chewing herbivores (*Helicoverpa armigera*) and piercing/sucking (fluid-feeding)
76 herbivores (*Rhopalosiphum padi*). We determined whether herbivory and stimulation of the JA
77 pathway with methyl jasmonate (MeJA) induced Si uptake and the activity of the JA and SA

78 pathways, whether Si modified these responses and how these factors affected herbivore
79 performance. We also determined whether Si supply shaped physical defences (leaf macro-
80 hairs). We predicted that the chewing herbivore would induce Si uptake and JA activity, but the
81 increase in JA concentrations would be lower in +Si plants because these plants possessed
82 constitutive physical Si defences. In contrast, aphids would not affect Si uptake or JA activity,
83 regardless of feeding intensity (i.e. single or multiple attacks), and aphids would be unaffected
84 by Si defences. Although there has been very little published work on the interactions between Si
85 and phytohormones and the consequences for different herbivore feeding guilds, the subject of
86 this study, there is now a substantive body of work on Si impacts on different types of
87 herbivores. Hence we conducted a meta-analysis of published literature to determine whether Si
88 adversely affected chewing and fluid-feeding arthropods to the same extent.

89

90 **Methods**

91 *Plants and Herbivores*

92 *Brachypodium distachyon* (Bd21-3), supplied by the French National Institute for
93 Agricultural Research (INRA, Versailles, France), were grown hydroponically using the system
94 and procedures outlined by Hall et al. (2020). The hydroponics system allows individual plants
95 to be grown in opaque cups filled with 330 mL nutrient solutions. Silicon inclusion for +Si plants
96 was achieved by adding liquid potassium silicate (K_2SiO_3) (Agsil32, PQ Australia, SA,
97 Australia) at a concentration of 2 mM (SiO_2 equivalent) to the nutrient solution and adjusted to
98 pH 5.5 using HCl to reduce the polymerisation of silicates. Silicon-free (-Si) plants had KCl
99 added to balance additional K^+ and Cl^- in the Si+ treatments and adjusted to pH 5.5 using HCl
100 (see Hall et al. 2020 for full details). Solutions were replaced weekly when cups were also

101 rotated within the glasshouse chamber. Plant propagation and experiments were conducted in
102 naturally lit glasshouse chambers maintained at 22/18°C Light:Dark on a 14:10 hour cycle.
103 Humidity was controlled at 50% ($\pm 6\%$). Conditions were monitored and regulated using the
104 PlantVisorPRO (Carel Industries, Padova, Italy) system.

105 Cultures of the phloem-feeding herbivore (*Rhopalosiphum padi* L.) were established
106 from a single parthenogenetic female obtained from laboratory cultures at Agriculture Victoria
107 Research (Horsham, VIC, Australia). Cultures were reared on barley plants (*Hordeum vulgare*
108 cv. 'Hindmarsh'). The chewing herbivore (*Helicoverpa armigera* Hübner), supplied by CSIRO
109 Agriculture & Food, Narrabri Australia, were individually hatched from eggs and fed on growing
110 media (see Johnson et al. 2019) at 20°C 15:9 hour photoperiod (Light:Dark) until required.

111 *Experimental Design and Procedures*

112 One hundred and eighty-six *B. distachyon* plants were grown hydroponically for six
113 weeks, half in -Si solution and half in +Si solution and used for different components of the
114 study (summarised in Fig. S1). For each Si treatment, 57 plants were assigned at random to five
115 treatments: (1) no herbivory, N = 15; (2) caterpillar herbivory, N = 10, (3) aphid herbivory, N =
116 10, (4) three bouts of aphid herbivory, N = 10 and (5) application of methyl jasmonate (MeJA),
117 N = 12. Caterpillar and aphid (single bout) herbivory treatments comprised applying a single
118 third-fourth instar *H. armigera* or four adult *R. padi* to each plant for 7d, respectively, when
119 plants were 5 weeks old. These herbivores were also used to measure the impacts of Si on
120 performance. For *H. armigera*, relative growth rates (RGR) were calculated [mass gained
121 (mg)/initial mass (mg)/time (days)] by measuring larval mass upon application and removal from
122 the plant. For *R. padi*, the total number of individuals were counted after 7d. The repeated bouts
123 of aphid herbivory treatment (4) comprised three periods of 3d of exposure to aphids, with 4d

124 intervening aphid-free days, starting when plants were three weeks old. All plants, including
125 those without herbivores, were caged using transparent cylinders with mesh apertures (similar to
126 those described in Johnson et al. 2019) fixed to the cups. The 12 plants from both Si treatments
127 assigned for MeJA treatment were sprayed at close proximity with 1 mM MeJA solution
128 prepared with 0.01% Tween 20. Twelve more plants from the two Si treatments were used as
129 controls by spraying with 0.01% Tween 20 in water (treatment 6 in Fig. S1).

130 The adaxial leaf surfaces of *B. distachyon* are typically covered with small prickle cells
131 and macro-hairs, with the latter potentially linked to Si supply (Glazowska et al. 2018). Three
132 leaves from each herbivore-free plant were selected at random and a 2×2 mm (4 mm²) section
133 from the middle of the leaf photographed at ×40 magnification. Leaf macro-hairs were
134 subsequently quantified. All plants (1–6 in Fig. S1) were harvested for analysis of Si, JA and SA
135 when plants were six weeks old. Plant tissue was immediately snap-frozen in liquid nitrogen and
136 stored at –80°C prior to chemical analysis (details of Si and JA/SA analysis is given in
137 supplemental material).

138 To determine whether leaf macro-hairs influenced the RGR of *H. armigera*, as previously
139 hypothesised (Hall et al. 2019), we physically removed a proportion of macro-hairs from 12 +Si
140 and 12 –Si plants, leaving a further 12 +Si and 12 –Si plants intact (7 and 8 in Fig. S1). Leaf
141 macro-hairs were removed by gently scraping the adaxial and abaxial leaf surfaces (the latter
142 possessed relatively few hairs) using a knife blade taking care to not damage the leaf. Forty-
143 eight fourth instar *H. armigera* were starved for 24 h and weighed before being placed
144 individually in a container with a tiller of fresh leaf material from all 48 plants. The cut end of
145 the tiller was inserted into a 1.5 mL tube with water to maintain turgor. Larvae were kept at 22
146 °C and allowed to feed for 48 h, after which time they were starved for a further 24 h to allow the

147 frass to be evacuated, before being reweighed. Herbivore RGR was calculated and leaf macro-
148 hairs were quantified (as described above) from all plants to determine the extent of macro-hair
149 removal relative to intact leaves.

150 *Statistical Analysis*

151 One-way ANOVAs with Si as a fixed factor were used to analyse leaf Si, leaf macro-hair
152 density, chewer RGR and aphid abundance. Two way ANOVAs were used to analyse impacts of
153 Si and MeJA on JA concentrations and Si and Si and leaf hair removal on chewer RGR.

154 Generalised linear models with Si and Herbivory/MeJA as fixed factors were used to analyse leaf
155 JA and SA concentrations with Poisson and gamma distributions, respectively. A Pearson's
156 correlation test was used to identify any relationship between JA and SA and between chewer
157 RGR and leaf macro-hairs. All analysis was conducted in either Genstat v19 (VSN International,
158 Hemel Hempstead, UK) or the R statistical platform (R Core Team 2018). Salient statistical
159 results are reported in text and figures, with full details and complete results reported in Table S1.

160 *Meta-analysis*

161 Full details of data collection, screening, meta-analysis and publication bias testing are
162 given in the supplemental material. In brief, we identified studies that quantified arthropod
163 herbivore performance responses when feeding on both +Si plants (e.g. Si supplemented or
164 plants containing naturally high Si concentrations) and -Si plants (e.g. with relatively lower
165 levels of Si than +Si plants). Herbivores were classified according to feeding behaviour; chewing
166 (mandibulate) or fluid-feeding. This constituted 95% of observations with the remaining 5%
167 coming from other feeding guilds.

168 A meta-analysis was conducted using the package *metaphor* (Viechtbauer 2010) in the R
169 statistical platform. The effect size (Hedges' d), the extent to which herbivores were affected,

170 was calculated for each pair of performance responses on +Si and –Si plant, respectively. This
171 measure of effect size compares two means using a pooled standard deviation and bias correction
172 and reflects the number of standard deviations by which the means differ (Hedges and Olkin
173 1985). The 95% confidence intervals were used to interpret whether Si had significant impacts
174 on herbivores (i.e. no overlap with zero) and differences between the two groups. Plots to the left
175 of zero indicate increasingly negative impacts of Si, whereas any plots to the right of zero would
176 reflect beneficial impacts of plant Si.

177

178 **Results**

179 *Si uptake, Phytohormones and Leaf Macro-hairs*

180 Si concentrations in –Si plants were below the levels of detection so statistical analysis
181 was conducted on +Si plants only. Leaf Si concentrations were significantly elevated when
182 plants had been subjected to chewing herbivory (Fig. 1A). Aphid herbivory had negligible
183 impacts on Si concentrations. Application of the chemical stimulant (MeJA), corresponding to
184 simulated chewing herbivory, also caused similar increases in leaf Si (Fig. 1A).

185 Chewers induced higher concentrations of JA (Fig. 1B), but this response was
186 substantially higher in –Si plants than +Si plants. Aphids had no impact on JA concentrations,
187 regardless of Si treatment. As might be expected with chemical stimulation of the JA pathway,
188 MeJA caused a very large increase in JA concentrations ($F_{1,34} = 211.73$, $P < 0.001$) but this was
189 much lower in MeJA treated +Si plants which was reflected in the significant interaction
190 between Si and MeJA treatments (Fig. 1B; $F_{1,34} = 14.70$, $P < 0.001$).

191 Si had no impacts on SA concentrations, though we observed that repeated bouts of aphid
192 herbivory stimulated SA synthesis (Fig. S2; Table S1). Moreover, chewers resulted in lower

193 concentrations of SA than herbivore-free plants. Compatible with antagonistic cross-talk
194 between SA and JA, we also observed a negative correlation between concentrations of the two
195 hormones ($r = -0.285$, $P = 0.024$). MeJA and Si had no significant impact on SA concentrations
196 (Table S1). Leaf surfaces, particularly the adaxial surface, possessed numerous non-glandular
197 macro-hairs (Fig. 3). Si supply increased the density of these macro-hairs by c. 18% to 3.14 per
198 mm^2 on +Si plants, compared to 2.61 per mm^2 on -Si plants ($F_{1,28} = 13.08$, $P < 0.001$).

199 *Herbivore Performance*

200 The RGR of chewing herbivores fell by over 70% when feeding on +Si plants ($72.81 \pm$
201 $14.08 \text{ mg mg}^{-1} \text{ day}^{-1}$) compared to those feeding on -Si plants ($317.29 \pm 43.72 \text{ mg mg}^{-1} \text{ day}^{-1}$)
202 ($F_{1,18} = 29.98$, $P < 0.001$). In contrast, aphid abundance was similar when feeding on either -Si
203 plants (15.40 ± 1.93) or +Si plants (12.90 ± 1.38) ($F_{1,18} = 1.11$, $P = 0.306$). Mean value \pm
204 standard error ($N = 10$) shown in all cases.

205 Partial removal of leaf macro-hairs reduced hair density (Fig. 4B and 4D) relative to
206 intact leaves (Fig. 4A and 4C). After macro-hair removal, Si continued to have negative impacts
207 on chewer RGR ($F_{1,44} = 239.86$, $P < 0.001$), with herbivores typically losing mass over 24 hours
208 (Fig. 4E). RGR was, however, significantly improved with removal of leaf macro-hairs ($F_{1,44} =$
209 53.95 , $P < 0.001$) to a similar extent on both -Si and +Si plants ($F_{1,44} = 0.86$, $P = 0.360$).
210 Furthermore, there was a significant negative correlation between chewer RGR and the macro-
211 hair density ($r = -0.293$, $P = 0.043$).

212 *Meta-analysis*

213 There were 478 herbivore responses from 88 studies that were suitable for inclusion in
214 the meta-analysis (see Supplemental Material and Fig. S3). Overall, Si had substantially more
215 defensive impacts on mandibulate (i.e. chewing) herbivores than on fluid-feeding herbivores

216 (Fig. 5). Si-based plant defences were around $2.5\times$ times more harmful for the performance of
217 chewing herbivores compared to that of fluid-feeders. Fluid-feeders were negatively impacted by
218 plant Si but the effect size was relatively small (-0.37) and in line with the non-significant effect
219 size generated in the present study (-0.45).

220

221 **Discussion**

222 This study has established that Si causes a reduction in JA concentrations in *B.*
223 *distachyon* in response to chewing herbivory. The fact that MeJA induced Si uptake
224 demonstrates that induced Si defences are regulated by the JA pathway. Crucially, the burst in
225 JA activity following MeJA application was lower in +Si plants than –Si plants which
226 establishes that lower JA in +Si plants was not simply due to reduced feeding / damage by
227 herbivores on these plants. Our results suggest that +Si plants are investing in physical defences
228 and while chewing herbivores continued to trigger the JA pathway, associated with further Si
229 uptake, it does not appear to be necessary to trigger this pathway to the same extent as in –Si
230 plants to maintain effective defences. In contrast, fluid-feeding herbivores neither induced Si
231 uptake or triggered the JA pathway and their performance was unaffected by Si. Meta-analysis
232 indicated that this may be a general pattern: fluid-feeders show some responses to the presence
233 of Si but are not affected by Si defence to the same extent as chewing herbivores.

234 Production of glandular (i.e. chemical secreting) trichomes is regulated by JA, whereas
235 non-glandular trichomes or macro-hairs can be formed independently of the JA pathway (Li et
236 al. 2004). In addition to forming discrete opaline phytoliths, Si can also augment trichomes and
237 macro-hairs on the leaf surface (Hartley et al. 2015) so while JA activity is required for Si uptake
238 it may not necessarily be directly involved in macro-hair formation. Moreover, once plant tissues

239 become silicified this is irreversible so plants possibly do not need sustained JA activity (and
240 continuous Si uptake) once physical defences are in place. Andama et al. (2020) also
241 demonstrated the importance of silicified non-glandular trichomes for herbivore defence. In
242 common with our study, physical removal of these trichomes improved herbivore performance.

243 Fluid-feeding aphids did not impact Si induction, whether feeding in single or multiple
244 bouts, and Si had no impacts on aphid performance suggesting that Si is not an effective defence
245 against aphids in this system. Many of the physical defences that Si confers probably have less
246 impact on fluid-feeders than chewing insects (Massey et al. 2006). Fluid-feeders do not need to
247 crush or macerate plant tissues and they can insert feeding stylets between physical structures
248 (e.g. phyloliths and macro-hairs). Moreover, the majority of fluid-feeding herbivores feed on the
249 phloem and therefore do not encounter Si which is transported in the xylem (Raven 1983, Ma
250 and Yamaji 2015). Nonetheless, there are some examples of aphids being negatively impacted by
251 Si (Keeping and Kvedaras 2008) and the current meta-analysis indicated that Si-enriched plants
252 were at least sub-optimal for this group.

253 The relationships between different physical and chemical plants defences have occupied
254 ecologists for decades (Koricheva et al. 2004). On the basis that plant defences are costly for the
255 plant to produce, some theories predict that defences should trade-off against one another
256 whereas others argue that plants deploy coordinated defence syndromes (Moles et al. 2013). In
257 an extensive global study comprising 261 species from 80 families, Moles et al. (2013) found
258 there was little consistent evidence for either proposition. They did, however, report that four of
259 the five significant defensive pairwise relationships were between ash content (a proxy for Si and
260 calcium oxalate defences) and other chemical defences. This could be compatible with a
261 defensive trade-off between Si and other chemical defences, and in support of this, Cooke and

262 Leishman (2012) provide evidence from a broad range of plant taxa showing negative trade-offs
263 between Si and carbon-based defences (phenolics and tannins).

264 Our observations that stimulation of the JA pathway leads to Si accumulation in *B.*
265 *distachyon*, whilst Si addition regulates lower levels of JA, is compatible with a potential trade-
266 off between defences. It would be metabolically costly for plants to simultaneously have
267 (demonstrably effective) physical defences in place *and* produce defensive secondary
268 metabolites. Our observation of how Si is integrated into plant defence signalling leads us to
269 hypothesise that Si could be a mechanism for delivering a coordinated defence syndrome. We
270 propose that Si may act as a form of cross-talk, similar to that between JA and SA (Thaler et al.
271 2012), that allows plants to regulate defensive responses in a coordinated manner, and to target
272 them against particular types of herbivore. In addition to this, we provide the first evidence, to
273 our knowledge, that the Si–JA interaction is specific to chewing herbivores and is not influenced
274 by fluid-feeding herbivores.

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278

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373

374 **Figure Legends**

375 **Fig. 1.** Leaf (A) Si and (B) JA concentrations in response to no herbivory, caterpillar herbivory,
376 aphid (single or multiple bouts) herbivory or application of MeJA. Lowercase letters indicate
377 significant differences between treatments arising from Fisher's LSD tests. Bold letters indicate
378 significant differences with plants with no herbivory (controls). Mean \pm standard error shown. (A)
379 N = 15, 10 and 12 for no herbivory, herbivore combinations and MeJA application, respectively.
380 (B) N = 8 for herbivores and N = 10 for MeJA.

381

382 **Fig. 2.** Confocal image of *B. distachyon* leaf surface showing macro-hairs. White bar = 250 μ m.
383 Images of -Si leaves that were (A) intact, (B) subject to hair removal and equivalent +Si leaves (C
384 and D, respectively). White bar = 1 mm. (E) Relative growth rates (RGR) of *H. armigera* when
385 feeding on such leaves over a 24hr period. Mean \pm standard error shown; N = 12.

386

387 **Fig. 3.** Effect size (\pm 95% confidence interval) of plant Si defence against arthropod herbivores
388 classified as mandibulate (chewing) or fluid-feeding.

389

Fig. 1.

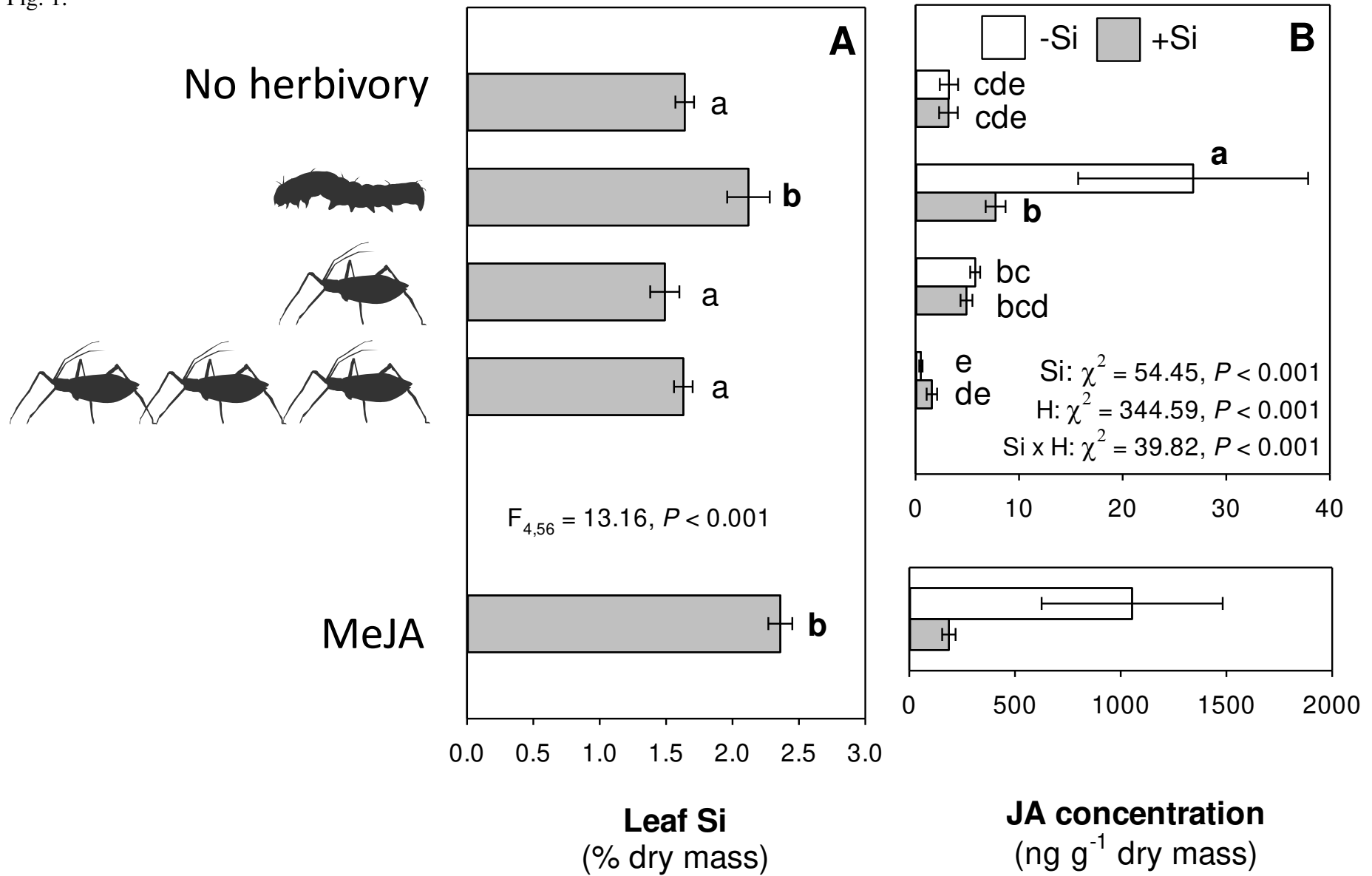


Fig. 2

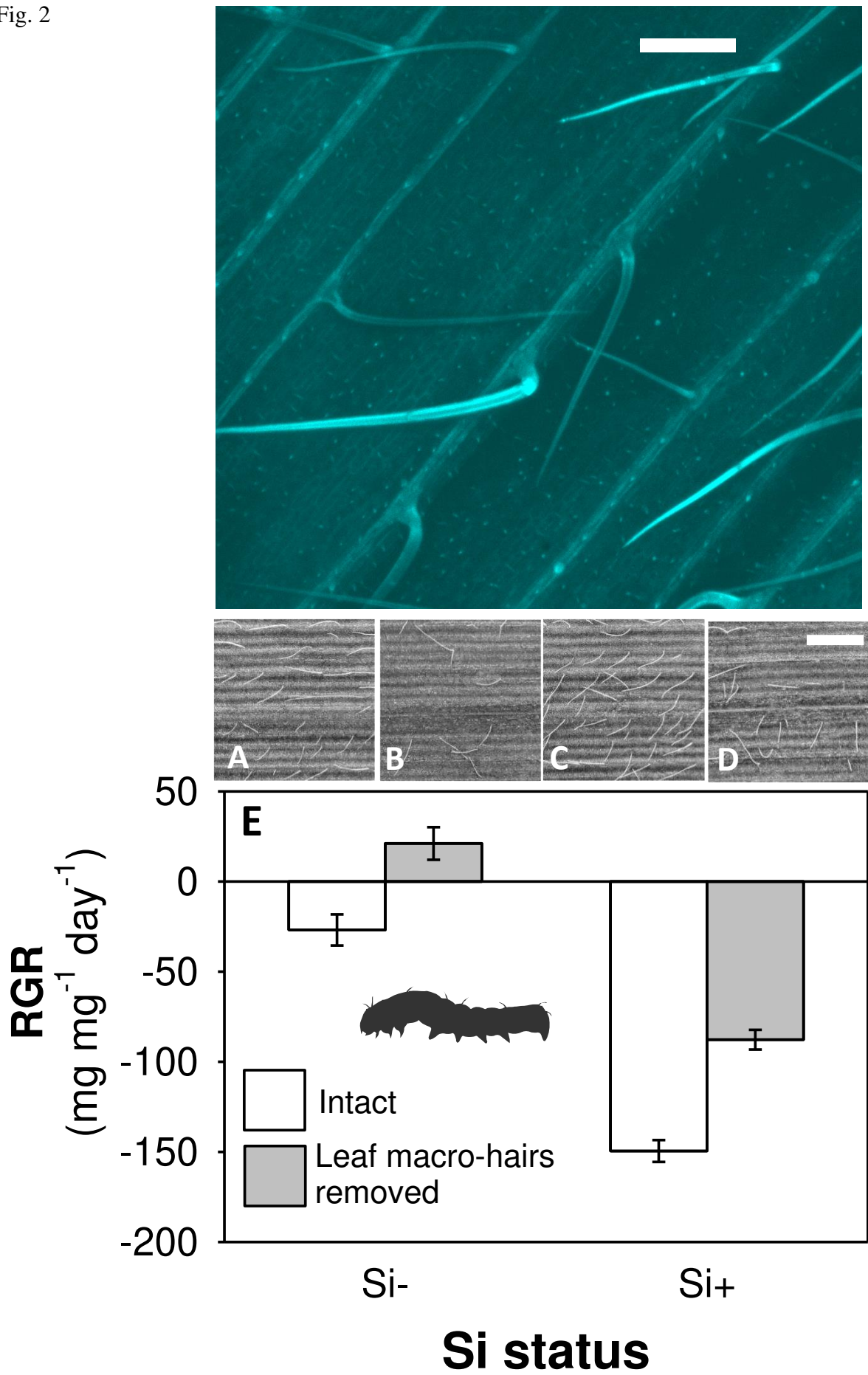


Fig. 3.

