

*Effect of humidity and temperature on the performance of three strains of *Aphalara itadori*, a biocontrol agent for Japanese knotweed*

Article

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Fung, C., González-Moreno, P., Pratt, C., Oliver, T. H., Bouchier, R. S. and González-Suárez, M. ORCID: <https://orcid.org/0000-0001-5069-8900> (2020) Effect of humidity and temperature on the performance of three strains of *Aphalara itadori*, a biocontrol agent for Japanese knotweed. *Biological Control*, 146. 104269. ISSN 1049-9644 doi: <https://doi.org/10.1016/j.biocontrol.2020.104269> Available at <https://centaur.reading.ac.uk/89912/>

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To link to this article DOI: <http://dx.doi.org/10.1016/j.biocontrol.2020.104269>

Publisher: Elsevier

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1 **Effect of humidity and temperature on the performance of three strains of *Aphalara***
2 ***itadori*, a biocontrol agent for Japanese Knotweed**

3

4 **Running title:** *Aphalara itadori* as a biocontrol for Japanese Knotweed

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28 **Declarations of interest:** none

29

30 **Article reference:** YBCON_104269

31 **Journal:** Biological Control

32 **Article accepted for publication:** 1 Apr 2020

33 **DOI:** 10.1016/j.biocontrol.2020.104269

34

35 **Highlights**

36

- 37 • Three strains of *Aphalara itadori* were tested under two environmental conditions
- 38 • More stressful environmental conditions slowed down psyllid development
- 39 • Biocontrol effectiveness was similar among strains, with no clear hybrid advantage

40

41 **Abstract**

42 Japanese knotweed (*Fallopia japonica*) is a highly damaging invasive species affecting UK
43 infrastructure and biodiversity. Under laboratory conditions, the psyllid *Aphalara itadori* has
44 demonstrated its potential to be a successful biocontrol agent for *F. japonica*. However, this
45 potential has not materialised in the field where long-term establishment of *A. itadori* has been
46 unsuccessful and faces the added challenge of climate change. Intraspecific variation (variation
47 among individuals of a species) has been shown to support establishment in alien species and
48 improve resilience to changing environmental conditions, here we propose it could improve
49 the performance of biocontrols. To test this possibility we compared the performance and
50 impact on *F. japonica* of three strains of *A. itadori* with different genetic backgrounds,
51 including a newly created hybrid. We hypothesize that genetic variability would be increased
52 in hybrids resulting in greater biocontrol effectiveness (greater impact on plant growth). We
53 also explored the potential influence of changing climate in performance, testing all strains
54 under two humidity conditions (with the same temperature). Contrary to our expectation, the
55 hybrid strain had the worst performance (slowest development rate and lower survival from
56 egg to adult emergence) under both environmental conditions. Exposure to different strains of
57 *A. itadori* did not result in consistent differences in plant growth, suggesting similar biocontrol
58 effectiveness among strains. Under the drier, more stressful, conditions plants exposed to *A.*
59 *itadori* had fewer leaves and accumulated less above-ground biomass. Overall, our results
60 suggest that genetic variability may not be the key to improve *A. itadori* biocontrol
61 effectiveness, but that predicted climate change, which anticipates drier and hotter summers in
62 the UK, could reduce the growth potential of *F. japonica* when exposed to *A. itadori*.

63

64 **Keywords:** Biological Control; Climate change; *Fallopia japonica*; Intraspecific Variation;
65 Invasive Species; Japanese Psyllid; Saturation Deficiency Index.

66 **Abbreviations**

67 LTLR: long-term laboratory-reared strain

68 STLR: short-term laboratory-reared

69 SDI: Saturation Deficiency Index

70

71 **1. Introduction**

72

73 Invasive species are a significant problem in the United Kingdom, where they are estimated to
74 cost the economy approximately £1.7 billion per annum (Booy et al., 2008; Williams et al.,
75 2010). Invasive species are both damaging to the UK's infrastructure and to the native
76 biodiversity. One of the most problematic invasive weeds in the UK is Japanese knotweed
77 (*Fallopia japonica* [Houttuyn] Ronse Decraene), a species native to Japan. The lack of fertile
78 *F. japonica* males in Britain, as determined from Random Amplified Polymorphic DNA
79 (RAPDs) analysis, suggests that all *F. japonica* in the UK is derived from a single clonal
80 individual that has reproduced through vegetative propagation (Hollingsworth and Bailey,
81 2000). This low genetic diversity however, has not hindered its invasive ability. *Fallopia*
82 *japonica* has become established in a wide-range of habitats, and grows asexually from small
83 fragments of underground root networks – rhizomes, weighing less than a gram (Bashtanova
84 et al., 2009; Hollingsworth and Bailey, 2000). These features, as well as its rapid growth rate,
85 make *F. japonica* highly invasive in the UK.

86

87 There have been varying attempts to eradicate or control *F. japonica*. Manual or chemical
88 removal can work at a local scale; however, the costs and time requirements make these
89 methods unfeasible as long-term or large-scale management solutions. Herbicide use in parks
90 and riparian areas where the plant is most prevalent has become less acceptable (Forman and
91 Kesseli, 2003). Biological control is often proposed as an effective alternative tactic for
92 invasive species, such as *F. japonica*. Reuniting an introduced weed with its host-specific
93 natural enemies from their country of origin has resulted in successful suppression of many
94 invasive weeds worldwide (Clewley et al., 2012; Schwarzländer et al., 2018). In comparison
95 to other control methods, biocontrol can be used everywhere and is generally cost effective and
96 environmentally friendly (Wittenberg and Cock, 2001).

97

98 The use of biocontrol agents for *F. japonica* in the UK has been explored by the non-profit
99 organisation CABI, UK, since 2003. Initially, candidate species were identified from the
100 Kyushu Island of Japan, the region from where the UK invasive *F. japonica* clones are thought
101 to have originated (Djeddour and Shaw, 2010). Out of the 186 candidate arthropod species

102 considered, *Aphalara itadori* Shinji (Hemiptera: Aphalaridae), otherwise known as Japanese
103 knotweed psyllid, was found to be the best agent, since laboratory studies showed it to be host-
104 specific (i.e. not affecting native plants) and highly damaging to *F. japonica*. However, despite
105 its effectiveness under laboratory conditions (Grevstad et al., 2013), the establishment of viable
106 populations in the field has been largely unsuccessful. A possible explanation for why field
107 releases have failed is a lack of genetic and phenotypic variability in the batches of *A. itadori*
108 that were released. Genetic bottlenecks are commonly implicated in the establishment failure
109 of biocontrol agents (see review by Fauvergue et al., 2012). It is not unusual in biocontrol
110 programs for host-range testing for specificity and safety to require a long period of laboratory
111 rearing. Indeed, in the UK, *A. itadori* was maintained in the laboratory from 2004 until its
112 approval for release in 2010 (Shaw et al., 2009). Because the released *A. itadori* came from
113 populations maintained under Japanese summer conditions at 22°C 13:11 hours day:night 50-
114 85% humidity for at least six years (~66 generations), they may have become conditioned to
115 the controlled environment room, as well as have potentially lost genetic diversity. This ‘colony
116 effect’ of laboratory reared animals has been seen in other insect species, such as in *Drosophila*
117 when undergoing laboratory selection experiments (Harshman and Hoffmann, 2000) and when
118 comparing wild to laboratory cultures of *Drosophila* (Sgrò and Partridge, 2000), and also in
119 *Anopheles gambiae* (Huho et al., 2007). As a result, the long-term laboratory-reared *A. itadori*
120 could have been ill-prepared for dealing with the variability in the natural environmental
121 conditions in the UK.

122

123 Intraspecific variation — the diversity of characteristics amongst individuals of a species
124 (Cianciaruso et al., 2009) — can be an important factor aiding in the establishment of alien
125 species (Forsman, 2014), but as mentioned above variability may be reduced in laboratory-
126 reared organisms. Plant and animal species with higher levels of intraspecific genetic and
127 phenotypic variation are more likely to establish successfully in new environments under
128 laboratory, semi-natural and natural conditions, with the largest effects seen in natural
129 experiments (Forsman, 2014). In addition, intraspecific variability can provide resilience to
130 changes in climatic conditions (Reusch et al., 2005; Sgrò and Hoffmann, 2004). Under climate
131 change, more variable populations are predicted to have an increased chance of containing
132 individuals with genotypes that allow population persistence (Oliver et al., 2015) whereas
133 locally adapted, less diverse populations are vulnerable because they have evolved traits to
134 suite only local stress factors (Benito Garzón et al., 2011).

135

136 The establishment of *A. itadori* may also have been affected by the interaction of different
137 climatic conditions. Hodkinson (2009) and pilot field experiments (CABI, unpublished data)
138 have shown that *A. itadori* population dynamics, and therefore their potential for establishment
139 in the UK, can be affected by expected rising temperatures and declining relative humidity. In
140 the UK, under climate change, conditions are likely to become more stressful due to a predicted
141 increase in temperature and decrease in humidity in the spring and summer (Murphy et al.,
142 2010) when *A. itadori* are most active after hibernation (Hodkinson, 2009). Therefore, effective
143 biocontrol requires consideration of how different environmental conditions could affect
144 effectiveness and resilience to future changes in climate.

145

146 For this study we compared the performance of the strain used in historic biocontrol releases
147 to two other strains with different genetic backgrounds. The first genetically different strain we
148 tested was from the same locality as original strain (Kyushu in South Japan) but had a shorter
149 laboratory-rearing history (2 years compared to 13 years). Using a newly collected wild type
150 strain would have been desirable but was not possible due the timing and cost of a new
151 collection and quarantine space. The second genetically different strain tested was a new hybrid
152 strain created from two distinct provenances of *A. itadori*. To create the hybrid we combined
153 males from Kyushu and females from Hokkaido (North Japan; Grevstad et al., 2013). The
154 Kyushu and Hokkaido strains of *A. itadori* are genetically distinct and both strain, as well as
155 the hybrid, can be distinguished using neutral molecular markers (Andersen et al., 2016). We
156 tested a hybrid as a potential approach to increase genetic variability and vigor (Birchler et al.,
157 2006; Szűcs et al., 2012). However, hybridization can also have negative effects which could
158 reduce the potential of this new hybrid strain (Heinze et al., 2019; Peer and Taborsky, 2005).
159 The performance and impact on *F. japonica* of the three strains was tested under two
160 environmental conditions that reflected standard laboratory growing conditions and a drier
161 environment reflective of climate change predictions.

162

163 **2. Material and methods**

164

165 *2.1. Aphanalara itadori* strains

166

167 We used three *Aphanalara itadori* strains. Two, the LTLR and STLR strains, were established
168 using adults collected from Kyushu, Japan (taken in 2004 and 2015 respectively). The hybrid
169 strain was created by mating LTLR strain males with females from a *A. itadori* line collected

170 in 2007 in Hokkaido, Japan and reared since that date at the Agriculture and AgriFood Centre
171 (AAFC) in Lethbridge, Canada. The crossing of lines was completed in December 2016 at
172 AAFC-Lethbridge under 16L:8D laboratory conditions. Second generation adult hybrids
173 (N~200) were shipped to the UK and reared in CABI under standard laboratory conditions
174 (see below). We used fourth generation hybrids for oviposition during the experiment. All
175 three strains were reared on knotweed in 100 x 90 x 100cm Perspex cages (average \pm SD:
176 $16.9^{\circ}\text{C} \pm 3.8^{\circ}\text{C}$, $47.2\% \pm 10.7\%$ RH and 14L:10D) in CABI's Egham quarantine greenhouse
177 facility.

178

179 *2.2. Experimental design and conditions*

180

181 We tested two environmental conditions that we then characterized using empirical estimates
182 of Saturation Deficiency Index (SDI), a measure of climate severity (Samways, 1987). In its
183 simplest form, SDI it is the difference between the saturation vapour pressure (SVP) at
184 maximum temperature, and the actual vapour pressure of a volume of air at maximum
185 temperature (Green and Catling, 1971; Samways, 1987). The value of SDI increases with rising
186 temperature and/or decreasing relative humidity. For our experiment, treatments were created
187 by changing humidity within experimental cages. Plants under high SDI conditions, reflective
188 of climate change predictions (hotter and drier), had dry capillary matting for the base of the
189 cage and a 40 x 50cm gauze covered hole at the back of the cage to increase ventilation. Plants
190 in low SDI conditions had wet capillary matting for the base of the cage, watered with 800ml
191 tap water every week, reflecting the standard laboratory growing conditions. We calculated
192 empirical SDI values for each treatment cage following Abteu and Melesse, (2013) and
193 Samways (1987):

$$194 \quad SDI = SVP \left(\frac{100 - RH}{100} \right) \text{ (Equation 1)}$$

195 where RH is relative humidity, and SVP is saturation vapour pressure calculated based on
196 temperature (T) as below:

$$197 \quad SVP = 0.611 e^{\left(\frac{17.27 \times T}{T + 237.7} \right)} \text{ (Equation 2)}$$

198 Humidity and temperature were recorded during the experiment at 30-minute intervals using
199 LogTag Haxo-8 dataloggers placed inside the sleeve of one randomly selected plant per cage.
200 We estimated SDI using the humidity and temperature recorded at each 30-minute interval. For
201 each day we then identified the three highest SDI values and calculated the arithmetic mean

202 per cage of those maxima over the duration of the experiment. This resulted in six SDI values
203 (one per cage). We averaged the three highest values instead of using the single highest value
204 to control for potential outliers. There are alternative methods of calculating SDI (see Green
205 and Catling, 1971), but we found results were equivalent with all methods (Table S1, Figure
206 S1).

207

208 Fifty-five days prior to the start of the first experimental batch, the rhizomes of 71 young *F.*
209 *japonica* of uniform genetic stock (collected from a single *F. japonica* patch with vegetative
210 reproduction) were cleaned and wet rhizome weights for each plant were obtained (average \pm
211 SD: 75.85g \pm 36.06g). Each rhizome was potted in an individual plastic pot (14.7cm diameter)
212 with a saucer (16.5cm diameter) and left to grow in a greenhouse under natural conditions
213 (average \pm SD: 21.0°C \pm 4.5°C, 51.6% \pm 12.4% RH and 14L:10D).

214

215 All experimentation was performed in quarantine glasshouses (average \pm SD: 21.0°C \pm 4.5°C,
216 51.6% \pm 12.4% RH and 14L:10D). Due to space constraints in the glasshouses, the experiment
217 was completed in three sequential batches over four months. For each batch, 14-15 days before
218 the start of the experiment, 18 plants were cut to the fourth node above ground on the main
219 stem and first node from the stem on branches, with additional stems cut to ground level. This
220 allowed us to standardise above-ground measurements of biomass. Cut *F. japonica* material
221 was collected and frozen, and dry weights later obtained for before and after above-ground
222 weight comparisons. Plants were then randomly assigned a *A. itadori* strain, and six plants from
223 each strain were placed into designated chambers for up to 8 days with 150 *A. itadori* adults to
224 allow oviposition ($n \approx 25$ *A. itadori* per plant).

225

226 After the oviposition period, the total number of eggs per plant was counted by searching the
227 top and bottom of all leaves and nodes using a hand lens. Plants with very high numbers of
228 eggs were removed from egg chambers earlier to avoid high egg density variation across
229 treatments (batch one: one STLR low SDI and one hybrid high SDI plant; batch two: one STLR
230 low SDI plant). Egg counts are minimum estimates because total counts would have required
231 damaging the plant, which would have prevented the experiment. We make the assumption
232 here that the number of visible eggs is proportionally related to the total number of eggs. Plants
233 were then randomly assigned to a low or high SDI treatment, resulting in three plant replicates
234 per strain per treatment per batch (experiment total: $n = 9$ plant replicates per strain per
235 treatment, total $n = 54$). We used 1m long insect sleeves supported by bamboo hoops for each

236 plant to prevent *A. itadori* from moving between plants (Figure S3). Each plant was placed in
237 a 16.5cm diameter saucer and irrigated twice a week manually to ensure *F. japonica* survival
238 irrespective of treatment. Total adult counts began 37 days after plants were placed in treatment
239 cages. Emergent adults were counted and removed using a manual aspirator every 6-7 days for
240 six weeks to allow all adults from the eggs laid prior to the experiment to emerge. Although
241 the nymphal stages cause the most damage to plants (Djeddour and Shaw, 2010), accurately
242 counting nymphs without removal is complicated, therefore we used adult counts to infer
243 survival to adult emergence. After all adults were counted, we obtained wet weights of above
244 ground and below ground plant biomass. Above ground plant material was then frozen and dry
245 weights were later obtained.

246

247 2.3. Response variables: *A. itadori* performance and plant growth

248

249 We used survival to adult emergence (henceforth referred to as ‘*A. itadori* survival’) and
250 development rates to assess *A. itadori* performance. *Aphalara itadori* survival was adjusted for
251 initial egg density, and was calculated as $100 * \frac{Adults}{Eggs}$, where *Eggs* was the total number of
252 eggs counted before moving the plants to the experimental treatments, and *Adults* was the total
253 number of emerged adults counted over the entire experiment for each plant. *Aphalara itadori*
254 development rate was evaluated by comparing the number of adults for each plant (expressed
255 as percentage of the total), counted at 1, 2 and 3 weeks after the first adult survival in each
256 cage. Counts after week 3 were not considered to avoid counting second generation offspring
257 emerging. One STLR plant from the low SDI treatment was removed as it had extreme adult
258 *A. itadori* numbers emerging compared to initial eggs counted.

259

260 Due to space limitations in the quarantine glasshouses, we could not assess how SDI treatments
261 affected plants without *A. itadori*. We evaluated impacts of *A. itadori* on *F. japonica* by
262 measuring differences in above and below ground biomass, number of leaves and stem height.
263 There was considerable variation in these traits between plants, thus, in the variables rhizome
264 weight, maximum height and leaf number, we did not compare absolute growth but instead
265 calculated relative growth as $100 * \frac{(Final-Initial)}{Initial}$, where *Final* was the measurement taken at
266 the end of the experiment and *Initial* was the measurement before the start of the experiment.
267 For the variable above-ground weight, the *Initial* was taken as zero (plants were potted as
268 rhizomes, without above ground material), and the *Final* was calculated as the sum of the

269 material that had been removed just prior to the experiment (to standardize plant size) and the
270 remaining material at the end. Both were measured as dry weights. Plant material was wrapped
271 in foil and placed into an oven at 70-90°C for 48h or until dried. As it was not possible to dry
272 rhizomes before the experiment without killing the plant, change in below ground biomass was
273 calculated using wet weights. The number of leaves was counted at the start and the end of the
274 experiment. Stem height was measured using a ruler from soil level to the tallest standing point
275 on the plant.

276

277 2.4. Data analysis

278

279 We evaluated the effect of strain and SDI on *A. itadori* survival, development and the four
280 measurements of *F. japonica* growth using linear mixed effect regression models fitted with
281 function ‘lmer’ from package *lme4* (Bates et al., 2015) in R version 3.4.3 (R Core Team, 2017).
282 Table S2 lists the fixed and random effects considered for each model. In summary, all models
283 included as a random factor the batch number (one, two or three) and, for *A. itadori* survival
284 and development, also observer ID (authors CF and CP, and Kate Constantine contributed to
285 egg counting). All models included SDI and strain as fixed predictors. In addition, models
286 assessing plant growth included as covariates: total number of adults to control for variation in
287 insect densities, and rhizome weight to control for initial plant conditions (except when
288 modelling rhizome weight). Models of *A. itadori* survival also included the total number of
289 eggs as a covariate. To model *A. itadori* development we used a B-splines analysis based on
290 count week to allow for non-linear changes in development. We tested models with additive
291 effects only, as well as with interactions between strain and SDI treatment. In the case of
292 development, Week was also tested for interactions (Table S2). Models with interactions were
293 only considered to be supported if interaction terms were significant (p-value < 0.05). We
294 evaluated model assumptions (normality and heteroscedasticity) plotting residuals from tested
295 models. We used post-hoc tests based on R function ‘diffsmeans’ and ‘lsmeansLT’ from
296 package *lmerTest* (Kuznetsova et al., 2017) to contrast among strains.

297

298 3. Results

299

300 3.1. *Aphalara itadori* performance

301

302 *Aphalara itadori* survival varied among strains ($F_{2, 36.17} = 12.49$, $P < 0.001$, $n = 18, 17$ and 18
303 for LTLR, STLR and Hybrid strains respectively; Table 1, Figure 2A). In particular, survival
304 from egg to adult emergence was significantly lower in hybrids (predicted mean [95%
305 confidence intervals]: 26.00% [10.99 – 41.01]) compared to LTLR (57.72% [41.86 – 73.57])
306 and STLR (54.79% [34.49 – 75.08]) strains, but LTLR and STLR did not differ ($P = 0.68$).
307 SDI did not significantly affect *A. itadori* survival ($F_{1, 46.86} = 1.66$, $P = 0.20$), but survival was
308 proportionally higher in plants with fewer eggs suggesting a density dependence effect ($F_{1, 46.82}$
309 $= 7.98$, $P = 0.007$).

310

311 The proportion of adults emerging generally decreased from the first to the third week, with
312 earlier emergence time under low SDI (higher humidity, $F_{1, 153} = 28.34$, $P < 0.001$; Table 1).
313 The LTLR strain had the fastest development rates, with notable difference under high SDI,
314 with the LTLR strain having peak emergence in the first week one compared to both the STLR
315 and the hybrid strain which displayed peak emergence during the second week (Table 1, Figure
316 1B). There was an interaction between STLR and SDI, with the majority of STLR adults
317 emerging sooner under lower SDI ($F_{2, 153} = 6.69$, $P < 0.001$).

318

319 3.2. Impacts on *F. japonica*

320

321 The considerable variation in plant growth recorded in all four traits was not consistently
322 associated with the *A. itadori* strains to which plants were exposed (Figure 2, Table 2). The
323 only significant effect of strain was detected in the change in plant height, where hybrids
324 (predicted mean [95% confidence intervals]: 352.21% [232.24 – 472.19]) had least effect in
325 suppressing plant growth (plants had greater percentage changes in height) compared to LTLR
326 (275.23% [155.23 – 395.22]) and STLR (286.40% [161.65 – 411.14]) which had similar
327 estimates ('lsmeansLT' estimates: $P = 0.08$ and 0.07 , for hybrids vs LTLR and STLR
328 respectively). For plant height, we also found evidence of a differential effect of *A. itadori*
329 strain conditional to SDI: the STLR strain was most effective at reducing maximum height
330 under high SDI values, but least effective under low SDI ($F_{2, 44.16} = 4.08$, $P = 0.019$, $N = 54$, 18
331 plants per strain; Figure 2C, Table 2). SDI influenced leaf number and above ground weight,
332 with plants having fewer leaves ($F_{1, 46.83} = 5.82$, $P = 0.020$) and smaller above ground weight
333 ($F_{1, 38.06} = 5.87$, $P = 0.020$) under higher SDI (low humidity).

334

335 Out of the four plant growth variables tested, leaf number was the only response variable which
336 was influenced by another predictor besides strain and SDI (other predictors: total eggs,
337 number of adults, week of emergence and initial rhizome weight; see Table A2 for when these
338 predictors were included in our models), where higher rhizome weights at the start of the
339 experiment were associated with more leaves ($F_{1, 47.01} = 9.29, P = 0.004$; Table 2). None of the
340 variables we tested explained change in rhizome weight (Table 2; Figure 1A).

341

342 **4. Discussion**

343

344 Our study aimed to improve biocontrol of *F. japonica* by exploring the effectiveness of
345 different *A. itadori* strains. We hypothesised that strains which had spent less time in the
346 laboratory (STLR and hybrid strain) would have undergone less selection pressure to perform
347 better under standard laboratory conditions, and therefore would perform better under altered
348 climatic conditions. Previous studies have shown that laboratory rearing may lead to reduced
349 genetic variability compared to wild stocks due to population bottlenecks and selection (Huh
350 et al., 2007; Sgrò and Partridge, 2000), and therefore laboratory stocks tend to become more
351 stress sensitive as selection for stress-related traits is relaxed (Hoffmann and Ross, 2018). Our
352 results did not consistently support our predictions suggesting longer time in laboratory culture
353 by itself is not affecting the performance of *A. itadori* biocontrol for *F. japonica*.

354

355 Among the strains, hybrids had lower survival and developed slower compared to the LTLR
356 strain. Although the hybrid was created from two genetically different strains (Andersen et al.,
357 2016), differences in the single-nucleotide polymorphisms (SNPs) may not have matched
358 differences in functional gene regions linked to the traits we were assessing. In addition,
359 although there have been studies which have shown improved hybrid fitness, for example in
360 ornamental pear tree *Pyrus calleryana* (Culley and Hardiman, 2009), hybridisation in our study
361 could have led to reduced, rather than improved, fitness. Between-population crosses from
362 Bremgartewald and Spilwald strains of the black timber bark beetle, *Xylosandrus germanus*,
363 were found to be less fit compared to inbred individuals (Peer and Taborsky, 2005). Hybrids
364 from populations of the intertidal copepod species *Tigriopus californicus* also exhibited the
365 negative effect of outbreeding depression, with hybrid fitness initially lower in terms of
366 survivorship and morphology (Hwang et al., 2011). In our study, the hybrid strain was created
367 from the combination of males from the Kyushu strain, which performs best on *F. japonica*
368 compared to other knotweeds, and females from the Hokkaido strain, which oviposit and

369 develop well on *R. sachalinensis* (Grevstad et al., 2013). It is possible that hybrid breakdown
370 may have occurred whereby the Hokkaido strain's adaptation and preference to living on *R.*
371 *sachalinensis* was expressed in the hybrids, explaining the low survival to adult emergence
372 observed in the hybrid strain compared to other strains. However, it is important to note that
373 the hybrid was equal to the other two strains observed in terms of reducing the plant growth
374 predictors assessed, and future work assessing more traits would further aid in determining the
375 performance of hybrid strains.

376

377 Our study found that *Aphalara itadori* development was slower under high SDI, which has also
378 been found for other psyllid species (see Hodkinson, 2009). Slower development could explain
379 why plants exposed to *A. itadori* under stressful low humidity levels (high SDI) had lower
380 growth in above-ground weight, height and number of leaves, compared to plants under high
381 humidity levels. The more damaging nymphal stage of *A. itadori* is extended under slower
382 development (Djeddour and Shaw, 2010) and therefore the per capita impact of individuals is
383 likely to increase, potentially making them more effective biocontrol agents under high SDI
384 conditions. Indeed, we found that the STLR strain developed slowest and had a greater impact
385 on plant height under high SDI. However, this benefit could be offset by there being fewer
386 generations per season, something that will need to be confirmed in future studies.

387

388 The findings that *A. itadori* survival was not influenced by SDI contrasts with other studies on
389 other *A. itadori* species that have shown that high SDI leads to lower survival (Hall and Hentz,
390 2001; Hodkinson, 2009; McFarland and Hoy, 2001). These differences may reflect variation
391 among species, but it is also possible that our drier conditions were not sufficiently stressful to
392 induce mortality. The experiments were done within a greenhouse where conditions limited
393 our ability to strictly control temperature and humidity.

394

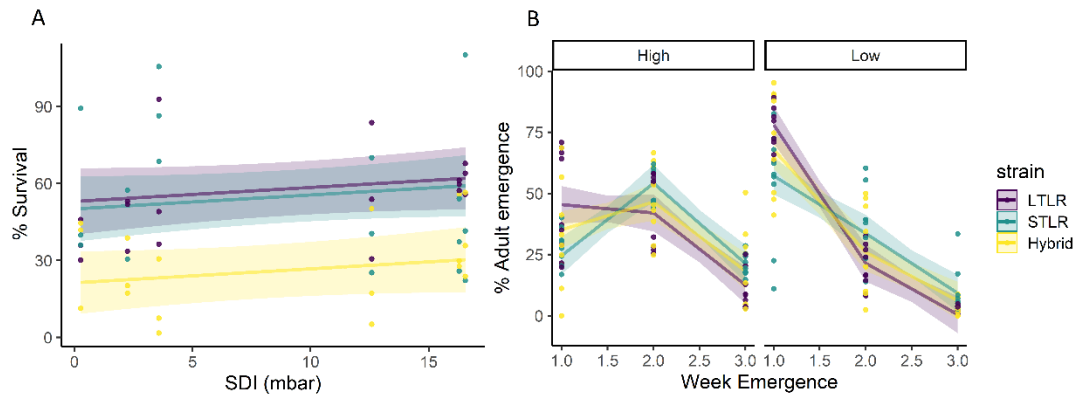
395 Due to space limitations in the quarantine area we could not assess how environmental
396 conditions affected plants without *A. itadori*. However, the reduced above-ground biomass and
397 number of leaves observed in plants under high SDI could reflect more stressful conditions for
398 the plants, especially as all plants were regularly watered, so only ambient humidity changed.
399 If plants by themselves were not affected by the more stressful ambient conditions in the
400 experiment, this suggests that *A. itadori* could be even more damaging when plant do suffer
401 from high stress conditions in the field.

402

403 Notably, we found no effects of strain or SDI on rhizome weight. This could be because both
404 insects and ambient humidity do not directly affect rhizomes, and nutrient availability in the
405 soil was sufficient to avoid rhizome depletion associated to above ground growth. Since *F.*
406 *japonica* is mainly spread by pieces of rhizome this highlights the challenge in developing an
407 effective biological control to reduce the spread of this invasive plant.

408

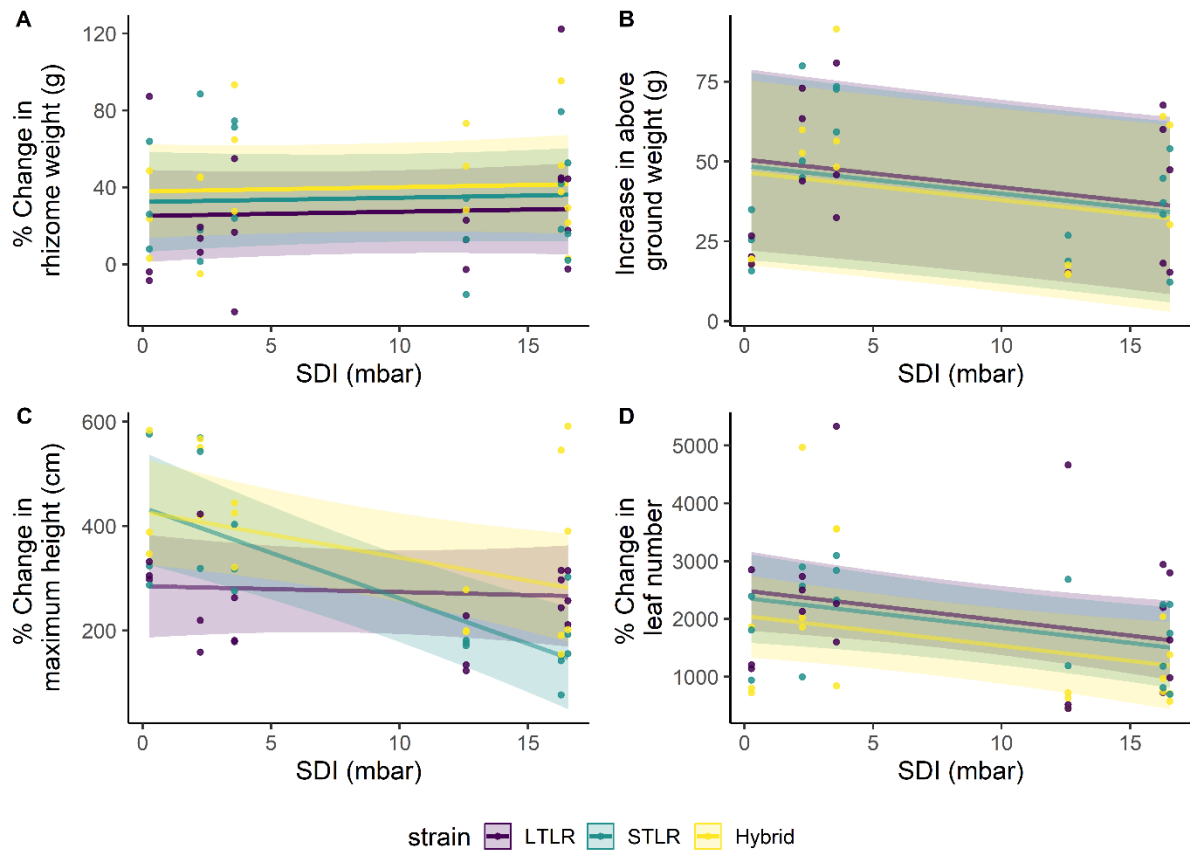
409 Overall, our results do not support a beneficial role of intraspecific variation in the biocontrol
410 effectiveness of *A. itadori*. Genetic work would be necessary to reveal if this is due to genetic
411 variability being different from our expectation (lower in LTLR and highest in hybrids).
412 Additional work under laboratory and field conditions would also be necessary to test a wider
413 range of climate conditions (as responses are likely to be non-linear), to evaluate cross-
414 generational changes including hybrid fitness after more generations, and to take into account
415 additional factors such as predator avoidance and overwintering performance. Effectively
416 controlling *F. japonica*, both above and below ground, is still the challenge ahead.



418

419 **Figure 1** Relationship between *Aphalara itadori* performance in terms of (A) percentage *A.*
 420 *itadori* survival to adult emergence versus Saturation Deficiency Index (SDI) in treatment
 421 cages and (B) *A. itadori* development rate per week. Data points show the observed survival
 422 of three *A. itadorii* strains (LTLR = Long-term laboratory reared; STLR = short-term
 423 laboratory reared and Hybrid strain) grown on *Fallopia japonica*. Lines show the predicted
 424 relationship with SDI from a linear mixed effects model with shaded areas indicating 95%
 425 confidence intervals.

426



427

428

429 **Figure 2** Relationship between growth of *F. japonica* versus Saturation Deficiency Index
 430 (SDI) in treatment cages. Data points show the observed survival to adult emergence of three
 431 *Aphalara itadori* strains (LTLR = Long-term laboratory reared; STLR = short-term
 432 laboratory reared and Hybrid strain) grown on *Fallopia japonica*. Plant growth was
 433 measured as either (A) rhizome weight, (B) above ground weight, (C) maximum height, and
 434 (D) leaf number. Lines show the predicted relationship with SDI from a linear mixed effects
 435 model with shaded areas showing 95% confidence intervals.

436

437 **Table 1** Coefficient estimates for the model predicting *Aphalara itadori* adult survival to
 438 adult emergence as a function of total number of *A. itadori* eggs, Saturation Deficiency Index
 439 value (SDI), and *A. itadori* strain (LTLR = Long-term laboratory reared; STLR = short-term
 440 laboratory reared and Hybrid strain); and adult development as a function of time (in weeks),
 441 Saturation Deficiency Index value (SDI), and *A. itadori* strain. We report best parameter
 442 estimates (β), their 95% confidence interval (CI), *P*-value, and the number of plants used in
 443 each analyses (*N*). The strain reference level (e.g. ‘LTLR’) is indicated in parentheses. The
 444 colon separating variable names indicates interaction terms. Significant variables are
 445 highlighted in bold.

Variable	β	Lower 95% CI	Upper 95% CI	<i>P</i> -value
Survival (<i>N</i> = 53)*				
Intercept (LTLR)	66.05	50.23	81.88	<0.001
SDI	0.54	-0.28	1.37	0.204
STLR	-2.93	-16.71	10.86	0.679
Hybrid	-31.71	-45.67	-17.76	<0.001
Total eggs	-0.02	-0.03	-0.01	0.007
Development (<i>N</i> = 54)				
Intercept (LTLR: Low)	78.05	69.42	86.69	<.001
STLR	-20.91	-30.29	-11.54	<.001
Hybrid	-10.23	-19.67	-0.78	0.035
High	-32.64	-40.26	-25.02	<.001
LTLR: Week 1-2	-56.54	-67.23	-45.85	<.001
LTLR: Week 2-3	-77.62	-88.31	-66.93	<.001
STLR: Week 1-2	33.07	19.98	46.16	<.001
STLR: Week 2-3	29.67	16.58	42.76	<.001
Hybrid: Week 1-2	14.72	1.63	27.81	0.029
Hybrid: Week 2-3	15.96	2.86	29.05	0.018
High: Week 1-2	53.17	42.48	63.86	<.001
High: Week 2-3	44.75	34.06	55.43	<.001
Total Eggs	0	-0.01	0.01	1

446 *One STLR plant had extreme adult *A. itadori* numbers emerging was removed from analysis

447

448 **Table 2** Summary of models predicting percentage change in *Fallopia japonica* factors as a
 449 function of Saturation Deficiency Index (SDI), *Aphalara itadori* strain (LTLR = Long-term
 450 laboratory reared; STLR = short-term laboratory reared and Hybrid strain), the total number
 451 of *A. itadori* adults produced at the end of the experiment, and the initial rhizome weight. We
 452 report best parameter estimates (β), their 95% confidence interval (CI), *P*-value, and the
 453 number of plants used in each analyses (*N*). The strain reference level (e.g. ‘LTLR’) is
 454 indicated in parentheses. The colon separating variable names indicates interaction terms.
 455 Significant variables are highlighted in bold.

Variable	β	Lower 95% CI	Upper 95% CI	<i>P</i> - value
Rhizome weight (<i>N</i> = 54)				
Intercept (LTLR)	27.32	-3.89	58.53	0.109
SDI	0.22	-1.03	1.47	0.729
STLR	7.29	-13.86	28.44	0.503
Hybrid	12.77	-12.45	37.98	0.326
Number of Adults	-0.01	-0.06	0.04	0.770
Above Ground Weight (<i>N</i> = 46)*				
Intercept (LTLR)	57.97	27.22	88.73	0.020
SDI	-0.87	-1.58	-0.17	0.020
STLR	-2.01	-13.79	9.77	0.740
Hybrid	-4.00	-18.61	10.61	0.594
Number of Adults	-0.02	-0.05	0.00	0.106
Initial Rhizome Weight	-0.00	-0.13	0.12	0.940
Maximum Height (<i>N</i> = 54)				
Intercept (LTLR)	340.49	204.08	476.90	<0.001
SDI	-1.14	-8.30	6.01	0.755
STLR	150.85	42.17	259.54	0.009
Hybrid	142.80	23.88	261.72	0.023
SDI: STLR	-16.27	-26.10	-6.45	0.002
SDI: Hybrid	-7.67	-17.33	2.00	0.127
Number of Adults	-0.15	-0.32	0.02	0.081
Initial Rhizome Weight	-0.12	-0.94	0.69	0.769

Leaf Number ($N = 54$)

Intercept (LTLR)	1622.59	493.61	2751.57	0.009
SDI	-51.75	-93.79	-9.71	0.020
STLR	-127.08	-833.21	579.04	0.726
Hybrid	-438.50	-1272.10	395.10	0.308
Number of Adults	-0.26	-1.88	1.36	0.754
Initial Rhizome Weight	12.48	4.45	20.50	0.004

456 *Eight *F. japonica* (three LTLR, one STLR and four Hybrid) had weights missing and were
457 removed from analysis

458

459 **Acknowledgments**

460 The authors would like to further thank Nikoli Thom, Kate Constantine and Sarah Thomas for
461 help with the experimental set-up, and Tom Johnson and Emma Gardner for help in advising
462 the statistical analysis. Thanks are also given to Richard Shaw and two anonymous reviewers
463 for comments on the manuscript.

464

465 **Funding**

466 This work was supported by the University of Reading; CABI; the Department for
467 Environment Food and Rural Affairs; and Agriculture and Agri-Food Canada.

468

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582

583 **Appendix A**

584 **Table A1** Alternative methods of calculating Saturation Deficiency Index value (SDI)
585 adapted from (Green and Catling, 1971). Maximum temperatures were the maximum
586 temperatures across the whole experiment.

SDI Methods

- 1 Mean of 3 highest daily maximum temperatures with the mean of the three
vapour pressures coinciding with the 3 highest maximum temperatures

 - 2 Mean of the 3 SDI values (millibars) coinciding with the 3 highest maximum
temperatures
-

587

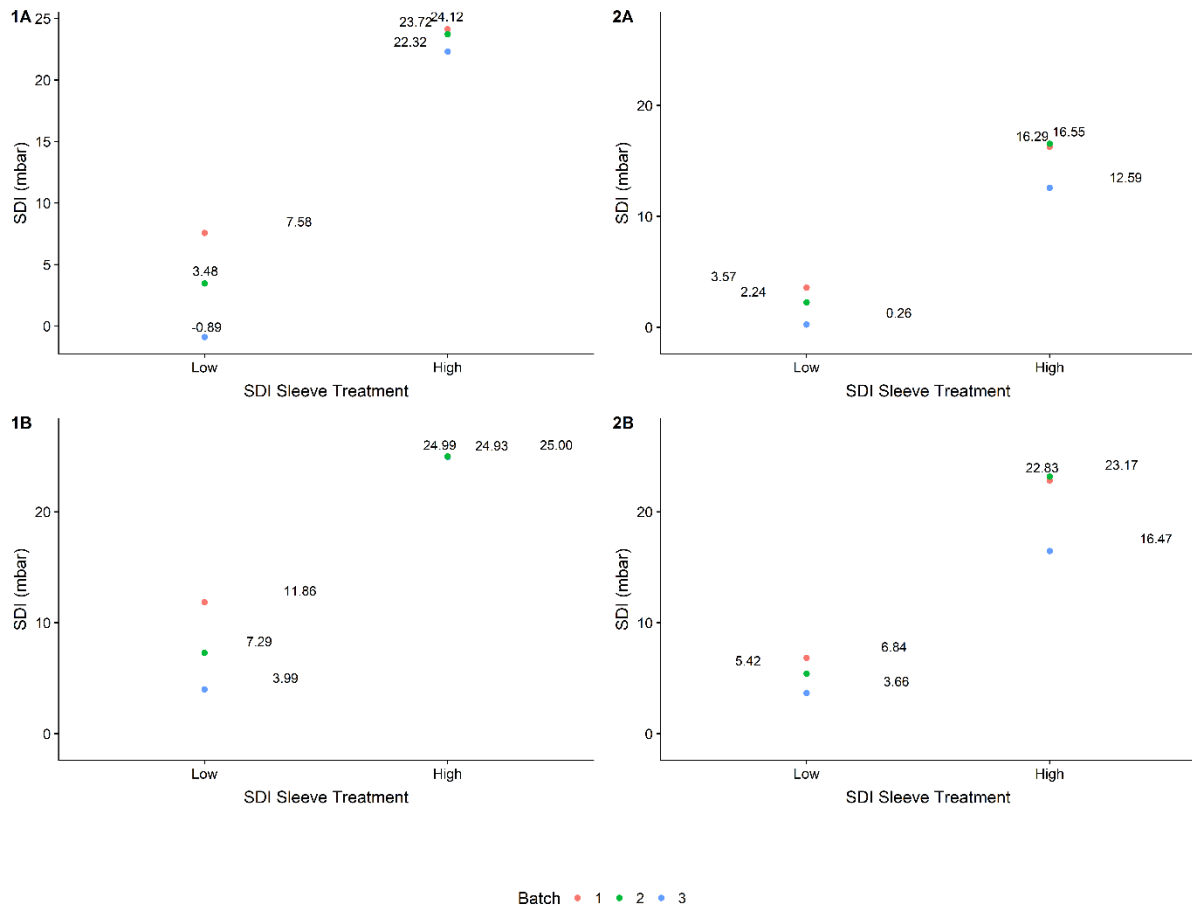
588

589 **Table A2** All models tested to analyse the effects of strain and Saturation Deficiency Index
590 value (SDI) on *Aphalara itadori* performance (survival to adult emergence and development)
591 and *A. itadori* impact on *Fallopia japonica* growth (rhizome weight, above ground weight,
592 maximum height and leaf number. * indicates tested interactions and models used are
593 highlighted in bold.

Model	Fixed predictors	Random Factors
<i>A. itadori</i> Performance		
Survival		
<i>S1</i>	Strain + SDI + total eggs	Batch + observer
<i>S2</i>	Strain*SDI + total eggs	Batch + observer
Development		
<i>D1</i>	Strain + SDI + week emerge + total eggs	Batch + observer
<i>D2</i>	Strain*SDI + week emerge + total eggs	Batch + observer
<i>D3</i>	Week emerge*(strain + SDI) + total eggs	Batch + observer
<i>D4</i>	strain*(SDI + week emerge) + total eggs	Batch + observer
Impact on <i>F. japonica</i>		
Rhizome Weight		
<i>R1</i>	Strain + SDI + total number of adults	Batch
<i>R2</i>	Strain*SDI + total number of adults	Batch
Above Ground Weight		
<i>A1</i>	Strain + SDI + total number of adults + before rhizome weight	Batch
<i>A2</i>	Strain*SDI + total number of adults + before rhizome weight	Batch
Maximum Height		
<i>H1</i>	Strain + SDI + total number of adults + before rhizome weight	Batch
<i>H2</i>	Strain*SDI + total number of adults + before rhizome weight	Batch
Leaf Number		
<i>L1</i>	Strain + SDI + total number of adults + before rhizome weight	Batch

L2

Strain*SDI + total number of adults + before rhizome weight Batch



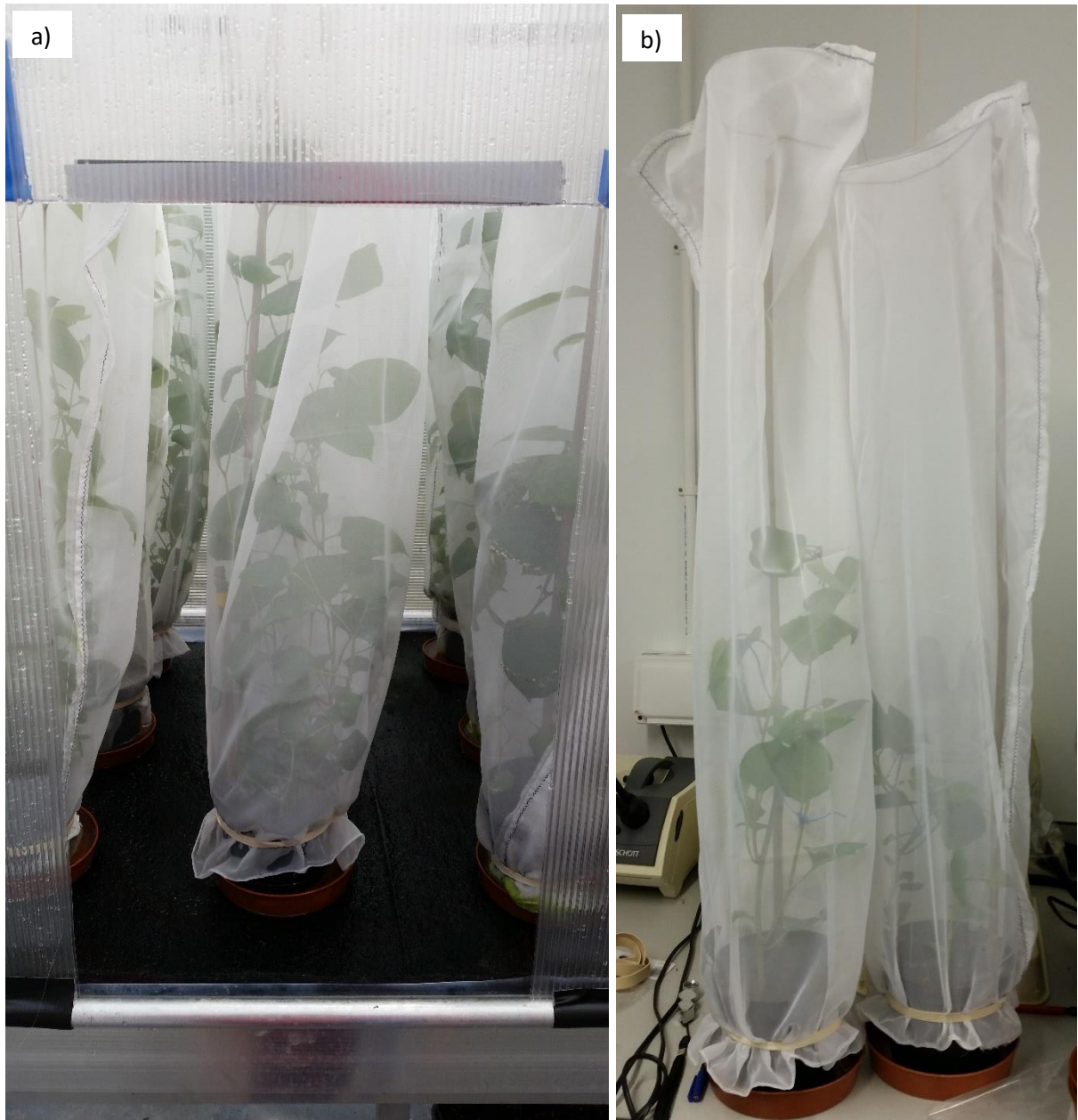
595

596 **Figure A1** Four methods chosen for calculating Saturation Deficiency Index value (SDI)
 597 adapted from (Green and Catling, 1971). Points are the calculated SDI values of dataloggers
 598 for each experimental batch. Each datalogger was placed in one sleeve within a treatment
 599 cage.

600 (1A) SDI was firstly calculated per day by taking the mean of the top three temperature
 601 values and its corresponding relative humidity values (RH). The final SDI value assigned to
 602 the batch was the average SDI for the whole experiment. (1B) SDI was firstly calculated per
 603 day by taking the mean of the top three temperature values and corresponding RH values.
 604 The final SDI value was then assigned by calculating the mean of the top three SDI values for
 605 the whole experiment. (2A) SDI values were calculated for each reading (30min) and the
 606 mean of the highest three SDI values was obtained. The final SDI value assigned to the batch
 607 was the average SDI for the whole experiment. (2B) SDI values were calculated for each
 608 reading (30min) and the mean of the highest three SDI values was obtained. The final SDI
 609 value assigned by calculating the mean of the top three SDI values for the whole experiment.

610

611



612

613 **Figure A2** Experimental *Fallopia japonica* plants. a) For the experiment, plants were placed

614 in a 16.5cm diameter saucer within a humidity cage with capillary matting. They were

615 irrigated twice a week manually; b) after egg counts, plants were covered in 1m long insect

616 sleeves, tied with elastic bands and supported by bamboo halos to avoid *Aphalara itadori*

617 escaping.

618

619

620