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

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

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Declarations of interest: none

Article reference: YBCON_104269

Journal: Biological Control

Article accepted for publication: 1 Apr 2020

DOI: 10.1016/j.biocontrol.2020.104269

Highlights

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

Abstract

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

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

Abbreviations

LTLR: long-term laboratory-reared strain

STLR: short-term laboratory-reared

SDI: Saturation Deficiency Index

1. Introduction

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

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

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

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

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

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

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

2. Material and methods

2.1. Aphalara itadori strains

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

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

2.2. Experimental design and conditions

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

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

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

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

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

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

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

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

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

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

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

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

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

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

2.4. Data analysis

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

3. Results

3.1. Aphalara itadori performance

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

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

3.2. Impacts on F. japonica

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

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

4. Discussion

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

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

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

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

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

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

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

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

Figures and Tables

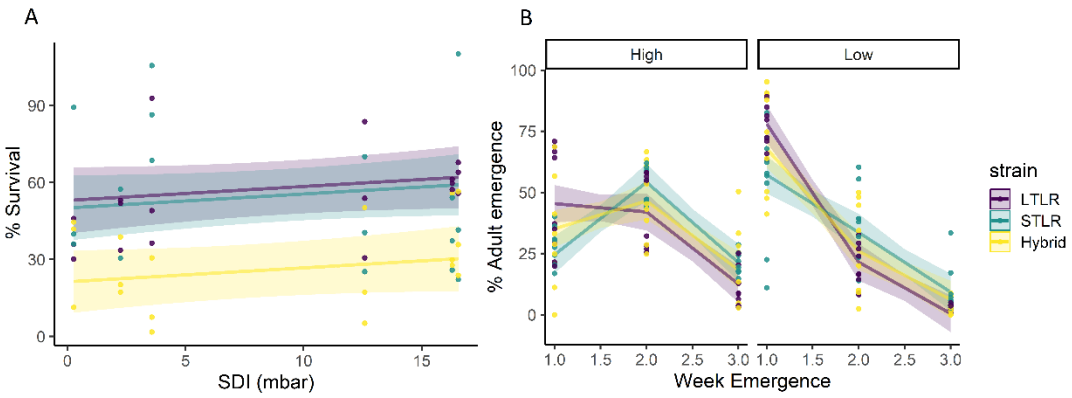

Figure 1 Relationship between *Aphalara itadori* performance in terms of (A) percentage *A. itadori* survival to adult emergence versus Saturation Deficiency Index (SDI) in treatment cages and (B) *A. itadori* development rate per week. Data points show the observed survival of three *A. itadorii* strains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

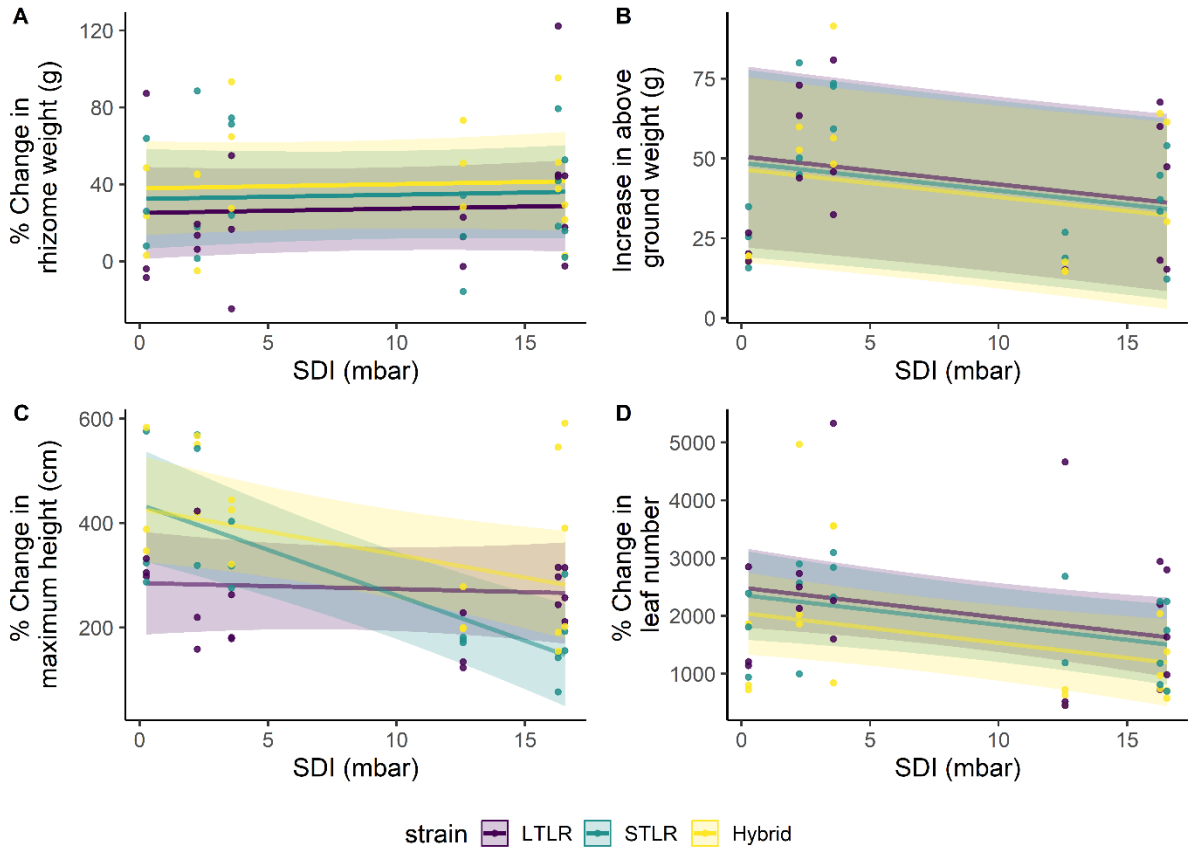

Figure 2 Relationship between growth of *F. japonica* versus Saturation Deficiency Index (SDI) in treatment cages. Data points show the observed survival to adult emergence of three *Aphalara itadori* strains (LTLR = Long-term laboratory reared; STLR = short-term laboratory reared and Hybrid strain)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

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

Variable	**β**	**Lower 95% CI**	**Upper 95% CI**	***P*-value**
Survival (*N* = 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 (*N* = 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

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

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

Variable	**β**	**Lower 95% CI**	**Upper 95% CI**	***P*-value**
Rhizome weight (*N* = 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 (*N* = 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 (*N* = 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**

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

Acknowledgments

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

Funding

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

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

L2	Strain*SDI + total number of adults + before rhizome weight	Batch

594

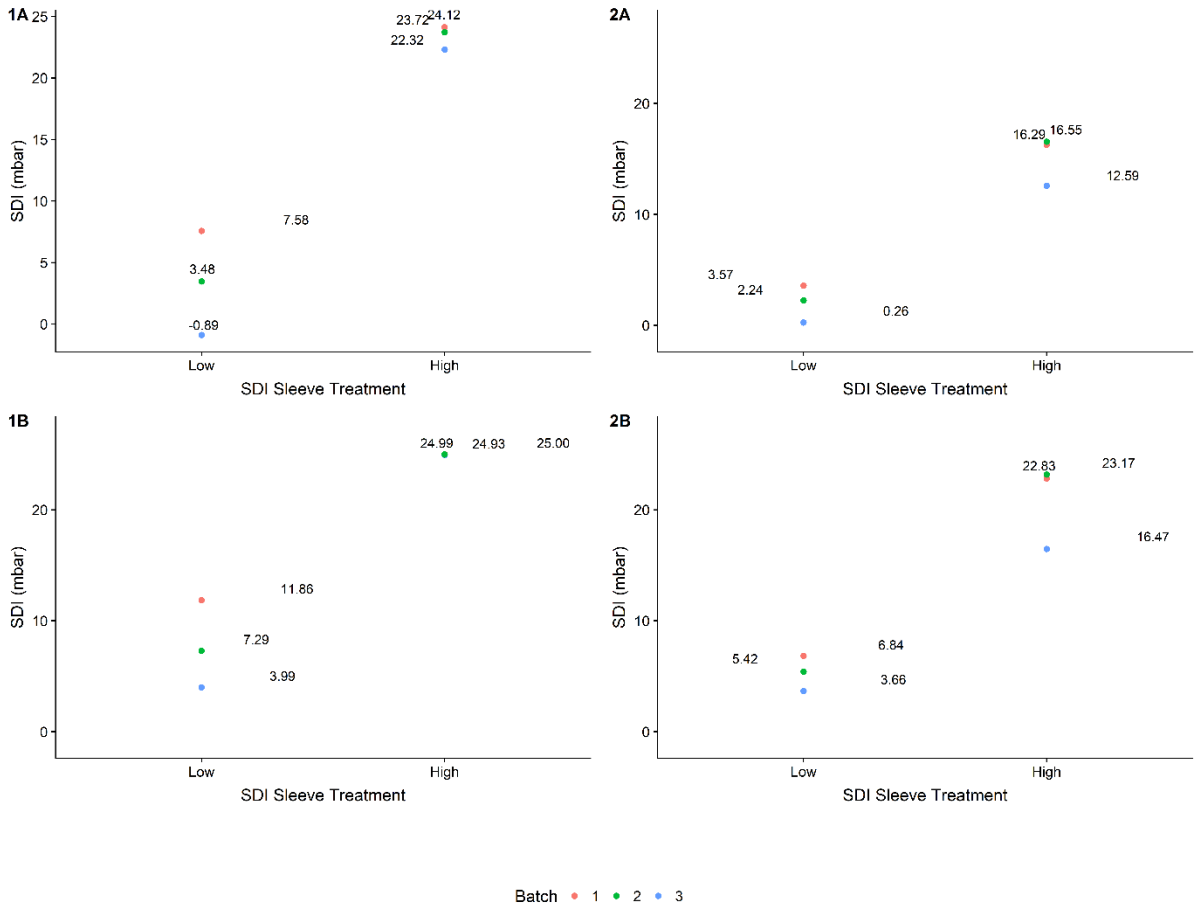

Figure A1 Four methods chosen for calculating Saturation Deficiency Index value (SDI) adapted from (Green and Catling, 1971). Points are the calculated SDI values of dataloggers for each experimental batch. Each datalogger was placed in one sleeve within a treatment cage.

(1A) SDI was firstly calculated per day by taking the mean of the top three temperature values and its corresponding relative humidity values (RH). The final SDI value assigned to the batch was the average SDI for the whole experiment. (1B) SDI was firstly calculated per day by taking the mean of the top three temperature values and corresponding RH values. The final SDI value was than assigned by calculating the mean of the top three SDI values for the whole experiment. (2A) SDI values were calculated for each reading (30min) and the mean of the highest three SDI values was obtained. The final SDI value assigned to the batch was the average SDI for the whole experiment. (2B) SDI values were calculated for each reading (30min) and the mean of the highest three SDI values was obtained. The final SDI value assigned by calculating the mean of the top three SDI values for the whole experiment.

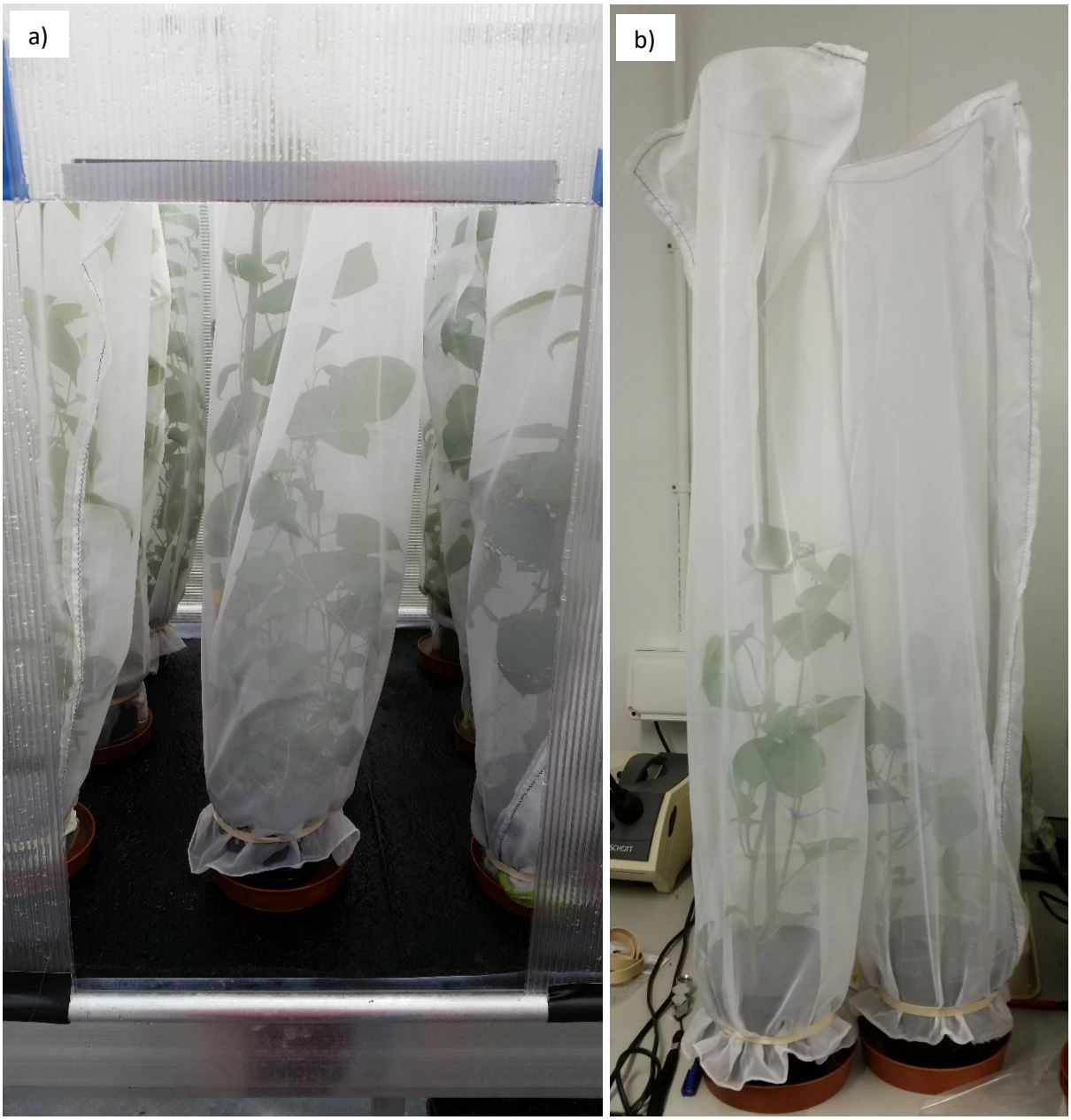

Figure A2 Experimental *Fallopia japonica* plants. a) For the experiment, plants were placed in a 16.5cm diameter saucer within a humidity cage with capillary matting. They were irrigated twice a week manually; b) after egg counts, plants were covered in 1m long insect sleeves, tied with elastic bands and supported by bamboo halos to avoid *Aphalara itadori* escaping.