Manipulating Phenotypic Plasticity to Improve Population Establishment of a Classical Biological Control Agent (the Psyllid, *Aphalara itadori* Shinji) for Invasive Knotweeds

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science, Forestry

Faculty of Forestry
University of Toronto

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Abstract

The psyllid Aphalara itadori was approved for release as a biocontrol agent for invasive knotweeds (Fallopia sp.) in Canada, but has had limited success establishing in the field. Establishing biocontrol agent populations in the field is a common problem and one unexplored possibility in improving establishment is the manipulation of an agent’s phenotype prior to release. We developed a series of laboratory bioassays to assess the psyllid’s fecundity and offspring fitness in response to host plants of varying quality. Results show an increase in fitness measures that appear unrelated to the host plant quality and more closely determined by temperature or humidity. This suggests phenotypic plastic responses in A. itadori populations are less sensitive to changes in their host and more sensitive to environmental variables. Determining which environmental factors drive changes in these fitness variables and rearing A. itadori in these conditions prior to release may improve population establishment.
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Introduction

Invasive non-native plants are a significant threat to biodiversity and economies worldwide by outcompeting and replacing native plants and/or preventing their re-establishment, thus altering the structure and function of an ecosystem (Hedja et al. 2009, Ehrenfeld 2010). This replacement of native species can cascade to higher trophic levels causing decreases in overall animal species richness and abundance (Vila et al. 2011) and making invasive non-native plants one of the major drivers of biodiversity loss worldwide (Pimental et al. 2005).

Managing non-native plant invasions is a significant economic issue; however, calculating exact economic damages from invasive non-native plants is inherently challenging with many ecological consequences and externalities across sectors being difficult to estimate (Colautti et al. 2006). In Canada, there are a reported 486 species of non-native invasive vascular plants causing $2.2 billion CDN in losses to Canadian agriculture alone (CFIA 2008).

Determining why a non-native species becomes invasive in its introduced range is a topic of much debate in invasion ecology. Non-native species introduced into new ranges are hypothesized to develop into problematic invasive species through one or any number of mechanisms: escape from predation (Meijer et al. 2016), novel defenses or predatory ability (Callaway and Ridenour 2004), high phenotypic plasticity (Davidson et al. 2011), rapid climate adaption (Colautti and Barrett 2013) or traits that favour high dispersal, growth rates, and fecundity (Moracova et al. 2015). Climate change and increasingly globalized trade are expected to introduce an even greater number of species into new territories, amplifying the chances for new invasive species to emerge in the future (Walther et al. 2009, Seebens et al. 2018).

The invasive knotweed complex

The invasive knotweed complex is a group of plants introduced to North America from eastern Asia comprised of three closely-related species of large rhizomatous perennial herbs in the Family Polygonaceae (Grevstad et al. 2018). The most notorious of the invasive knotweeds is Japanese knotweed (*Fallopia japonica* Houtt.), known by common names such as Mexican bamboo, false bamboo, Japanese bamboo, and donkey rhubarb (Kabat et al. 2006). Also included
in the complex are giant knotweed (*Fallopia sachalinensis* Ronse Decr. 1988) and a hybrid between giant and Japanese knotweed, Bohemian knotweed (*Fallopia x bohemica* Chrtek & Chrková) (Grevstad et al. 2018). The knotweed complex is also synonymized as *Polygonum* or *Reynoutria* (Beerling et al. 1994), and sometimes the morphologically similar Himalayan knotweed (*Persicaria wallichii* Greuter and Burdet), found in a sister clade to the three other species, is included in this invasive knotweed complex (Sanchez et al. 2011).

In this thesis, the invasive knotweed complex will be defined as only including *F. japonica*, *F. sachalinensis*, and *F. x bohemica*. All three species can reach 3-4m in height and form dense clusters of thick hollow bamboo-like stems with a papery ochrea or sheath around the stem nodes (Freeman and Reveal 2005). *Fallopia sachalinensis* has large (15-30 cm x 7-25 cm), heart-shaped leaves while *F. japonica* has much smaller (5-12 cm x 5-8 cm) leaves with truncate bases (Freeman and Reveal 2005). Bohemian knotweed (*Fallopia x bohemica*) has leaves showing intermediate characteristics of both parental plants (Freeman and Reveal 2005).

Japanese knotweed was originally imported to Europe from Japan to be sold as an ornamental during the 1840s; later it was exported to North America in the 1860s-70s by horticultural enthusiasts (Del Tredici 2017). Giant knotweed was presumably introduced via a similar path as there is evidence of the plant’s use in gardens from the same period (Del Tredici 2017). Since it was first introduced, Japanese knotweed has been allowed to proliferate across countries and borders, disrupting and overtaking a variety of ecosystems. Widespread dispersal has been aided in a large part by commercial nurseries selling Japanese knotweed in the 1850s, which was then compounded through the sharing of cuttings and disposal of rhizomes in the soil (Bailey & Conolly 2000). In particular, Japanese knotweed has garnered attention for its aggressive growth and inextirpable qualities, causing the plant to be listed as one of the world’s worst invasive alien species (ISSG 2013).

Knotweed invasions are present in many parts of Europe, Australia, and New Zealand. In the USA, invasive knotweeds are found in 41 states and recognized as a noxious or prohibited species in eight states (USDA-NCRS 2018). In Canada, invasive knotweeds are known to be present in all provinces, with the possible exception of Saskatchewan (USDA-NCRS 2018). Favourable, warm and humid climates make southern Ontario and British Colombia the most
vulnerable provinces to knotweed invasion and spread. Currently, B.C. has the largest known knotweed infestation in the country with an estimated 50% of the suitable area in the province already invaded (Bourchier and Van Hezewijk 2010). *Fallopia japonica* is present in the majority of southern Ontario municipalities (EDDMapS: Japanese Knotweed. 2018), but the actual extent of the infestation is unknown. Potentially over 50% of the total land area in southern Ontario is climatically suitable for knotweed establishment and the size of the invasion is expected to continue to grow (Bourchier and Van Hezewijk 2010).

Invasive knotweeds are gynodioecious, occurring as both a hermaphrodite and a male sterile form in their native ranges (Bailey 2013). Within the United Kingdom, all *F. japonica* are thought to be a single clonal copy of one male-sterile (female) plant that has largely spread vegetatively (Hollingsworth and Bailey 2000). A large proportion of the *F. japonica* in North America also appears to be the same male-sterile clone (Gaskin et al. 2014), but with greater genetic diversity found with some producing viable seed (Grimsby and Kesseli 2010). The most likely origin of this variation is due to a separate introduction of a hermaphroditic *F. japonica*, introduced around the same time period (Del Tredici 2017). *Fallopia sachalinensis* occurs at a higher rate in North America than Europe, and exists in hermaphroditic and female forms, but low genetic diversity suggests one of its main mechanisms of spread is also through vegetative propagules (Gaskin et al. 2014). While initially considered uncommon in North America, there is growing recognition that *F. x bohemica* is widely present; i.e. one study sampling invasive knotweeds from Oregon, Washington State, and B.C. found the majority of sampled plants were bohemian knotweed, attributing success to its ability to spread vegetatively and by seed (Gaskin et al. 2014). *Fallopia x bohemica* is also the most genetically diverse taxon due to its ability to backcross with both *F. japonica* and *F. sachalinensis*, as well as to intercross with itself (Gammon et al. 2007).

Within its home range, *F. japonica* is an early colonizer of volcanic slopes and is a common species in herbaceous communities (Beerling et al. 1994), however in their invaded territories, invasive knotweeds are known to colonize a wider variety of habitats, including disturbed areas, riparian areas, and forest margins (Beerling et al. 1994). *Fallopia japonica* has also been shown to be more vigorous, grow taller, and have a greater leaf area in their invaded range compared to their native range (Maurel et al. 2013).
Ecological impacts from invasive knotweeds have been studied across a variety of phyla. In riparian areas, stands of *F. japonica* are thought to promote animal habitat generalists to the exclusion of habitat specialists; the latter requiring specific niche conditions not met by *F. japonica* (Serniak et al. 2017). One of the consequences of this habitat shift is that invasion by knotweed has been shown to lead to declines in invertebrate biodiversity (Kappes et al. 2007, Gerber et al. 2008, Topp et al. 2008, Stoll et al. 2012). Conversely, there are species that benefit from the introduction of invasive knotweeds, including invertebrate detrivores and fungi, which are supported by the uncommonly high production of dense leaf litter in knotweed stands (Lavoie 2017). Thus, it appears that knotweed patches may be fundamentally altering the structure of an invaded ecosystem by initiating a trophic shift from one based on primary producers to detrivores (Kappes et al. 2007).

The ability of invasive knotweeds to affect ecosystem structure and function also extends to the overall plant community. Due to the height of its growth and the number of stems produced, the plant forms a dense canopy excluding most other plant species (Beerling et al. 1994, Siemens and Blossey 2007). Long, linear tracts of homogenous knotweed stands are common in invaded riparian areas; established stands have been observed covering areas over 500 m² in the U.K. (Beerling et al. 1994). This competitive exclusion is augmented further by the high leaf litter density, which itself can smother other seedlings and alter soil composition through the addition of organic matter and a resulting thicker A-horizon (Maurel et al. 2010). In addition, the increased leaf litter in knotweed stands is of lower quality, having a much greater carbon to nitrogen ratio than for native woody species (Urgenson et al. 2009). Invasive knotweeds have also been shown to competitively exclude other plant species through allelopathy (Siemens and Blossey 2007, Murrell et al. 2011, Dommanget et al. 2013), however, the magnitude of this effect has recently come into question when comparing results to controlled experiments in the field (Parepa and Bossdorf 2016). The ability of knotweeds to outcompete established native plants in invaded ranges prevents new recruitment and subsequently homogenizes the ecosystem. This process has been observed in forest understories where *F. japonica* has become the dominant understory species, preventing the recruitment of new tree saplings (Wilson et al. 2017).
Socio-economic impacts of invasive knotweeds have also been documented. Knotweed in riparian areas can erode banks, clog drainage systems, and block access to waterways for recreational purposes (Payne and Hoxley 2012). In particular, Japanese knotweed has a very high profile in the U.K. where it has been the target of extensive new reports which have raised public concern. Reports of the cost and difficulty of removing Japanese knotweed, compounded by the potential for its roots to damage foundations and infrastructure, has resulted in much publicized cases of plummeting home prices for infested properties (Payne and Hoxley 2012). The concern over the possibility of property damage has even led to mortgage refusals for those looking to buy knotweed-infested homes (Payne and Hoxley 2012, Robinson et al. 2016). Costs for knotweed removal to the U.K. economy are estimated at £166 million per year (i.e. ~$294 million CDN) (DEFRA, 2015), averaging £50 to £200 (~$90-$350 CDN) per square metre (Williams et al. 2010). Given these social costs, the previously described ecological costs to native species, and the potential area for spread in B.C. and Ontario, it is expected that current and future impacts from invasive knotweeds will be considerable.

**Challenges of knotweed management**

It is the regenerative ability of knotweeds that makes invasive knotweeds particularly pernicious weeds (i.e. unwanted plants). New plants regrow from sections of rhizome as small as 0.7g (Brock and Wade 1992), but they can also root and establish through stem cuttings (De Waal 2001). *Fallopia japonica* is able to re-shoot after multiple stem cuttings throughout the growing season with as many as four cuts in a single year being required to cause a net depletion of below-ground biomass in the rhizome (Seiger and Merchant 1997). Completely depleting nutrient stores within the rhizome can take multiple years of dedicated stem removal. Digging the plant runs into difficulties as completely removing the rhizome is challenging and managing the removed materials need to be handled carefully to prevent further propagation (McHugh 2006).

The use of herbicides in managing invasive knotweeds has met similar difficulties as mechanical control. Foliar application of herbicides such as glyphosate, imazapyr, and synthetic auxins have been shown to be effective at reducing knotweed stems in the short term but have had difficulty affecting nutrient stores in the rhizome, which in turn allows for regrowth (Bashtanova et al.
Multiple consecutive years and a concentrated effort are needed to fully eliminate knotweed patches with herbicides alone (Hagen and Dunwiddie 2008, Rudenko and Hulting 2010). Furthermore, herbicide application suffers from logistical constraints such as broadcast spraying, which is indiscriminate and can affect non-target species, while stem injection is costly and limited by stem size (Hagen and Dunwiddie 2008, Payne and Hoxley 2012).

There is a large disparity between the amount of herbivory knotweeds experience in their invaded range compared to their native range (Krebs et al. 2010). In fact, escape from enemies in their invaded range has been given as one explanation for their success and proliferation here in North America (Krebs et al. 2010). The lack of predators in introduced habitats (Grevstad et al. 2012), the difficulty of chemical and mechanical control, the widespread availability of additional suitable habitats (Barney et al. 2006, Bourchier and Van Hezewijk 2010), and the potential for current and future damage has made invasive knotweeds an excellent candidate to investigate another management option, classical biological control.

Classical biological control (biocontrol) entails the use of host-specific, co-evolved natural enemies from the native range of an invader to suppress and help manage their populations in the new region (Messing and Brodeur 2018). Biocontrol agents used to control invasive plants have a history of success in reducing target plant size, reproductive ability, and density (Stiling and Cornelissen 2004, Clewley et al. 2012). These agents can be a highly cost-effective measure to control weeds with estimates of benefit:cost ratios for successful agents ranging from 2:1 to 4000:1; this compares to returns of approximately 3:1 for many chemical or mechanical programs (Culliney 2005).

**Biological control using the psyllid *Aphalara itadori* Shinji**

The psyllid *Aphalara itadori* Shinji (Aphalaridae: Aphalarinae) is a classical biological control agent being studied for the management of invasive knotweeds in Europe and has recently been approved for release in Canada. Psyllids (Hemiptera: Sternorrhyncha: Psylloidea) are an insect superfamily of more than 3800 species closely related to whiteflies and aphids (Burckhardt and Ouvrard 2012). They are characterized by their saltatorial hind legs (from which their common name of “jumping plant lice” is derived) and feed by inserting sucking mouthparts into plant phloem tissue where they inject saliva (often containing bacterial endosymbionts) to improve the

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nutritional composition of ingested sap (Thao et al. 2000). Feeding results in excess sugar and water excreted as honeydew or as a waxy secretion called “lerp” by psyllids adults and nymphs.

The vast majority of psyllids have highly host-specific relationships with perennial dicots (Hodkinson 2009) and are found throughout tropical, subtropical, and temperate regions around the world (Ouvrard 2018). Psyllids reproduce sexually and their life cycle is comprised of eggs, followed by five nympha instars and a winged adult (Hodkinson 2009). Several species are important agricultural pests, including the citrus psyllid *Diaphorina citri* Kuwayama, which causes citrus greening disease (Huanglongbing) through the transmission of the *Candidatus Liberibacter asiaticus* bacterium (Grafton-Cardwell et al. 2013), and the potato psyllid, *Bactericera cockerelli* Sulc, which causes Zebra chip disease by damaging solanaceous crops through direct feeding and transmission of the bacterial pathogen *Candidatus Liberibacter psyllaurous* (Butler and Trumble 2012).

*Aphlara itadori* is native to Japan, Korea, and the Kurile and Sakhalin Islands (Burckhardt and Lauterer 1997) and was first described in 1938 where it was originally placed in the genus *Psylla* (Shinji 1938); it was later moved to *Aphalara* by Sasaki (1954) (Ouvrard, 2018). The psyllid completes its entire development and feeding on all life stages of knotweeds, namely *Fallopia japonica*, *Fallopia sachalinensis*, and *Fallopia x bohemica*.

Adult *A. itadori* are ~ 2-2.5 mm in length from head to wing tip, with a female-biased size dimorphism, where females are ~ 0.5 mm longer than males. Sexing adult *A. itadori* can be done under low magnification using male and female genitalia. In females, there is an obvious sword-like ovipositor on an elongated abdomen while males have a shorter abdomen with a pair of parameres extending beyond a rounded sub-genital plate. Both males and females are similar in appearance with light brown bodies and semi-transparent wings that have well-defined dark brown patches (Fig. 1). Females have a lifetime fecundity of ~ 600-700 eggs over a 30 to 40-day production period (Shaw et al. 2009). Complete development from egg to adult takes between 28-42 days, with a mean ± SE of 32.9±0.8 days at 22±1.5 °C (Shaw et al. 2009). All life stages feed on phloem sap with the greatest amount of feeding occurring during the nymphal stage. Nymphs are free-living and can relocate to parts of the plant that facilitate feeding such as soft, newly-emerging leaves and new stems.
Psyllids in the *Aphalara* genus overwinter as an adult in diapause by relocating to an overwintering shelter plant or conifer bark and leaf litter in autumn (Hodkinson 2009). We expect *A. itadori* to use a similar strategy in Canada and in field trials near Lethbridge, AB the psyllid has successfully overwintered surrounded by mulch chips, albeit at relatively low numbers.

Two distinct biotypes of *A. itadori* are known to exist on *Fallopia* host plants, varying in their developmental success and reproductive potential. One such population of *A. itadori*, sourced from Hokkaido in northern Japan, readily lays eggs on all three invasive knotweeds, but can only reach adulthood on *F. sachalinensis* (Grevstad et al. 2013). Conversely, a second biotype, one sourced from Kyushu in southern Japan, readily oviposits and successfully develops on all three knotweeds; however, the developmental success of the Kyushu psyllid on *F. sachalinensis* is much more variable than that of the Hokkaido biotype. Lab testing has shown that both biotypes are able to halt the growth of knotweed, reducing above- and below-ground biomass by more than 50% in a 50-day period on their preferred knotweed hosts (Grevstad et al. 2013). A single nucleotide polymorphism (SNP) array for each biotype has been developed to accurately identify populations post-release (Andersen et al. 2016). It is suggested that both Hokkaido and Kyushu populations may be required to control invasive knotweed populations in release programs (Grevstad et al. 2013).

Prior to approval of release in the UK and Canada, host-range testing was conducted on a wide variety of plant species native to North America and Europe, including important agricultural
and ornamental species closely related to invasive knotweeds from these continents. In the UK trials where *A. itadori* was given a choice of host plants to feed on, it showed a strong preference to oviposit on *F. japonica*, *F. sachalinensis*, and their hybrids (Shaw et al. 2009). It also oviposited on several non-target species during these trials, however in the majority of cases, the total number of eggs laid on the latter was a fraction of those laid on the invasive knotweeds, and none of the eggs developed through to adulthood on the non-knotweed hosts (Shaw et al. 2009). Given the weak developmental performance, high adult mortality, and the low ornamental importance of the non-target plant species, the potential impact of *A. itadori* on non-target plants was considered negligible, and the southern biotype was approved for release in the UK during 2010 (Pratt et al. 2013).

Further *A. itadori* host-specificity testing was completed for North American flora that added 70 plant species and varieties, including rare and at-risk species, that were identified by a USDA-APHIS Technical Advisory Group on biological control of weeds (Grevstad et al. 2012). North American species were chosen based on their relatedness to the three *Fallopia* spp., the majority being from the same family (Polygonaceae) (Grevstad et al. 2012). Further testing was done against three plant species identified as potential marginal hosts, including an introduced groundcover (*Muehlenbeckia axillaris*), a native vine (*Fallopia cilinodis*), and the agricultural crop buckwheat (*Fagopyrum esculentum*) (Grevstad et al. 2013). Both psyllid biotypes laid only minimally on *F. cilinodis* and *F. esculentum*, and neither could sustain themselves beyond the first generation. Only the southern biotype (Kyushu) was able to produce multiple generations on *M. axillaris*, however this line died out and psyllid damage on this plant was found to be minimal compared to that caused by common garden pests such as aphids, scale, and spider mites. In choice experiments, both psyllid biotypes laid > 90% of their eggs on *F. japonica* or *F. sachalinensis* compared to the three non-target plants suggesting that there would only be a very low risk of releasing either *A. itadori* biotype in North America as summarized in the 2012 petition (Grevstad et al. 2012). Based on this petition, and a comprehensive review of the risk data, the Canadian Food Inspection Agency (CFIA) approved *Aphalara itadori* for cage release to control invasive knotweeds in Canada during 2014, and then in open releases during the summer of 2016. With this approval, research focus has recently shifted to the introduction and establishment of *A. itadori* populations on invasive knotweeds under field conditions.
Analysis on climate matching for *A. itadori* sourced from Japan has shown that the closest climatic regions in Canada are found in coastal and interior B.C., southern Ontario, Quebec, and parts of Atlantic Canada (Fig. 2). Given that B.C. and Ontario have the greatest current distribution of invasive knotweeds and are at the highest risk for further spread based on the favourable climate match (Bourchier and Van Hezewijk, 2010), these provinces have been given priority as targets for knotweed releases in Canada.

Caged field releases of the Kyushu *A. itadori* strain have been ongoing since 2014 near Nelson, BC, Ottawa, ON, and in Lethbridge, AB. In releases thus far, populations have only established at very low numbers or failed to establish at all. Work here has shown that the psyllids will indiscriminately lay large numbers of eggs on *F. japonica* in the field, however the overwhelming majority of the eggs fail to reach adulthood. Dead 1st- and 2nd-instar nymphs are often found in dissections of field plants, and <10% of potential adults are found five days post-release (Fig. 3). Few individual adults seem to successfully overwinter in Canada with only those released in a diapause state managing to survive. Similar trends are seen in the releases sites in the UK where individual adults are seen to overwinter but fail to establish as permanent populations (Pratt et al. 2013).

**Establishment of classical biological control agents**

Despite the successful establishment of many biocontrol agents, population establishment rates for agents can be low (Zalucki and van Klinken 2006, Fowler et al. 2008). One of the key questions in understanding establishment for any biocontrol agent is in finding an optimal release size (Memmott et al. 1998, Grevstad 1999, Shea and Possingham 2000). In the case of *A. itadori*, the rearing of large numbers of the insect has been relatively easy and resulted in numerous releases with several thousand individuals. Thus, it is unlikely that release densities can explain the low rate of establishment in this system.

Allee effects (i.e. difficulty in mate finding) are sometimes identified as a potential barrier to establishment of biocontrol agents (Hopper and Routh 1993, Grevstad 1999, Fowler et al. 2008), however, this is also an unlikely explanation for the poor establishment of *A. itadori* as when
Figure 2. Climate match for the Kyushu (southern) and Hokkaido (northern) biotypes of *Aphalara itadori* in North America based on climate from their source location in Japan. Red regions indicate a stronger match to the climate where source populations are found. Climate matching analysis done with Climex ® “match climates” analysis (adapted from Grevstad et al. 2012).

Figure 3. Number of adult *Aphalara itadori* located during a caged release field trial in Lethbridge, Alberta during the summer 2016. On 16 June, ~ 2500 laboratory-reared adults were released and another 460 adults were released on 20 July from a laboratory colony.
caged in the field, adults aggregate and lay large numbers of eggs on the plants. Predation in introduced territories may also impede biocontrol population establishment (Zalucki and Van Klinken 2006). Due to the Asiatic origin of *A. itadori*, it is unlikely that there will be high rates of predation or parasitism for populations of *A. itadori* in Canada and neither has been observed thus far in field releases.

A more likely cause for low establishment rates of psyllid populations is their maladaptation to field conditions, or rather their adaptation to captive conditions. Classical biocontrol agents usually have spent many generations in the laboratory before they are approved for release as they are tested against a range of potential non-target hosts in the lengthy approval process. In the case of *A. itadori*, the Canadian approval process took approximately 7 years, and the Kyushu population originally collected in 2004 had been reared in the UK before a sample of this population was then brought to Canada in 2007. Such long-term laboratory rearing encourages selection for survival under static laboratory conditions which may leave agents maladapted to the stochastic field conditions they will experience upon release (Nunney 2003, Woodworth et al. 2002). This maladaptation is made even worse by the low genetic variability usually present in these typically small populations used to start the original colonies (Nunney 2003). The captive population of *A. itadori* currently being reared at Agriculture and Agri-Food Canada (AAFC) was started from a population of < 100 individuals and reared for over a decade under laboratory conditions. As well, there have been several near crashes in populations during this time resulting in potential genetic bottlenecks. The result is a strong likelihood that the difficulties observed establishing *A. itadori* population in the field can be attributed to laboratory adaptation, exacerbated by a lack of genetic variability.

Rearing techniques to avoid genetic issues in biological control agents have been proposed, with recommendations that include; maintaining multiple, separate lines, rearing fewer laboratory generations, mimicking field conditions in the lab, maintaining high populations to avoid genetic bottlenecks, and sourcing new agents from the field (Nunney 2003, Fowler et al. 2008). Unfortunately, these techniques to conserve genetic diversity run into logistical constraints due to limited rearing space, the potentially decade-long release approval process, the difficulty of collecting replacement agents from the field, and population crashes from stochastic events. To address laboratory adaptation for *A. itadori*, a new population would have to be sourced from
Japan with its associated costs and importation difficulties. This is made even more difficult as *A. itadori* is uncommon and cryptic in its home range (Grevstad et al. 2012). Further, using a known captive colony of biocontrol agents is desirable to minimize risks because it is of known taxonomic identity, genetic history, and is pest and disease free (De Clerck-Floate et al. 2014).

Given these barriers, it is unlikely that the potential genetic paucity of the captive population of *A. itadori* can be easily addressed. However, it may still be possible to influence traits in the current laboratory population to improve establishment in the field. One unexplored possibility to help improve captive populations of biocontrol agents having difficulties establishing in the field is to explore their inherent capacity for adaptive phenotypic plasticity.

**The role of phenotypic plasticity**

Phenotypic plasticity is the ability of an organism’s genotype to express a different phenotype based on the environmental conditions it has been exposed to (Pigliucci et al. 2006) and includes the modification of an organism’s developmental path so that it can adapt to its environment. Such “developmental plasticity”, as termed by Nettle and Bateson (2015), may be influenced by both maternal and latent effects, where “maternal effects” are phenotypic changes that a mother passes onto her offspring based on her experiences and “latent effects” are experiences at earlier stages of development that alter phenotype later in life (O’Connor et al. 2014). There is growing evidence that traits expressed early in life as a result of environmental conditions can last multiple generations and play a role in conditioning organisms to succeed in future environmental conditions (Jablonka et al. 1995, Connor et al. 2014). Because these forms of developmental plasticity can prepare an organism for its future habitat, they may also be important for determining a specie’s range expansion.

Laboratory experiments have shown that populations of flour beetles (*Tribolium castaneum* Herbst) exposed to low quality food as larvae performed better on low quality food as adults compared to larvae given high quality food that resulted in better adult performance on high quality food (Van Allen and Rudolf 2013). The effects on the carrying capacities in these populations of flour beetles was observed over several generations of the experiment (Van Allen and Rudolf 2013) suggesting that larval food conditions can lead to a phenotypic change promoting the colonization of different adult habitats. There is similar evidence from female
western bluebirds (*Sialia Mexicana* Swainson) where mothers developing under poor resource conditions lead to dispersing phenotypes in their offspring; this may account for the recent large range expansion of this species (Duckworth 2009). Such studies demonstrate the clear impact of developmental plasticity and explain how it can lead to the colonization of new environments by promoting phenotypes selected for specific environments. Unfortunately, there is a dearth of studies examining this phenomenon, and even fewer studies assessing its application in the field.

Classical biological control agents are organisms that are absent from the territories where they are being introduced. In this respect, biocontrol agents are analogous to a species expanding its environmental range into new territory. By exposing a biocontrol agent to selected field-like conditions before it is released, improved phenotypes could be selected thereby promoting increased survival and establishment in the early critical stages of a release. Viewing a biocontrol release establishment program through the lens of adaptive phenotypic plasticity is a novel approach. Traditionally, the focus has been on maintaining population genetic diversity rather than exploring the limits already present within a population. Providing specific conditions that will condition an organism to overcome barriers in a new environment could provide the extra nudge needed to help it establish and could act as another tool in biocontrol theory.

It is important to note that not all phenotypic plasticity is adaptive, and that due to some limiting factor, an expressed phenotype may in fact be deleterious (Pigluicci et al. 2006). For example, one phenotype in a population of starlings with poor flight capacity was expressed when experiencing conditions of higher nest competition (O’Hagan et al. 2015). It is apparent then that in order for developmental plasticity to be adaptive, the phenotypic change(s) initiated by environmental cue(s) must provide a clear fitness advantage over individuals in the given environment who did not receive the same cue (Nettle and Bateson 2015).

**Research goals**

The current thesis was designed to explore the potential for exploiting phenotypic plasticity in the psyllid, *A. itadori*, in order to improve its establishment after release for classical biological control of Japanese knotweed. Of particular interest was the question whether traits that would provide a fitness advantage to *A. itadori* in its introduced range could be induced in lab rearing prior to release. In iterative laboratory experiments exposing psyllid pairs and their offspring to
rearing environments in a series of factorial experiments, I explore the impact the maternal environment on individual fitness of their offspring.

The experiments presented here use manipulated differences in knotweed host plants to determine environmental conditions that limit the success of the psyllid upon release. Mechanical damage prior to release of the psyllid could be an important component of an integrated pest management program for invasive knotweeds. If damaged knotweed plants are fatal to *A. itadori*, then it might be possible to induce psyllid resistance to the plants’ chemical defenses through manipulating its plasticity during development? Here, I explore this question by first testing the response of the psyllids to damaged and intact plants, predicting that adult psyllids exposed to mechanically damaged Japanese knotweed plants in the lab would show high mortality, possibly caused by induced defenses. Second, I test the psyllids’ responses to older and younger knotweed plants with the expectation that field-grown knotweed might present a barrier to psyllid feeding, and therefore establishment, as it is significantly taller, with larger, tougher leaves compared to plants grown in the lab and used for psyllid rearing. The question here is whether psyllids reared on tougher older plants condition their offspring for better survival on plants of the same condition.

If experimental conditions can cause a plastic developmental response in *A. itadori*, then we would predict that offspring will have greater fitness when exposed to the same conditions experienced by their parents compared to offspring of parents not exposed to the same conditions. If this prediction holds, then the release strategy for *A. itadori* could be enhanced by rearing psyllids under conditions for a generation prior to being released that maximizes their fitness and establishment after release. Standardized quality measurements for biological control agents (directly or indirectly related to fitness) include: survival rates, fecundity, development time, size, and sex ratios (Grenier and De Clerq 2003). This experimental framework will also allow us to explore how the psyllid performs when exposed to a variety of environmental conditions, and thereby allow a better understanding as to what drives *A. itadori* population dynamics in general. Finally, this research will contribute to a broader knowledge about those variables important to the establishment of biocontrol agents after long-term lab rearing and ultimately how to improve the efficacy of future biological control programs.
Materials and Methods

Plant and insect material

The Japanese knotweed, *Fallopia japonica*, used throughout the thesis was originally sourced from a wild population in Nelson BC (IAPP #112369), and was confirmed the same genotype as that dominant in the United Kingdom. *Fallopia japonica* was selected for all experiments since this species had been used in most of the previous research and field releases. The added advantage of this species is that the majority of these plants are clonal and thus, likely have reduced variability to improve testing of the biocontrol agent responses. The plants were grown in 12.5 cm-diameter pots with a modified Cornell soilless mix (see Appendix 1 for soilless mix composition). Pots were planted with 3g- to 5g-sections of Japanese knotweed rhizome split from a laboratory stock and grown under a long day length 16:8 LD cycle at 22.0°C with 32W-4100K fluorescent tube lighting.

A laboratory population of southern strain (Kyushu) *A. itadori* that had been maintained in quarantine at AAFC’s Insect Microbial Containment Facility in Lethbridge, Alberta since 2007 was used as the test agents. Insects were kept at 22°C and reared on *F. japonica* plants (grown in the same method above) in vented 0.3m x 0.3m x 0.6m-plexiglas cages.

Preliminary host quality experiments

A preliminary experiment was performed by manipulating the quality of Japanese knotweed host plants in the laboratory to measure the fitness response of *A. itadori*. The intent here was to determine the best timing for psyllid releases based on plant age and to see whether a simple pre-treatment of mechanical damage would influence psyllid performance.

Knotweed plants were subjected to three different treatments prior to being exposed to psyllids: 1) a juvenile plant grown for two weeks; 2) a mature plant grown for 6 weeks; and 3) a damaged or cut plant grown for 4 weeks and then the growth cut, removed, and allowed to regrow for 2 weeks. Six replicates were set up for each treatment. A total of 40 *A. itadori* adults were released into each cage and left for a period of 96h, after which they were removed. Eggs laid over the exposure period were counted and allowed to develop through to adulthood. It was expected that
juvenile or cut plants would act as more desirable host plants than older uncut plants due to the
greater amount of soft, newly-emerged foliage re-sprouting and available for feeding by
vulnerable early-instar nymphs. High psyllid mortality (later attributed to high psyllid densities)
here necessitated a second, identical experiment be performed, which helped to also develop a
bioassay protocol that was based on using only separate pairs of psyllids in all test designs.

**Bioassay experimental design**

All experiments following preliminary experiments were performed using the same general
framework to test the responses of individual psyllid pairs from the laboratory population on
cultivated Japanese knotweed hosts. Host plants were exposed to one of two treatments before
plants were placed individually inside 55cm x 28cm, low-density, polyethylene, wicketed bags
with 0.5 mm-holes for ventilation (Fig. 4). One adult female and two adult male *A. itadori* were
introduced to each bagged plant and allowed to mate and oviposit for 96 hours, after which they
were removed. Eggs laid on the foliage were counted and allowed to complete development to
adulthood. Once emerged, F1 adults were collected and set up in a full factorial design. One
female and two male F1 adults were placed on a newly-bagged Japanese knotweed plant, either
with the same treatment they were exposed to in their natal environment or to a new treatment.
Again, F1 adults were allowed to oviposit on individual plants for 96 hours, after which they
were removed and their offspring (F2) left to develop. Daily checks for adult emergence were
done after 4 weeks. Adults emerging from each replicate were retained sexing and morphological
measurement.

Measurements taken for each replicate in all experiments included; total number of eggs laid,
time until first observed oviposition (24h, 48h, 72h, and 96h), and morphological measurements
of the ovipositing female. Egg measurements included; proportion developing into adults, sex
ratio of emerged adults, and developmental time.

Morphological measures of the ovipositing female psyllids were used to assess their relative
fitness since previous studies have shown that larger body size is linked with greater fitness in
many insect populations (Kingsolver and Huey 2008). Morphological trait measurements were
Figure 4. Example of potted Japanese knotweed (*Fallopia japonica*) in vented polyethylene bag used as an experimental unit in bioassay experiments. Plant is approximately 8-weeks old grown from 3-5g sections of rhizome from laboratory stock at AAFC, Lethbridge, AB.

Based on Hodkinson and White (1979) and included; right forewing length, right forewing width, right forewing Rs vein length, thorax width, thorax length, and right hind leg tibial length (Fig. 5). These morphological features were chosen based on a combination of traits identified as good indicators to differentiate between populations of other psyllid species (Lashkari et al. 2012, Paris et al. 2016, Vargas-Madriz et al. 2013). Female psyllids were photographed at 100x magnification using a Dino-Lite Edge 5MP digital microscope and measured with DinoCapture 2.0 software.

Relationships for each measured variable were modelled using generation number *parent natal environment*treatment as the prediction terms to determine whether different combinations of rearing environment, host plant treatment, and overall generational trends influenced the selected trait variables. Experiments took place during a 16:8 LD light cycle, and temperature and relative humidity (RH) were recorded using a HOBO UX100 datalogger inside one cage each plant treatment.
Figure 5. Morphological measurements of *Aphalara itadori* adult females taken for all bioassay experiments. Photos show: a) right hind leg tibial length, b) right forewing length, width, and Rs vein length, and c) thorax width and length. Photographs and measurements taken with Dino-Lite Edge 5MP digital microscope.

**Experiment 1: Damaged versus intact host plants**

Induced chemical defense compounds are widespread among plants (Karban et al. 1997) and may be activated by both simulated and real herbivory (Strauss et al. 2002). It is possible that the high mortality observed in the preliminary experiments here may have been driven by the higher concentrations of defense compounds in those knotweed plants that had been damaged by greater...
 psyllid feeding. Damaged knotweed also results in more new growth of stems and leaves, and this in turn seems to be preferred by developing nymphs. Thus, the first experiment conducted was to determine whether damaged knotweed plants were fatal to *A. itadori*, and if so, whether resistance to chemical defenses could be induced as a result of psyllid developmental plasticity.

Plants were damaged by removing all stems from a rhizome in each pot except for a single sprout that itself had half of its leaves removed. Intact plants were left unaltered. The F0 psyllids from the laboratory colony were separated and one female and two males were placed onto 24 damaged and 24 intact knotweed hosts (N=48) to oviposit. Once eggs developed into F1 adults, these adults were transferred to new knotweed hosts (also either damaged or intact) to oviposit. This resulted in a 2x2 full factorial design containing the F1 psyllids’ natal habitat (damaged or intact) and the host on which they oviposited (damaged or intact). Each natal habitat and oviposition habitat combination had 10 replicates (N = 4x10).

A third set of experiments was carried out to determine whether the rearing environment of a psyllid’s grandparent (its parent’s parent) could affect its fitness. Emerging F2 adults from the eggs laid by the F1 adults were placed on a new damaged and intact plant as in the previous experiments resulting in a 2x2x2 factorial design containing F1 psyllid natal habitat (damaged, intact), F2 psyllid natal habitat (damaged, intact), and the host on which F2 adults were ovipositing (damaged, intact). This design was set to provide 48 replicates of Japanese knotweed with 6 replicates of each treatment (N= 8 combinations x6), however, due to low emergence in some treatment-natal-history combinations, an even distribution of all combinations was not possible. Models included treatment, generation, natal environment and an interaction term between treatment and natal environment to test for natal environment induced fitness changes (Table 1).

In this experiment, it was predicted that oviposition, egg-to-adult survival, and body size would be lower for psyllids feeding on damaged than undamaged knotweed hosts. However, it was also expected that reduced fitness from damaged host plants could be overcome if a F1 psyllid’s parent had also been reared on a damaged versus an undamaged plant.
Table 1. Factors and corresponding levels used in models predicting Aphalara itadori fitness responses in Experiment 1. Each F0 A. itadori used in the experiment came from the same natal environment (laboratory colony) and F1 and F2 A. itadori were reared on either damaged or intact Japanese knotweed (Fallopia japonica) plants.

<table>
<thead>
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<th>Factor</th>
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<tr>
<td>Generation</td>
<td>F0, F1, F2</td>
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<tr>
<td>Parent natal treatment</td>
<td>Colony, Damaged, Intact</td>
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<tr>
<td>Treatment</td>
<td>Damaged, Intact</td>
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Experiment 2: Juvenile versus mature host plants

Many plants allocate defenses differently throughout their ontogeny. Vulnerable early stages are expected to be more intolerant to herbivory and limited in their potential to produce mechanical defenses, but may be well chemically defended, whereas older plants may eschew chemical defenses for less costly mechanical defenses (Boege and Marquis 2005). As field samples from mature knotweed stands often return with dissected early-instar A. itadori nymphs, I would hypothesize that older Japanese knotweed plants are able to present barriers to immature psyllids.

Two knotweed treatments were used to examine this question: 1) juvenile plants grown for 3 weeks with stems recently emerging from the soil and 2) mature plants grown for 8 weeks that had larger leaves and longer stems. F0 Colony psyllids (1 female and 2 males) were placed on 22 juvenile and 22 mature plants (N=44). Once eggs developed into F1 adults, these adults were transferred to new juvenile or mature host plants for fresh foliage. The experiment was a 2x2 full factorial design with one factor being the F1 psyllids’ natal habitat (juvenile or mature host) and the other the host on which were subsequently offered for oviposition (juvenile or mature). Each natal and ovipositional habitat combination had 10 replicates for a total sample size of forty. Models included treatment, generation, natal environment and an interaction term between treatment and natal environment to test for natal environment induced fitness changes (Table 2).

Here, I predicted that oviposition, egg-to-adult survival, and body size from psyllids on mature knotweed hosts would all be lower than on juvenile plants, but that the reduced fitness expected
on the mature host plants could be overcome when a psyllid’s parent was reared on a damaged plant.

Table 2. Factors and corresponding levels used in models predicting *Aphalara itadori* fitness responses in Experiment 2. Each F0 *A. itadori* used in the experiment came from the same natal environment (laboratory colony) and F1 *A. itadori* were reared on either juvenile or mature Japanese knotweed (*Fallopia japonica*) plants. Psyllids from each generation and natal treatment were exposed to either juvenile or mature knotweed resulting in six possible generation*parent natal treatment*treatment combinations.

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<td>Generation</td>
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<tr>
<td>Parent natal treatment</td>
<td>Colony  Juvenile  Mature</td>
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<td>Treatment</td>
<td>Juvenile  Mature</td>
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**Experiment 3: Hardened leaves**

Knotweed plants that were four weeks old were placed in a greenhouse for an additional four weeks to induce leaf hardening (increased sclerophyll). These were compared to a 2nd cohort of knotweed plants that were grown for 8 weeks in the laboratory. Increased sunlight penetrating the greenhouse glass increases the exposure of plants to UV-B radiation and this hardens leaves by thickening the cuticle and epidermal layer (Jansen et al. 1998). Leaf-mass-area (dry leaf mass/leaf area) was used to determine differences between leaf hardness in the different plant treatments. Leaf-mass-area is known to correlate strongly with other mechanical properties related to herbivory resistance, such as leaf hardness and thus can be used as a surrogate for them (Hanley et al. 2007). Leaf area was calculated with Easy Leaf Area V2 software (Easlon and Bloom 2014) and then the leaves were dried in a drying oven for 72h at 70°C before weighing dry mass. Leaf-mass-area in the greenhouse plants increased by a mean of 27% compared to plants grown in our typical laboratory conditions.

The performance of F2 psyllid adults resulting from the mature knotweed in Experiment 2 were used to compare to that of the standard colony psyllids (F0) reared on hardened and unhardened knotweed plants. This resulted in a 2x2 full factorial design containing psyllid natal habitat (colony vs mature knotweed plant) and the host on which they were subsequently offered for
oviposition (hardened vs unhardened) (Table 3). Each natal habitat and ovipositional habitat combination had 12 replicates for a total N=48.

Table 3. Factors and corresponding levels used in models predicting Aphalara itadori fitness responses in Experiment 3. Each F0 A. itadori used in the experiment came from the same natal environment (laboratory colony) and F2 A. itadori were reared on mature Japanese knotweed (Fallopia japonica) plants from Experiment 2. Psyllids from each generation and natal treatment were exposed to either hardened or unhardened knotweed resulting in four parent natal treatment*treatment combinations.

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<td>Parent natal treatment</td>
<td>Colony, Mature</td>
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<tr>
<td>Treatment</td>
<td>Hardened, Unhardened</td>
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In this experiment, I expected to see lower oviposition, egg-to-adult survival, and body size for psyllids reared on hardened knotweed hosts than on hosts from unhardened plants, and that the reduced fitness for those reared on hardened host plants could be overcome by F2 psyllids reared on tough, mature plants.

Experiment 4: Psyllid density manipulation

Patterns in the data were not adequately explained by the insect treatments and individual habitat history based on the three previous bioassay experiments. These experiments showed a clear increase in body size and total number of eggs for psyllids reared one generation in the bagged experiments compared to those reared in colonies. Rearing density was substantially lower in the bagged bioassay than in colonies suggesting that access to competition may be driving body size. Thus, in this experiment the number of psyllids introduced into the bagged experimental units was manipulated to determine whether psyllid population densities would affect select biological parameters.

The bioassay framework was the same as the previous experiments except the number of psyllids introduced to individual bags was manipulated. Treatments were: 1) 1 female:2 males, 2) 5 females:5 males, and 3) 10 females:10 males. All psyllids were sourced from the laboratory colony population of A. itadori with each treatment replicated 20 times (N=60). In addition to the variables measured in Experiments 1, 2, and 3, ovipositional preference was recorded based on:
1) the number of eggs laid according to leaf size, 2) whether oviposition occurred on the upper or lower half of the host, and 3) for plant structure (leaf top, leaf bottom, stem nodes). Leaf size was separated into small (<2.5 cm at base), medium (2.5-5 cm at base), and large (>5 cm). All knotweed hosts were grown for 4 weeks. Consistent with the definition by Craig et al. (1989), oviposition preference is defined in this thesis as non-random oviposition on host plants where preferred oviposition sites are those that experience higher numbers of eggs oviposited.

**Statistical analysis**

All statistical analyses were performed in R version 3.3.0. Egg counts were analyzed in each experiment for significant differences between treatments and natal histories using generalized linear models (GLMs) with a Poisson error distribution and log link function. If data were found to be over-dispersed a quasi-Poisson distribution was used instead. Where significant results were found, multiple comparisons were done using the general linear hypotheses (glht) function from the multcomp package in R (Hothorn et al. 2017) with a Tukey’s HSD. Significant differences between treatments and natal histories for sex ratio and egg-to-adult success were analyzed using GLMs with a binomial error distribution and logit link function. If data were found to be over-dispersed, a generalized linear mixed model (GLMM) was corrected with a randomly generated intercept as outlined in Warton and Hui (2011). GLMMs were generated using the glmer function from the lme4 package in R (Bates et al. 2009). Morphological measurements and developmental times were compared using a univariate analysis of variance (ANOVA) between treatments and natal histories. Where significant differences were found, post-hoc comparisons done using Tukey’s HSD.

Ovipositional preferences for each density were examined in the psyllid density manipulation experiment. A Chi-square test was used to compare egg distributions to expected distributions. The expected distributions of eggs were pooled across all replicates at each density (i.e. one, five and ten females). Expected distributions for leaf size data were calculated as the number of leaves from each leaf size/total number of leaves, while expected vertical distribution was assumed to be equal for both the upper and lower half of the knotweed host plant (0.5). Expected distributions for plant structure preferences (i.e. leaf abaxial, leaf adaxial, or stem node) were based on ovipositional preferences recorded from Experiments 1, 2, and 3.
Based on the results from all experiments, I modelled variation in egg counts to determine which factors were driving the observed differences. Experiments were modelled both individually and with all data pooled to examine overarching trends. Selection for the egg count model used Quasi-AIC (QAIC) from the MuMIn package in R (Barton 2018) with a quasi-Poisson error distribution to account for over-dispersed data. Explanatory variables included treatment, developmental % RH, developmental temperature, psyllid age, and body size.
Results

Preliminary host quality tests

In the initial experiment, adult psyllids introduced onto knotweed host plants experienced significantly higher mortality during the 4-day oviposition period on juvenile and damaged plant treatments, compared to mature plant treatments (GLM, mature plant mortality, z=-6.271, df=18, p<0.001) (Fig. 6). A follow-up experiment with the same treatments, but also controlling for psyllid age, was conducted and the same pattern was seen with significantly more adults dying on damaged and juvenile plants during the oviposition period than on mature plants (GLM, mature plant mortality, z=-8.766, df=18, p<0.001) (Fig. 6). The proportion of eggs successfully developing into adults was significantly higher on mature plants in the initial experiment than in juvenile or cut treatments (GLM, mature plant egg-to-adult success, z=8.527, df=16, p<0.001), but was significantly lower in the second experiment than both juvenile and cut treatments (GLM, mature plant egg-to-adult success, z=-9.267, df=16, p<0.001) (Fig. 7). A heavy infestation of thrips in the second experiment may have contributed to the low psyllid emergence.

High adult mortality on cut and juvenile host plant treatments in both preliminary experiments led to questions whether results were driven by the host plant treatments. Thus, the bioassay using individual bagged plants was developed to address this high mortality by dramatically reducing psyllid density and exposing the insects to hosts with similar age and condition.

Experiment 1: Damaged versus intact plants

Oviposition – The number of eggs laid increased significantly in both the F1 and F2 generations compared to the F0 generation (laboratory colony) of psyllids (Table 4, Fig. 8). There was no significant difference between the number of eggs laid by F1 and F2 females (z= -0.484, df=126, p>0.05), nor did the damaged and intact plant treatments have a significant impact on oviposition. There was no significant interaction between treatment and natal environment (p>0.05).
Figure 6. Mean mortality of adult *Aphalara itadori* (+/- SE) when placed on either juvenile, cut, or mature Japanese knotweed (*Fallopia japonica*) treatments in preliminary host quality experiments 1 and 2. Letters beside each point indicate significant differences at P<0.05 between treatments within each experiment according to Tukey’s HSD.

Figure 7. Proportion of *Aphalara itadori* eggs successfully developing to adults placed on either juvenile, cut, or mature Japanese knotweed (*Fallopia japonica*) treatments in preliminary host quality experiments 1 and 2. Letters beside each point indicate significant differences at P<0.05 between treatments within each experiment according to Tukey’s HSD.
Similarly, psyllid natal history had no significant impact on the number of eggs she oviposited (Table 4). The percentage of eggs that successfully survived until adulthood was higher in F0 eggs (53.0 ± 4.4%) than in F1 eggs (42.0 ± 5.0%) or F2 eggs (37.2 ± 3.7%), however no significant differences were found (Table 5). There was also no significant difference in egg-to-adult success when accounting for both natal history and host plant treatment as well as their interaction term (p>0.05).

**Table 4.** GLM results of Experiment 1 quasipoisson model testing whether *Aphalara itadori* oviposition is affected by host plant treatment, generation number, natal environment and the interaction term between treatment and natal environment. Asterisk indicates significance of P<0.05.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Levels</th>
<th>Coefficient</th>
<th>SE</th>
<th>t</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>Intact</td>
<td>-0.082</td>
<td>0.237</td>
<td>-0.347</td>
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<td>0.729</td>
</tr>
<tr>
<td>Generation</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>135</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>0.919</td>
<td>0.304</td>
<td>3.023</td>
<td>135</td>
<td>0.003*</td>
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<tr>
<td></td>
<td>F2</td>
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<td>135</td>
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<td>-</td>
<td>-</td>
<td>135</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Intact</td>
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<td>0.229</td>
<td>-0.197</td>
<td>135</td>
<td>0.844</td>
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<tr>
<td>Treatment*Natal env</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>135</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Intact*Colony</td>
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<td>-0.413</td>
<td>135</td>
<td>0.681</td>
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<tr>
<td></td>
<td>Intact*Intact</td>
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<td>0.35</td>
<td>-0.986</td>
<td>135</td>
<td>0.326</td>
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</tbody>
</table>

**Sex ratio** – Significantly more males than females emerged from F0 eggs (50.0 ± 0.06 %) than F2 eggs (38.1 ±% (Table 6), but host plant treatments and parental natal environment had no significant impact on sex ratios and no significant interactions were found between the two terms (GLMM, z=0.370, df=75, 0.711).
Table 5. GLMM results of Experiment 1 binomial model testing whether *Aphalara itadori* egg-to-adult success is affected by host plant treatment, generation number, natal environment and the interaction term between treatment and natal environment. Significance of P<0.05 used.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Levels</th>
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<th>z</th>
<th>df</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Treatment</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>117</td>
<td>-</td>
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<tr>
<td></td>
<td>Intact</td>
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<td>0.078</td>
</tr>
<tr>
<td>Generation</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>117</td>
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</tr>
<tr>
<td></td>
<td>F1</td>
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<tr>
<td></td>
<td>F2</td>
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<td>1.602</td>
<td>117</td>
<td>0.109</td>
</tr>
<tr>
<td>Natal Env</td>
<td>Damaged</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>117</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Intact</td>
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<td>0.425</td>
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<td>117</td>
<td>0.544</td>
</tr>
<tr>
<td>Treatment*Natal Env</td>
<td>Damaged*Damaged</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>117</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Intact*Colony</td>
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<td>0.954</td>
<td>117</td>
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</tr>
<tr>
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<td>0.627</td>
<td>1.650</td>
<td>117</td>
<td>0.099</td>
</tr>
</tbody>
</table>

Table 6. GLMM results of Experiment 1 binomial model testing whether *Aphalara itadori* sex ratio is affected by host plant treatment, generation number, natal environment and the interaction term between treatment and natal environment. Asterisk indicates significance of P<0.05.

<table>
<thead>
<tr>
<th>Factor</th>
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<th>df</th>
<th>p</th>
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</thead>
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<td>Treatment</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>117</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Intact</td>
<td>-0.114</td>
<td>0.185</td>
<td>-0.614</td>
<td>117</td>
<td>0.539</td>
</tr>
<tr>
<td>Generation</td>
<td>F0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>117</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>-0.411</td>
<td>0.226</td>
<td>-1.811</td>
<td>117</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>-0.504</td>
<td>0.222</td>
<td>-2.272</td>
<td>117</td>
<td>0.023*</td>
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<td>Natal Env</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>Intact</td>
<td>-0.374</td>
<td>0.194</td>
<td>-1.929</td>
<td>117</td>
<td>0.054</td>
</tr>
<tr>
<td>Treatment*Natal Env</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>117</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Intact*Colony</td>
<td>0.114</td>
<td>0.301</td>
<td>0.379</td>
<td>117</td>
<td>0.705</td>
</tr>
<tr>
<td></td>
<td>Intact*Intact</td>
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<td>0.279</td>
<td>1.01</td>
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<td>0.314</td>
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Figure 8. Mean number of eggs laid (±SE) for three generations of *Aphalara itadori* on bagged Japanese knotweed host during 96 h exposure period in bioassay Experiment 1. F0 *A. itadori* was sourced from laboratory colony, F1 and F2 *A. itadori* were reared for 1 and 2 generations on bagged Japanese knotweed hosts, respectively. Different letters indicate significant difference between means at P<0.05 according to Tukey’s HSD.

**Developmental time** – Egg-to-adult developmental time was not significantly different between generations (Table 7), but F2 psyllids did have longer developmental times (33.1±0.293 days) than F0 (31.4±0.351 days) or F1 (31.5±0.404 days) psyllids. There was no significant effect of host plant treatment or parent natal environment on developmental times or from their interaction term.

**Morphometrics** – The size of different morphological characters changed between almost all psyllid generations (F0 females vs F1, F2, and F3 females) (Table 8). All features were 1.8% to 8.2% larger in F1, F2, and F3 females. The largest intergenerational increases in this experiment were thorax width (mean increase, F1: 4.1%, F2: 6.90%, F3: 8.2%) and the smallest increases were seen in Rs wing vein length (mean increase, F1: 1.87%, F2: 2.12%, F3: 3.9%). No significant effect on any morphological feature was found from treatment or parent natal environment models (tibia length $F_{(3,78)}=0.679$, p=0.567; wing width $F_{(3,79)}=1.838$, p=0.147; wing length $F_{(3,79)}=0.194$, p=0.900; Rs vein length $F_{(3,79)}=0.303$, p=0.823; thorax width $F_{(3,78)}=1.838$, p=0.147; thorax length $F_{(3,78)}=1.069$, p=0.367). No significant interactions were found (p>0.05).
Table 7. Experiment 1 linear model testing whether *Aphalara itadori* egg development time is affected by host plant treatment, generation number, natal environment and the interaction term between treatment and natal environment. Asterisk indicates significance of P<0.05.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Levels</th>
<th>Coefficient</th>
<th>SE</th>
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<th>df</th>
<th>p</th>
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</thead>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<td>Intact</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>102</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F1</td>
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<td>-1.525</td>
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<td>F2</td>
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<td>0.631</td>
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<td>0.503</td>
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<td>Natal Env</td>
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<td>-</td>
<td>-</td>
<td>102</td>
<td>-</td>
</tr>
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<td>0.236</td>
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<td>Treatment*Natal Env</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
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<td>1.010</td>
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Table 8. Morphological measurements (mm) (mean ± SE) of female *Aphalara itadori* from each generation within Experiment 1. F0 A. itadori were sourced from laboratory colony, F1, F2, and F3 A. itadori were reared for 1, 2, and 3 generations on bagged Japanese knotweed hosts, respectively. Bonferroni correction (α/n tests) was used to adjust for multiple comparisons. Asterisk denotes significance of P<0.05/6 according to Tukey’s HSD.

<table>
<thead>
<tr>
<th>Generation</th>
<th>F0</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wing length</td>
<td>2.20±0.0102</td>
<td>2.25±0.0110*</td>
<td>2.26±0.0086*</td>
<td>2.29±0.009*</td>
</tr>
<tr>
<td>Wing width</td>
<td>1.091±0.0069</td>
<td>1.125±0.0048*</td>
<td>1.123±0.0052*</td>
<td>1.128±0.0052*</td>
</tr>
<tr>
<td>Rs vein length</td>
<td>1.225±0.0068</td>
<td>1.248±0.0063</td>
<td>1.251±0.0060*</td>
<td>1.273±0.0074*</td>
</tr>
<tr>
<td>Thorax width</td>
<td>0.609±0.0040</td>
<td>0.634±0.0033*</td>
<td>0.651±0.0045*</td>
<td>0.659±0.0038*</td>
</tr>
<tr>
<td>Thorax Length</td>
<td>0.641±0.0050</td>
<td>0.668±0.0049*</td>
<td>0.660±0.0045</td>
<td>0.664±0.0046*</td>
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<tr>
<td>Tibia Length</td>
<td>0.461±0.0032</td>
<td>0.473±0.0025*</td>
<td>0.473±0.0034</td>
<td>0.477±0.0023*</td>
</tr>
<tr>
<td>n</td>
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<td>36</td>
<td>45</td>
<td>48</td>
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</table>
Experiment 2: Juvenile versus mature plants

Oviposition – Overall F1 psyllids laid significantly more eggs than F0 psyllids (F0: 22.85±2.889 vs F1: 72.5±5.172) (Fig. 9, Table 9), but no significant ovipositional differences were found between the host plant treatments for both F1 and F0 psyllids. No significant interactions were found between treatment or natal environment (p>0.05).

Table 9. GLM results from Experiment 2 quasipoisson model testing whether Aphanara itadori oviposition is affected by host plant treatment, generation number, natal environment and the interaction term between treatment and natal environment. Asterisk indicates significance of P<0.05.

<table>
<thead>
<tr>
<th>Factor</th>
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<td>-</td>
<td>-</td>
<td>87</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>juvenile</td>
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<td>0.0398</td>
<td>-0.359</td>
<td>87</td>
<td>0.721</td>
</tr>
<tr>
<td>Generation</td>
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<td>-</td>
<td>-</td>
<td>87</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>1.039</td>
<td>0.364</td>
<td>2.858</td>
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<td>0.005 *</td>
</tr>
<tr>
<td>Natal Env</td>
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<td>-</td>
<td>-</td>
<td>87</td>
<td>-</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Treatment*Natal Env</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>87</td>
<td>-</td>
</tr>
<tr>
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<td>0.063</td>
<td>1.901</td>
<td>87</td>
<td>0.061</td>
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</table>

Differences in egg-to-adult success were not significant between F1 and F0 psyllids, but host plant treatments significantly affected the proportion of egg-to-adult success (Table 10). Treatment and parental natal history had a significant positive effect on the developmental success of eggs laid on juvenile knotweed for those whose parents also developed on juvenile knotweed; this was in contrast to eggs laid on mature knotweed (Fig. 10). While not significant, developmental success of eggs laid on mature knotweed was positive if parents developed on mature knotweed (p>0.05).
Figure 9. Mean number of eggs laid (±SE) for two generations of *Aphalara itadori* on bagged Japanese knotweed host during 96 h exposure period in bioassay Experiment 2. F0 *A. itadori* was sourced from laboratory colony. F1 *A. itadori* were reared on bagged Japanese knotweed hosts. Different letters indicate significant difference between means at P<0.05 according to Tukey’s HSD.

Table 10. GLMM results of Experiment 2 binomial model testing whether *Aphalara itadori* egg-to-adult success is affected by host plant treatment, generation number, natal environment and the interaction term between treatment and natal environment. Asterisk indicates significance of P<0.05.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Levels</th>
<th>Coefficient</th>
<th>SE</th>
<th>z</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>mature</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>84</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>juvenile</td>
<td>1.361</td>
<td>0.463</td>
<td>2.939</td>
<td>84</td>
<td>0.003 *</td>
</tr>
<tr>
<td>Generation</td>
<td>F0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>84</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>0.704</td>
<td>0.498</td>
<td>1.424</td>
<td>84</td>
<td>0.154</td>
</tr>
<tr>
<td>Natal Env</td>
<td>mature</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>84</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>juvenile</td>
<td>0.182</td>
<td>0.524</td>
<td>0.346</td>
<td>84</td>
<td>0.729</td>
</tr>
<tr>
<td>Treatment*Natal Env</td>
<td>mature*mature</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>84</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>mature*colony</td>
<td>-1.602</td>
<td>0.623</td>
<td>-2.547</td>
<td>84</td>
<td>0.011 *</td>
</tr>
<tr>
<td></td>
<td>juvenile*mature</td>
<td>-1.511</td>
<td>0.783</td>
<td>-1.928</td>
<td>84</td>
<td>0.050 *</td>
</tr>
</tbody>
</table>
Figure 10. Interaction plot between the proportion (mean ± SE) of successfully developed eggs laid by F1 *Aphalara itadori* psyllids reared on juvenile or mature knotweed from juvenile or mature knotweed plants (Experiment 2) using the “effects” package in R (Fox 2018).

**Sex ratio** - Male to female sex ratio was not significantly different between generations (Table 11), host plant treatment or based on F1 psyllid natal environment and no significant interactions were found between natal environment and treatment (p>0.05)

**Developmental time** - F1 eggs (34.8 ± 0.389 days) took significantly longer to develop than F0 eggs (32.4 ± 0.419 days) (Table 12). Treatment and parental natal environment and their interaction term were not found to have a significant influence on egg development times.

**Morphometrics** - Nearly all morphological features were significantly larger in F1 psyllids than F0 psyllids, and all features were significantly larger in F2 than F0 psyllids (Table 13). All features were 1.7 to 6.9% larger in F1 and F2 than F0 psyllids, with the largest gain in the length of the Rs wing vein (F1=3.10% mean increase versus F2=6.86%), and the least in thorax length (F1=1.69% mean increase versus F2=2.92%). Host plant treatment and psyllid natal environment had no significant effect on body size (p>0.05).
Table 11. GLMM results of Experiment 2 binomial model testing whether *Aphalara itadori* sex ratio is affected by host plant treatment, generation number, natal environment and the interaction term between treatment and natal environment. Asterisk indicates significance of $P<0.05$.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Levels</th>
<th>Coefficient</th>
<th>SE</th>
<th>z</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>mature</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>71</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>juvenile</td>
<td>0.100</td>
<td>0.169</td>
<td>0.591</td>
<td>71</td>
<td>0.554</td>
</tr>
<tr>
<td>Generation</td>
<td>F0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>71</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>0.315</td>
<td>0.177</td>
<td>1.778</td>
<td>71</td>
<td>0.075</td>
</tr>
<tr>
<td>Natal Env</td>
<td>mature</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>71</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>juvenile</td>
<td>0.231</td>
<td>0.187</td>
<td>1.660</td>
<td>71</td>
<td>0.090</td>
</tr>
<tr>
<td>Treatment*Natal Env</td>
<td>mature*mature</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>71</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>mature*colony</td>
<td>0.124</td>
<td>0.242</td>
<td>0.511</td>
<td>71</td>
<td>0.609</td>
</tr>
<tr>
<td></td>
<td>juvenile*mature</td>
<td>-0.114</td>
<td>0.348</td>
<td>0.327</td>
<td>71</td>
<td>0.744</td>
</tr>
</tbody>
</table>

Table 12. Experiment 2 linear model testing whether *Aphalara itadori* egg development time is affected by host plant treatment, generation number, natal environment and the interaction term between treatment and natal environment. Asterisk indicates significance of $P<0.05$.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Levels</th>
<th>Coefficient</th>
<th>SE</th>
<th>t</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>mature</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>72</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>juvenile</td>
<td>0.915</td>
<td>1.113</td>
<td>0.822</td>
<td>72</td>
<td>0.414</td>
</tr>
<tr>
<td>Generation</td>
<td>F0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>72</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>1.971</td>
<td>1.023</td>
<td>1.95</td>
<td>72</td>
<td>0.049*</td>
</tr>
<tr>
<td>Natal Env</td>
<td>mature</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>72</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>juvenile</td>
<td>-1.111</td>
<td>1.21</td>
<td>-0.918</td>
<td>72</td>
<td>0.362</td>
</tr>
<tr>
<td>Treatment*Natal Env</td>
<td>mature*mature</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>72</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>mature*colony</td>
<td>-1.724</td>
<td>1.372</td>
<td>-1.257</td>
<td>72</td>
<td>0.213</td>
</tr>
<tr>
<td></td>
<td>juvenile*mature</td>
<td>0.252</td>
<td>1.752</td>
<td>0.144</td>
<td>72</td>
<td>0.886</td>
</tr>
</tbody>
</table>
Table 13. Morphological measurements (mm) (mean ± SE) of female Aphalara itadori from each generation within Experiment 2. F0 A. itadori were sourced from laboratory colony, F1, F2, and F3 A. itadori were reared for 1, 2, and 3 generations on bagged Japanese knotweed hosts, respectively. Bonferroni correction (α/n tests) was used to adjust for multiple comparisons. Asterisk denotes significance of P<0.05/6 according to Tukey’s HSD. Asterisk denotes significance of P<0.05 according to Tukey’s HSD.

<table>
<thead>
<tr>
<th>Generation</th>
<th>F0</th>
<th>F1</th>
<th>F2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wing length</td>
<td>2.21±0.0110</td>
<td>2.27±0.0010*</td>
<td>2.32±0.0140*</td>
</tr>
<tr>
<td>Wing width</td>
<td>1.086±0.0060</td>
<td>1.127±0.0062*</td>
<td>1.146±0.0076*</td>
</tr>
<tr>
<td>Rs vein length</td>
<td>1.224±0.0073</td>
<td>1.262±0.0072*</td>
<td>1.308±0.0090*</td>
</tr>
<tr>
<td>Thorax width</td>
<td>0.640±0.0052</td>
<td>0.670±0.0043*</td>
<td>0.668±0.0053*</td>
</tr>
<tr>
<td>Thorax Length</td>
<td>0.650±0.0058</td>
<td>0.661±0.0037</td>
<td>0.669±0.0039*</td>
</tr>
<tr>
<td>Tibia Length</td>
<td>0.455±0.0028</td>
<td>0.469±0.0025*</td>
<td>0.482±0.0027*</td>
</tr>
<tr>
<td>n</td>
<td>43</td>
<td>38</td>
<td>22</td>
</tr>
</tbody>
</table>

Experiment 3: Hardened leaves

In this experiment, half of the psyllids used were reared on mature knotweed in Experiment 2 (i.e. F2 psyllids) and half were from the regular laboratory colony (F0). For statistical analysis, the natal environment of the parental psyllid corresponded to the generation number given. Therefore, only one simplified generation*treatment model was used for this experiment.

Fitness measurements

Oviposition - F2 psyllids laid significantly more eggs than F0 psyllids (F2 = 60.67 ± 6.66 eggs, F0 =12.21 ± 2.90) (Table 14, Fig. 11). The treatment did not have a significant effect on oviposition nor was there any significant interaction between generation number or treatment (p>0.05). The percentage of eggs that reached adulthood successfully was not significantly different between generations or host plant treatments and no significant interaction between the two was seen (p>0.05) (Table 15).
Table 14. GLM results from Experiment 3 quasipoisson model testing whether *Aphalara itadori* oviposition is affected by host plant treatment, generation number and their interaction term. Asterisk indicates significance of P<0.05.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Coefficient</th>
<th>SE</th>
<th>t</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (unhardened)</td>
<td>-0.454</td>
<td>0.499</td>
<td>-0.910</td>
<td>43</td>
<td>0.368</td>
</tr>
<tr>
<td>Generation (F2)</td>
<td>1.350</td>
<td>0.355</td>
<td>3.811</td>
<td>43</td>
<td>0.005 *</td>
</tr>
<tr>
<td>Treatment*Generation</td>
<td>0.528</td>
<td>0.544</td>
<td>0.971</td>
<td>43</td>
<td>0.337</td>
</tr>
</tbody>
</table>

Figure 11. Mean number of eggs laid (±SE) for two generations of *Aphalara itadori* on bagged Japanese knotweed host during 96 h exposure period in bioassay Experiment 3. F0 *A. itadori* was sourced from laboratory colony, F2 *A. itadori* were reared on”mature” Japanese knotweed hosts from Experiment 2. Different letters indicate significant difference between means at P<0.05 according to Tukey’s HSD.

Table 15. GLMM results from Experiment 3 binomial model testing whether *Aphalara itadori* egg-to-adult success is affected by host plant treatment, generation number and their interaction term. Significance of P<0.05 was used.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Coefficient</th>
<th>SE</th>
<th>z</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (unhardened)</td>
<td>0.354</td>
<td>1</td>
<td>0.352</td>
<td>47</td>
<td>0.725</td>
</tr>
<tr>
<td>Generation (F2)</td>
<td>-0.501</td>
<td>0.902</td>
<td>-0.555</td>
<td>47</td>
<td>0.579</td>
</tr>
<tr>
<td>Treatment*Generation</td>
<td>-1.062</td>
<td>1.319</td>
<td>-0.805</td>
<td>47</td>
<td>0.421</td>
</tr>
</tbody>
</table>
**Sex ratio** - Eggs from F0 psyllids laid on unhardened host plants produced significantly fewer males than F1 psyllids laid on plants grown in the greenhouse or on non-greenhouse plants and F0 eggs on greenhouse plants (Table 16, Table 17). However, these data were based on very few samples (n=8) because many eggs did not successfully develop. Variation was seen in the number of replicates across each experimental treatment for the same reason.

**Table 16.** GLMM results from Experiment 3 binomial model testing whether *Aphalara itadori* sex ratio success is affected by host plant treatment, generation number and their interaction term. Asterisk indicates significance of P<0.05.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Coefficient</th>
<th>SE</th>
<th>z</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (unhardened)</td>
<td>0.657</td>
<td>0.410</td>
<td>-2.224</td>
<td>43</td>
<td>0.026 *</td>
</tr>
<tr>
<td>Generation (F2)</td>
<td>-0.912</td>
<td>0.323</td>
<td>2.036</td>
<td>43</td>
<td>0.042 *</td>
</tr>
<tr>
<td>Treatment*Generation</td>
<td>0.534</td>
<td>0.461</td>
<td>1.158</td>
<td>43</td>
<td>0.247</td>
</tr>
</tbody>
</table>

**Table 17.** Mean sex-ratio (no. males/no. total adults) ±SE from *Aphalara itadori* developing from eggs laid across generations and treatments in Experiment 3.

<table>
<thead>
<tr>
<th>Generation</th>
<th>Treatment</th>
<th>Sex ratio ±SE</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0</td>
<td>Hardened</td>
<td>0.610±0.103</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Unhardened</td>
<td>0.263±0.068</td>
<td>8</td>
</tr>
<tr>
<td>F2</td>
<td>Hardened</td>
<td>0.679±0.065</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Unhardened</td>
<td>0.690±0.056</td>
<td>9</td>
</tr>
</tbody>
</table>

**Developmental time** - Generation and treatment had no significant effect on egg development time (34.50 ± 0.517 days; F(3,34)=1.366, p=0.27, Table 18).

**Table 18.** Experiment 3 linear model testing whether *Aphalara itadori* egg development time is affected by host plant treatment, generation number and their interaction term. Significance of P<0.05 was used.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Coefficient</th>
<th>SE</th>
<th>t</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (unhardened)</td>
<td>0.611</td>
<td>1.564</td>
<td>0.391</td>
<td>34</td>
<td>0.698</td>
</tr>
<tr>
<td>Generation (F2)</td>
<td>-2.056</td>
<td>1.419</td>
<td>-1.449</td>
<td>34</td>
<td>0.157</td>
</tr>
<tr>
<td>Treatment*Generation</td>
<td>1.333</td>
<td>2.111</td>
<td>0.631</td>
<td>34</td>
<td>0.532</td>
</tr>
</tbody>
</table>
**Morphometrics** - Morphological features were not compared directly in this experiment as each psyllid introduced had a separate natal history (i.e. F0 psyllids were reared in typical laboratory conditions and F2 psyllids from Experiment 2 reared on mature knotweed). Morphological data from F2 psyllids were used for comparison to previous generations in Experiment 2 instead.

**Overall Morphometrics**

**Body size and oviposition** - Egg counts were compared to the size of various morphological features to determine whether body size predicted the number of eggs laid (GLM). Pearson’s correlations show high r-values between all morphological features (r=0.49-0.81; df=251) and all features had a significant positive effect on the number of eggs laid (wing length $F_{(1,249)}=19.95$, p<0.001; wing width $F_{(1,249)}=22.55$, p<0.001; Rs vein length $F_{(1,249)}=19.45$, p<0.001; thorax width $F_{(1,249)}=32.38$, p<0.001; thorax length $F_{(1,249)}=19.15$, p<0.001; and tibia length $F_{(1,249)}=6.57$, p=0.011). A Quasi-AIC (QAIC) analysis was used to determine which feature or set of features best explained oviposition. All data were pooled across the Experiments 1, 2 and 3 to see whether an over-arching pattern emerged. The best overall model had 2-variables which included thorax width and wing width and was only marginally better than a 3-variable model that included wing width, thorax width, and length (Table 19).

**Table 19.** Quasi-AIC output for five best models predicting the number of eggs *Aphalara itadori* oviposit based on size of morphological features measured in bioassay Experiments 1, 2, and 3. Morphological features used as predictors included: thorax width, thorax length, right hind tibia length, and right wing width, length, and Rs vein length.

<table>
<thead>
<tr>
<th>Model Inputs</th>
<th>df</th>
<th>QAIC</th>
<th>ΔQAIC</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thorax width+Wing width</td>
<td>3</td>
<td>302.6</td>
<td>0.105</td>
<td></td>
</tr>
<tr>
<td>Thorax length+Thorax width+Wing width</td>
<td>4</td>
<td>302.8</td>
<td>0.22</td>
<td>0.094</td>
</tr>
<tr>
<td>Thorax length+Thorax width</td>
<td>3</td>
<td>303.1</td>
<td>0.51</td>
<td>0.081</td>
</tr>
<tr>
<td>Thorax length+Thorax width+Tibia length+Wing width</td>
<td>5</td>
<td>304.1</td>
<td>1.45</td>
<td>0.051</td>
</tr>
<tr>
<td>Rs vein length+Thorax length+Thorax width</td>
<td>4</td>
<td>304.1</td>
<td>1.46</td>
<td>0.051</td>
</tr>
<tr>
<td>Intercept only</td>
<td>1</td>
<td>337.1</td>
<td>34.49</td>
<td>0</td>
</tr>
</tbody>
</table>
**Body size trends** - Increases in body size were almost uniform across the generations for bioassay Experiment 1 and 2 (Table 8 and Table 13). Increases in psyllid body size were not adequately explained by treatment nor by the natal environment of the parental generation. One potential reason for this uniformity in larger body size might be explained by the difference in density of psyllids; the colony cages had hundreds of adult and juvenile psyllids in them compared to the bagged knotweed Experiments 1, 2 and 3 where there was a very low density of psyllids.

Using the number of adults that successfully developed from bagged knotweed plants as a surrogate for psyllid density, comparisons were made between realized adult body size and density. Pooling results of Experiments 1, 2, and 3, no significant relationships were found between psyllid density and any of the morphometric measures (wing length $F_{(1,106)}=0.875$, $p=0.352$; wing width $F_{(1,107)}=1.145$, $p=0.2871$; Rs vein length $F_{(1,107)}=0.183$, $p=0.670$; thorax width $F_{(1,106)}=0.018$, $p=0.893$; thorax length $F_{(1,106)}=0.226$, $p=0.635$; and tibia length $F_{(1,105)}=2.327$, $p=0.130$).

**Experiment 4: Density manipulation experiment**

**Oviposition** - Plants with five females had significantly more eggs than plants with only one female (GLM $t(60)=4.187$, $p<0.001$), and plants with ten females had significantly more eggs than both plants with five females (GLM $t(60)=3.849$, $p<0.001$) and one female (GLM $t(60)=6.381$, $p<0.001$) (Fig. 12A). However, eggs laid per female were not significantly different between either density (GLM ten females: $t(60)=0.716$, $p=0.477$, five females: $t(60)=0.272$, $p=0.787$) (Fig. 12B).

**Egg-to-adult success** - A significantly lower proportion of eggs successfully developed to adulthood in plants with ten females than in plants with one or five females (one female = 0.18 ± 0.04% successfully developed, five females = 0.24 ± 0.02%, GLM $z=-0.580$, $p=0.562$, ten females = 0.14 ± 0.02%; GLM $z=-2.461$, df=51 $p=0.014$).
Sex ratio – The proportion of males to females from eggs laid on plants did not significantly differ between the three treatments (one female = 0.68 ± 0.059% males; five females = 0.65 ± 0.050% males; GLM z = -0.093, df=47 p = 0.926; ten females = 0.649 ± 0.037% males GLM z = -0.247, df=47 p = 0.805).

Developmental time - No significant difference in egg-to-adult developmental time was seen between the three treatments (one female: 40.6±1.38 days, five female: 40.65±0.448, ten female: 40.7±0.424, F(2,47)=0.00476, p=0.995). Developmental time was noticeably longer here compared to the previous three experiments (approximately 5-7 days longer) likely due to lower room temperatures in this experiment (20.05°C here compared to 21-22.5°C in past experiments).

Morphometrics - A random selection of F1 psyllids was made from the low-density treatment (one female) and the high-density treatment (ten females) based on the number of F1 adults that had emerged. Specifically, plants with very low numbers of F1 adults and very high numbers of F1 adults were chosen to help determine whether low psyllid densities in the previous three experiments were responsible for the larger body sizes in psyllids reared in the bagged host plants. Unfortunately, only one high density replicate produced more F1 adults than replicates from the three of the previous bioassay experiments (n=91 adults). In Experiments 1, 2, and 3 one adult female was chosen at random from each knotweed replicate. In Experiment 4, multiple adult females from the plant that produced 91 adults were measured (n=10) as it was the best approximation of a high density natal environment with Experiment 4.

Of the six morphological measurements taken, only adult density was inversely related to tibial length (F(1,28)=6.879, p<0.05), albeit the tibial lengths from this replicate (91 adults) varied considerably (tibial length ranged from 0.418 to 0.479mm) (Fig. 13).

Oviposition - Chi-square goodness-of-fit tests were used to compare the number of eggs laid at each psyllid density to determine whether population density influenced oviposition site choice behaviour. Expected distributions were derived from single females on each plant in the density experiment and then compared to the expected distributions of eggs from those replicates with
Figure 12. Mean total number of eggs laid by one, five, and ten *Aphalara itadori* females on *Fallopia japonica* host plants of females over 96 h exposure period. (A) mean number of eggs per female laid at each density (B) mean number of eggs divided by total number of females. Different letters indicate significant difference between treatments at P<0.05 according to Tukey’s HSD.
Figure 13. Right hind tibial length measured from F1 female *Aphalara itadori* compared to the number of F1 adults that developed with their bagged Japanese knotweed (*Fallopia japonica*) replicate in Experiment 4.

five and ten females. Compared to plants with one female, psyllids were significantly more likely to lay more eggs on the lower 50% of knotweed hosts than the top 50% when there were five females ($X^2=28.573$, df=3, $p<0.001$) and significantly less likely when there were 10 females ($X^2=12.957$, df=3, $p<0.001$). Plants with five females also laid significantly more eggs on medium sized leaves (2.5-5cm base) ($X^2=98.435$, df=3, $p<0.001$), than the preferred large leaves (>5cm base) on plants with one female while plants with 10 females laid a significantly higher proportion of eggs laid on large leaves ($X^2=138.56$, df=3, $p<0.001$). Plants with five and ten psyllids showed a significantly greater preference for leaf undersides than plants with one female ovipositing a proportionally greater number of total eggs (five females: $X^2=163.51$, df=3, $p<0.001$; ten females: $X^2=218.83$, df=3, $p<0.001$) and at proportionally more individual leaf undersides (five females: $X^2=34.681$, df=3, $p<0.001$; ten females: $X^2=86.864$, df=3, $p<0.001$). Five of the six measures of ovipositional preference were significantly different for replicates.
with five females, whereas four of the six measures were significantly different for replicates with ten females.

The number of potential ovipositional sites on each host plant was made using counts of the number of abaxial and adaxial surfaces, as well as number of stem nodes. Greater than 75% of the potential sites were not used for egg-laying at the different psyllid densities. Plants with ten females per plant had a significantly higher number of sites used for egg-laying compared to those with only five females (GLM z=4.205, p<0.001) and female psyllids from both treatments oviposited at a significantly higher number of sites on the plants than when there was only one 1 female per plant (GLM 10 females: z=7.365 p<0.001, 5 females: z=4.205, p<0.001). However, when dividing the total sites laid at per female, single females laid at significantly more of the potential sites on the plant compared to plants with five or ten females (GLM 5 females: z=-4.358, p<0.001, 10 females: z=-5.066, p<0.001).

**Further modelling**

Given the results from the density experiment, increases in oviposition across the generations and related to increases in body size remained inadequately explained. Thus, a model including temperature, humidity, and an estimate of psyllid parental age was developed to help further understand the data patterns. A Quasi-AIC analysis was conducted on the total number of eggs with the following predictor variables: 1) mean developmental temperature, 2) mean developmental % relative humidity (RH), 3) mean temperature during oviposition, 4) mean RH during oviposition, 5) host plant treatment, 6) parent natal environment, and 7) psyllid maximum age.

Mean developmental temperature and RH were recorded during psyllid oviposition, both for the parent and offspring generations (i.e. 96 h). Temperature and RH were calculated from data loggers placed on the soil in one or two bagged plant replicates for each experiment, providing a total of five different developmental temperatures and RH and 6 different ovipositional temperatures and RH. Temperature and RH were included in the model as an interaction term.

Host plant treatments were the same treatments used on Japanese knotweed plants in Experiments 1, 2, and 3 (i.e. damaged, intact, juvenile, mature, hardened, and unhardened). The
natal environment of the parental generation was defined as the habitat in which a psyllid was reared, while psyllid maximum age was calculated as the maximum age (in hours) that a psyllid could be based on the last time adults were removed from the cage, (i.e. adults were removed from a cage on day 1 maximum age would be 24 hours and adults found in the same cage on day 4, psyllid maximum age would be 72 hours). Psyllid maximum age was more accurately measured for F0 psyllids as coordinating large emergence numbers for experiment set-up was easier, than for F1 and F2 females.

QAIC results indicated that the optimal model should include maximum psyllid age, mean developmental temperature and RH, their interaction term, and mean ovipositional RH (Table 20). There was a clear positive relationship between developmental RH and oviposition and a negative relationship between developmental temperature and oviposition. The same patterns are not observed for ovipositional period RH and temperature (Fig. 14).

Two GLM analyses were carried out to calculate a pseudo $R^2$ and estimate model fit; one included ovipositional temperature and RH and the other ovipositional temperature and RH. For the logistic regression model, I used McFadden’s pseudo $R^2$,

$$R^2 = 1 - \frac{\log\text{likelihood full model}}{\log\text{likelihood null model}}$$ (McFadden 1979),

which provided a pseudo $R^2$ of 0.377 for the model including all terms, and a pseudo $R^2$ of 0.361 for the model without ovipositional temperature and RH. McFadden’s pseudo $R^2$ values do not correspond to typical $R^2$ values, thus a pseudo $R^2$ of 0.2 - 0.4 indicates a good fit for the model. Including ovipositional temperature and RH data did not improve the model fit, thus the model with developmental temperature and RH, their interaction term, and maximum psyllid age was considered to be the best fit (Table 21).

As morphological features were positively correlated with egg-laying, the final model was tested further for wing width and thorax width since these features predicted egg-laying best in the previous models (see section Body Size Trends). The model was significant for wing width ($F(4,246)=11.39$, $p<0.001$, $R^2=0.143$), but predictor variables were not significant due to the high
Table 20. Quasi-AIC output for four best models predicting the number of eggs *Aphalara itadori* oviposit with mean developmental temperature, mean developmental % relative humidity (RH), mean temperature during oviposition, mean RH during oviposition, host plant treatment, parent natal environment, and psyllid maximum age as predictors. Analysis was from pooled psyllid data from Experiments 1, 2, and 3 (n=251).

<table>
<thead>
<tr>
<th>Model</th>
<th>d</th>
<th>QAIC</th>
<th>ΔQAIC</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max psyllid age+mean dev. temp.+mean dev. RH+mean Oviposit RH+mean dev. Temp.*mean dev. RH</td>
<td>6</td>
<td>32</td>
<td>2.8</td>
<td>0.242</td>
</tr>
<tr>
<td>Max psyllid age+mean dev. temp.+mean dev. RH+mean Oviposit RH+mean oviposit temp.</td>
<td>6</td>
<td>323.9</td>
<td>1.04</td>
<td>0.144</td>
</tr>
<tr>
<td>Max psyllid age+mean dev. temp.+mean dev. RH+mean Oviposit RH+mean oviposit temp.+mean dev. Temp.*mean dev. RH</td>
<td>7</td>
<td>324.2</td>
<td>1.39</td>
<td>0.121</td>
</tr>
<tr>
<td>Max psyllid age+mean dev. temp.+mean dev. RH+mean Oviposit RH+mean oviposit temp.+mean oviposit Temp.*mean oviposit RH</td>
<td>7</td>
<td>324.3</td>
<td>1.44</td>
<td>0.118</td>
</tr>
<tr>
<td>Intercept only</td>
<td>1</td>
<td>460.0</td>
<td>137.11</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 21. GLM results for *Aphalara itadori* oviposition prediction model identified by Quasi-AIC analysis. Asterisk indicates significance of P<0.05

<table>
<thead>
<tr>
<th>Factor</th>
<th>Coefficient</th>
<th>SE</th>
<th>t</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max age</td>
<td>0.001</td>
<td>0.0006</td>
<td>3.113</td>
<td>226</td>
<td>0.002 *</td>
</tr>
<tr>
<td>Development temp.</td>
<td>-0.534</td>
<td>0.079</td>
<td>3.185</td>
<td>226</td>
<td>0.001 *</td>
</tr>
<tr>
<td>Developmental RH</td>
<td>0.167</td>
<td>0.494</td>
<td>3.38</td>
<td>226</td>
<td>0.001 *</td>
</tr>
<tr>
<td>Dev. RH*Dev. Temp.</td>
<td>-0.072</td>
<td>0.021</td>
<td>-3.34</td>
<td>226</td>
<td>0.001 *</td>
</tr>
</tbody>
</table>

correlation between variables (p>0.05). The model was also significant for thorax width and mean developmental temperature, mean developmental RH, and their interaction term, but maximum psyllid age did not affect thorax width (F(4,246)=11.39, p<0.001, R²=0.172).
Figure 14. Number of eggs laid by *Aphalara itadori* based on their A) mean %RH during development, B) mean temperature during development, C) mean %RH during 96 h oviposition period, and D) mean temperature during 96 h oviposition period. Ovipositional data was pooled across all psyllids in Experiment 1, 2, and 3 (n=251). Boxes show median (centre line), 1st and 3rd quartile (box edges), and maximum and minimums (whiskers).
Discussion

Evidence of phenotypic plasticity

The central questions in this thesis were to determine: 1) To what degree phenotypic plasticity exists for traits that would favour biocontrol establishment in a laboratory colony of A. itadori?; 2) If phenotypic plasticity exists, then can a phenotypic response be elicited through experimental manipulation either of the host plant or rearing conditions?; 3) Does adaptive phenotypic change carry over between psyllid generations?; and 4) Will phenotypic responses will help psyllid populations establish in the field?.

In terms of the first question, there is consistent evidence from my bioassay experiments that psyllid body size will change in relation to their rearing environment. Larger body size is linked to both greater fecundity, survival, and mating success (Kingsolver and Huey 2008), and while I found support for increased fecundity with increasing body size, the relationships were not tight. Given that the bioassays conducted here only examined a relatively short (96-h) ovipositional period, it is unlikely that the real benefit from increased body size was not realized. Still, increased body size and egg-laying would be expected to improve the successful establishment of a biocontrol agent and would be a desired attribute to foster during production rearing.

Host plant treatments to address the second question were chosen based on the expectation that they were in some way limiting to psyllids and their offspring (based on past experiments and observations in the field). However, I found limited evidence of phenotypic change in psyllid traits based on their exposure to the selected test plant treatments. Rather it was the change in rearing conditions of the polyethylene bag and room temperature that appeared to drive the increase in body size. Rearing psyllids in polyethylene bags at lower temperatures and potentially at lower densities may be the key to attaining larger individuals for release in future biocontrol programs.

The bioassay experiments followed successive generations to address the third question and determine whether the conditions the parent generation was exposed to impacted the phenotype of their offspring. To test this we would expect a significant interaction term between host plant treatments and the natal environment of the parents to be found in the fitness models, indicating
increased fitness in offspring reared in the same environment as their parents. Unfortunately, limited evidence was found linking the natal environment of the parents to the phenotypic performance of their offspring on host plant treatments. The limitation here was likely because the host plant treatments induced little discernable phenotypic change in the parent generation to pass onto their offspring. However, there were phenotypic changes that appeared to be driven within psyllid generations. Psyllids reared in the polyethylene bags had a larger body size than those reared in the lab cages, suggesting that the natal rearing environment had a greater latent effect on psyllid phenology than either maternal or parental effects. Separating what is and what is not a maternal effect is inherently difficult as the influence of a female on her offspring can begin even before she reaches sexual maturity (Bernardo 1996). Although the current experiments provide evidence that the conditions psyllids experience early in life will influence their phenotypic outcome, I saw no evidence that these same conditions will influence their offspring: phenotypic changes in psyllid body traits remained stable for at least three generations (F1 to F3), as did related increases in oviposition when psyllids were reared in polyethylene bags. In terms of maximizing fitness for a biocontrol agent, it is desirable that it can reliably attain large body size and high oviposition. The phenotypic changes I observed here appear to carry over between psyllid generations, however it is unclear how long they will last once these conditions are removed (i.e. development in polyethylene bags versus released into the field). It is possible that such changes would be lost as quickly as they were gained, but the population increases from increased fitness in a single generation could carry over to multiple successive generations a phenomenon noted in other studies of phenotypic plasticity (Rossiter 1991, Van Allen and Rudolf 2013).

It is uncertain whether the results seen here can address the fourth question on whether phenotypic responses are adaptive under release conditions. If host plant treatments were found to have a substantial effect on psyllid fitness, then stronger conclusions could be made about the actual factors that might limit population establishment in the field. For example, if psyllids reared on the “mature plants” in Experiment 2 had poor survival and small body size as adults, then one could conclude that older knotweed plants might limit psyllid from establishing in the field. Increased body size and egg-laying could be expected to improve population establishment in the field, however in the absence of what we know to actually be limiting, no concrete
conclusions can be made. Still, obtaining reliable increases in psyllid body size before release for even a single generation would improve chances of permanent establishment.

While the central questions addressed in this thesis were ultimately unclear a number of notable trends were found in the measured variables. Very similar trends were seen in the major biological parameters measured for psyllids in the first experimental bioassays (i.e. oviposition, egg-to-adult success, sex ratio, egg development time, and body size). In most cases, significant results were driven by differences across generations rather than by differences in the host plants or by the natal environment of the psyllids. In Experiment 1 (Damaged vs. Intact plants), neither treatment nor natal environment had any effect on these variables, whereas differences between the generations were significant. In Experiment 3 (Hardened Leaves), no significant differences were observed between treatments, but differences between the generations were significant for oviposition and sex ratio. Experiment 2 (Juvenile vs. Mature) was the only bioassay where the host plant treatments produced significant differences in both oviposition and egg-to-adult success while generation was significant for body size, oviposition and development time.

**Oviposition and egg-to-adult success**

The number of eggs oviposited in the 96-h exposure period was significantly higher in F1 and F2 generations for all three experiments. While host plant treatments and parental natal history did not impact psyllid oviposition in Experiment 1 and 3 there is evidence that psyllids reared on mature host plants from Experiment 2 would oviposit a greater number of eggs on mature host plants over juvenile host plants.

Natal habitat preference induction (NHPI) is a response that favours certain habitats based on an organism’s natal environment (Davis and Stamps 2004). If a NHPI were present we would expect *A. itadori* that could be induced to lay more eggs on certain qualities of knotweed host based on their natal environment. This would be beneficial to the predictability of establishing field population of *A. itadori* and would be indicative of phenotypically plastic responses this thesis was designed to explore. Increased oviposition on mature host plants over juvenile hosts from psyllids raised on mature hosts could point towards a NHPI. However, the conclusions that can be made about a NHPI in Experiment 2 are weakened by low replication in the data collected on psyllids raised on mature knotweed ovipositing on juvenile knotweed (n=7) and the high
variability of oviposition within these replicates (mean eggs laid +/- 1 SD= 49.429 +/-39.409). Further, psyllids reared on juvenile host plants showed no significant preference between mature or juvenile host plants when ovipositing. As no NHPI was found in Experiment 1 and 3 as well, it appears that there is little to weak evidence for changes in oviposition frequency based on host plant quality within these experiments. Further oviposition choices testing could be done using a more robust sample size with psyllids reared on mature knotweed to determine whether a NHPI is present.

On the other hand, there is more evidence of a NHPI in terms of successful egg-to-adult development. In Experiment 2, psyllid offspring reared on both juvenile and mature host plants were more likely to reach adulthood if reared on hosts of the same quality as their parent. In particular, the proportion of eggs that developed successfully nearly doubled for psyllids reared on juvenile plants when laid on juvenile plants (47.1% success) compared to those laid on mature plants (24.4% success). The same trend was seen for psyllids reared on mature plants, although the gap in increased success was considerably smaller (39.6% success on mature plants and 34.0% success on juvenile plants) and not significant. Once again, there was low replication for psyllids reared on mature knotweed and ovipositing on juvenile knotweed (n=7), as several females died accidentally in the initial 96-h exposure period.

A greater number of eggs reached adulthood successfully in F1 and F2 psyllids than in F0 psyllids, but the proportion of eggs successfully developing into adults was F1 and F2 generations than in F0 psyllids. Part of the difference in this proportional success appeared to be driven by some plants with high numbers of eggs from F1 and F2 psyllids yet no adult emergence (F1 = 11.47 and F2 = 15.1% plants with eggs but no adults). There was also a larger proportion of eggs from F1 and F2 psyllids with very low (< 20%) adult emergence (F1 = 12.6%, F2 = 12.5%, and F0 = 4% with < 20% egg-to-adult success). Dissections of plants with eggs but no adult emergence showed unhatched eggs suggesting that some females actually failed to mate. Unmated A. itadori females have previously been observed ovipositing on knotweed hosts, possibly to create room for developing eggs (personal observation).

Unfertilized ovipositional behaviour is also seen in other insects. Wenninger and Hall (2007) have shown that the Asian citrus psyllid, Diaphorina citri Kuwayama will lay unfertilized eggs if
unmated as will the psyllid *Psylla pyricola* Förster, albeit this behaviour has only been reported near the end of the lifespan (Hodkinson 1974). Unfertilized egg-laying occurs in many insect orders and can account for a large proportion of overall oviposition (Rivet et al. 1989, Zeng et al. 2017). Reasons for unfertilized oviposition are unclear but may be to ensure that ovarioles can continue to produce eggs if the female mates later in life (Rivet et al. 1989), or that they provide nutritional benefit to offspring that will eventually hatch from fertilized eggs (Zeng et al. 2017).

In the bioassay experiments, not all females laid eggs that failed to hatch, and *Aphalara itadori* nymphs are not known to predate on unfertilized eggs making it unlikely that they enhance offspring nutrition. It is more likely that this behaviour is related to ensuring continued egg production over the lifetime of the female. With a recorded lifetime fecundity of 600-700 eggs, the ability of a female psyllid to produce eggs continuously over her life would be needed, and due to size constraints, this would necessitate that eggs are laid as they develop. In this way, females may be gambling on finding a mate later to maximize their fecundity rather than stop egg production, and this would explain why I saw a greater number of unfertilized eggs as females aged.

**Psyllid age**

Most psyllid species do not have a complete cohort of eggs post-eclosion, and instead ovarioles develop mature eggs over the course of their adult life (Burts and Fischer 1967, Wenninger and Hall 2007). It seems probable then that older females introduced onto plants later would more likely engage in unfertilized egg “dumping” when they have not mated in order to deplete the surplus of unlaid eggs that would continue to develop.

Unfortunately, the data surrounding psyllid age in this experiment is approximate, calculated as the maximum possible age from the time psyllids had last been removed from a cage. The mean maximum age for psyllids in the F1 and F2 generations was older than F0 psyllids at the time of their 96 h oviposition since emergence could not be as tightly controlled as it was for F0 psyllids (mean maximum age for F0 = 72 h; F1 = 134 h; and F2 = 146 h). Based on the estimated ages that we do have, there is no obvious pattern relating psyllid age to non-hatching egg-laying. The largest number of unhatched eggs was 94, oviposited by one F2 female who could have been at
most 96 h old post-eclosion. The most common maximum female age across the three bioassays was 96 h (in approximately 37% of all replicates). While maximum psyllid age was not directly related to egg-to-adult success, I did find a positive relationship between maximum psyllid age and eggs laid.

Based on the data collected in these experiments, psyllids as young as 36 h post-eclosion seem to be able to begin oviposition. Based on preliminary dissections of female *A. itadori*, it appears that several unmated females between only 0 and 24 h old contain no mature eggs, having instead small ovarioles that are difficult to distinguish. Instead, older females (unknown age) randomly selected for dissection showed clearly differentiated ovarioles, that had mature eggs present, and a spermatheca containing a spermatophore (Fig. 1). This may possibly explain why I observed low oviposition rates in younger females since they would not have time to begin maturing eggs. Further work is needed dissecting mated and unmated females of different ages to determine when eggs mature, when peak egg loads are present, and the role of mating in egg development and oviposition. Also, of interest is the question whether multiple matings are needed to sustain oviposition, or whether mating is even needed to trigger egg development.

Female psyllids of species other than *A. itadori* studies here are known to have diverse mating strategies, some of which are ready to mate within 24 h post-eclosion (Guédot et al. 2012) while others take several days (Burts and Fischer 1967). Guédot et al. (2012) found that even after mating, some female psyllids may not begin laying eggs for several days, and Wenninger and Hall (2007) showed that some females may mate repeatedly over the course of their adult life while others mate only once. Variation exists in absorption of the spermatophores as well, with some species absorbing them in only 4 days for presumably for nutritional purposes while others take longer (Wenninger and Hall 2008). Here, individual *A. itadori* females were observed mating multiple times (personal obs.), but whether they require multiple matings or whether they absorb spermatophores is unknown. It is also still unclear at what age adult females are prepared to mate or to begin oviposition.
Figure 15. Dissection of *Aphalara itadori* females showing eggs and ovarioles, and spermatheca. A) female 0-24 hours old, ovarioles non-distinguishable; B) female unknown age, with multiple mature eggs (red arrows) and full spermatheca (blue arrow); and C) female unknown age, several eggs in early stage of development (green arrow) and ovarioles easy to differentiate with full spermatheca (blue arrow).

My work will help inform questions around psyllid age, oviposition, and egg viability although these aspects have not been dealt with explicitly in this thesis. Maternal age reportedly shares a negative relationship with offspring fitness, where fitness decreases with offspring size (Fox 1993) and egg viability (Hercus and Hoffmann 2000). In my bioassay experiments here, older psyllids laid more eggs, but this did not seem to have any effect on offspring size nor egg-to-adult success. The absolute maximum age a female psyllid used in these experiments could have
been was 18 days old (n=1), with the majority being a maximum of 5 days or less (81%). Given the approximate 60-day lifespan of *A. itadori*, it is possible that the negative impacts of maternal age were not captured in the present study. Further, female psyllids used in my study may have been too young to reach peak egg production as was the case for another psyllid species where peak egg oviposition occurred in the middle of the adult lifespan, tapering off at young and old extremes (Burts and Fischer 1967). Overall, it appears that the higher number of eggs laid by the F1 and F2 psyllids than F0 psyllids, and the higher proportion of unfertilized eggs, was due to their advanced age.

**Impact of host plant treatment**

The host plant treatments in the three initial bioassays had limited to almost no effect on psyllid illogical parameters. The only exception was the greater number of eggs laid on mature knotweed by F1 females reared on mature knotweed and the increased egg-to-adult success for eggs oviposited on juvenile knotweed by adults reared on juvenile knotweed in Experiment 2. Unfortunately, this bioassay suffered from low replication and high variation making it difficult to be conclusive.

Plant host quality in herbivorous insects has wide ranging consequences for their individual life histories, including developmental time and body size (Awmack and Leather 2002). If a particular host plant condition was detrimental to psyllid fitness, then we would expect this would be reflected in longer developmental times and/or smaller body sizes for psyllids. Developmental times and body sizes were not significantly different for F2 psyllids in Experiment 1 or 2 regardless of the natal history for the F1 parent or the host plant age. This suggests differences found in oviposition and egg-to-adult success were more likely driven by chance variation from low replication rather than detrimental or positive impacts from the treatments themselves.

Results from Experiment 3 are of particular importance when examining the establishment of *A. itadori* field populations. Of all the plant treatments tested here, the “hardened” plants exposed to sunlight in the greenhouse were the most similar to plants found in the field. The higher leaf-
mass-area found in these plants exposed to greenhouse sunlight was expected to provide a physical barrier to feeding by early-instar psyllids, and this might also help to explain the high nymphal mortality found in field samples. As no significant differences were found in oviposition or egg-to-adult success between psyllids feeding on hardened and unhardened plants, despite a 27% increase in leaf-mass-area in the hardened plants, there is no evidence that leaf hardness causes high nymphal mortality in the field.

The difference in leaf-mass-area in hardened plants may be a poor approximation of leaf-mass-area of plants found under field conditions. Gammon (2009) found the leaf-mass-area of *Fallopia japonica* knotweed in New England field plots ranged between 0.0057 and 0.0200 g/cm$^2$, which is 1.8 to 6.25 times higher than the leaf-mass-area found in the greenhouse hardened plants in my bioassay. If this is the case, then leaf hardness may have not been high enough here to stop feeding by early-instar psyllids.

The preliminary experiments examining psyllid response to juvenile, mature, and damaged plants suggested that damaged and juvenile plants may be causing high adult mortality. Thus, subsequent bioassay experiments were designed to test this. No significant adult mortality was seen on damaged plants in Experiment 1 nor on juvenile plants in Experiment 2. Egg-to-adult success was also not reduced on damaged plants in Experiment 1 suggesting that the high mortality seen in the preliminary experiments was the result of high psyllid densities not host plant condition.

**Impact of psyllid density**

Experiment 4 was set up to examine the impact of psyllid density on adult body size and oviposition based on the assumption that both parameters would likely as psyllids were reared for generations on bagged knotweed host plants. Across many phylla including insects, high population densities lead to increased resource competition, and this is associated with smaller body size (Currie 1993). Smaller body size has been linked to lower fecundity in many insect groups (Honek 1993, Kingsolver and Huey 2008). The relationship between population density and individual body size has been observed in other species of psyllids (Hodkinson 1979), and
thus I expected that the different psyllid densities between the laboratory colony cages and the individual bagged plants would be an important explanatory variable for adult body size.

In general, knotweed hosts in the bagged experiments had much smaller populations (<100 nymphs) than those in the laboratory cages, where the latter contained hundreds of nymphs and adults. Thus, I predicted that psyllid nymphs developing at high population density would have reduced adult body size and there was some evidence to support this in terms of shortened hind tibial lengths. However, there was large variation in this measurement, and no significant relationship was seen for the remaining five morphological characters. The highest density of psyllids reared in Experiment 4 was 91 adults, which was higher than in previous experiments but significantly lower than in the laboratory colony cages. It is possible that psyllid densities were not high enough in this experiment to detect an effect on body size, however psyllid ovipositional behaviour clearly did change in response to nymphal density.

Psyllids at higher densities laid more eggs on plants than those at lower densities, although the mean number of eggs laid per psyllid did not increase regardless of density. This suggests that psyllids in this experiment may be making an ovipositional choice independent of density. Psyllids are known to reduce oviposition rates at higher densities presumably to limit offspring resource competition (Clark 1963, Hodkinson 2009). It is possible psyllid densities were not high enough to cause a behavioural change in oviposition as females did not lay fewer eggs at higher densities in Experiment 4.

More eggs were laid on the upper side of leaves in Experiment 4 than in Experiment 1, 2, and 3 or in the field trials. Though at a much lower frequency, Aphalara itadori will oviposit at stem nodes and on the underside of leaves. I found that plants with five or ten female psyllids laid a proportion of eggs on leaf undersides than on upper surfaces and that they also laid significantly more compared to those plants with only one female. If one assumes that solitary females on plants choose to oviposit in the most optimal sites for their offspring, then it appears that higher psyllid densities lead to females choosing sub-optimal sites for egg-laying (i.e. leaf undersides).

When alone on a plant, female psyllids distributed their eggs relatively evenly across the whole host plant, whereas when females were at five and ten per plants, they tended to aggregate their eggs (i.e. laid at few sites per psyllid), even though they laid more eggs overall. Past studies have
shown that multiple psyllids will aggregate their oviposition, possibly as a result of pheromone production by females to attract other females (Blair 2014, Broom 2009). Both of these authors found however that such aggregation only occurred at low psyllid densities (< 20 females), with dispersion favoured at higher density. Insects aggregate eggs for predator satiation, facilitating mate-finding (Aukema and Raffa 2004), overcoming plant defenses, and/or improving nutritional composition (Sharma and Raman 2017). It is possible that in A. itadori, aggregation allows psyllid nymphs to overcome plant defenses and therefore improve nutritional composition, which in turn may have played a role in the increased egg-to-adult success females where there were five per plant (24% of eggs reached adulthood) compared to one female per plant (18% reached adulthood).

Interestingly, eggs with ten females per plant had the lowest success rate with only 14% reaching adulthood. The reasons for this are unclear. For all treatments here, egg-to-adult success was much lower than in previous experiments possibly due to a slightly lower temperature (= 20.05°C compared to 21-22.5°C in previous experiments). Nava et al. (2007) reported reduced egg and nymph viability in the psyllid, Diaphorina citri Kuwayama with only a 2% difference in the same temperature range, no where near the approximately 25-35% drop seen egg success rate in Experiment 4 eggs compared to psyllids in the previous bioassays.

The high adult mortality observed in the preliminary host quality experiments could have been a result of feeding on either juvenile or damaged plants, as there was far less plant material for egg-laying and this may have led to a density-related dispersal response. No other host plants were available for psyllids under these conditions and attempting to disperse causing starvation. Although psyllids were only exposed to plants for 96 h here, starvation has been observed to occur quickly in adult psyllids deprived of knotweed host material. This response is consistent with the findings of Blair (2009) who noted dispersal onto new host plants at high densities of A. itadori. This dispersal response fits with overall views of density dependent dispersal decisions, however it is not consistent with the hypothesis suggested above that female psyllids aggregate eggs in order to improve the outcome for their offspring. Organisms are thought to disperse from high populations when the benefits of aggregating (i.e. mating finding, access to resources) outweigh the costs from competition (Fowler 2009). In my experiments, aggregation occurred at population sizes used in Experiment 4 (five and ten females per plant), while dispersal appeared
to occur in at population sizes of twenty females per plant in the preliminary host quality experiments.

**Changes in body size**

Only body size differed in all the comparisons between F0 and F1, F2, and F3 psyllid generations (Experiments 1 and 2) with size increasing between the F0 generation and the latter three generations. No significant relationship was seen between psyllid body size and the condition of the host plant it was reared on or between the environment that the offspring’s parent was reared on. As noted above, the lower population density in the rearing environments of the F0 psyllids (i.e. colony cage) vs. F1, F2 and F3 psyllids (i.e. bagged bioassay unit), there is only very limited direct evidence supporting this hypothesis when comparing body sizes in F1, F2, F3 psyllids where natal environment density was known.

An alternative possibility for larger body sizes in F1, F2, and F3 psyllids is their exposure to different temperature regimes than F0 psyllids. Temperatures were slightly lower in the experimental bioassay room (21.2 - 22.62°C) than in the room where the psyllid lab colony was kept (23.2°C), and this may help explain the observed differences. Insects are known to show smaller adult body size when reared at higher developmental temperatures (Davidowitz et al. 2004, Kingsolver and Huey 2008, Clissold and Simpson 2015), and it has been suggested that this is due to the differential impact of rearing temperature on important life history phenologies such as developmental time (i.e. time to moulting) and growth rate (Kingsolver and Huey 2008). If higher temperature regimes affect developmental time more than growth rate, then this means that one would expect body size to be reduced in adult insects (Kingsolver and Huey 2008). It is possible that this explanation might account for the larger body size in the F1, F2, and F3 than F0 psyllids seen here as rearing temperatures of the former three generations were lower than that of the F0 psyllids. Unfortunately, temperature data were limited to only one or two temperature and RH loggers per experiment in this study, yet despite this, we still saw a significant relationship between temperature and adult wing and thorax widths. Interestingly, these same morphological features were shown to best predict overall adult psyllid fecundity in Experiments 1, 2, and 3.
even though developmental temperature only accounted for 4.4% of the observed variability in determining adult wing width and 3.8% of variability in adult thorax width.

Another factor that may have influenced the large difference in body size between the rearing conditions of generation F0 and other generations was relative humidity. Relative humidity was considerably lower in the Plexiglas colony cages (38.9%) compared to the bagged experimental plants (69.3 - 74.5%). This differential is partly due to the higher temperatures in the colony cages, but also likely to the size of the cage and improved airflow from cage vents than in the more constricted in the bagged plants. High relative humidity has been observed to be important in the survival of adult psyllids (McFarland and Hoy 2001), and especially for nymphs who must avoid desiccation (Hodkinson 1979). Nymphs of A. itadori here seemed to seek high RH, as they were often found feeding near the base of stems in the lab where RH was highest and in contact with damp soil from regular watering. However, despite this preference for higher RH, no change in survival was found in the analysis.

To date, there is no direct evidence in the literature establishing a causal link between RH and body size, and this suggests that the increases in body size seen here between the generations are unlikely to be related to RH. The most likely explanation then is that these increases are partially due to the lower experimental temperatures for F1, F2, and F3 generations compared to those in the laboratory cages of the F0 psyllids. As discussed previously, high psyllid densities have been found to result in smaller adults and colony densities were not adequately captured in the density bioassay. Thus, it is possible that the low psyllid densities in the bioassay replicates are the missing explanatory variable for changes in body size.

Sex ratio

Sex ratios in psyllids are known to be approximately 1:1 (Hodkinson 2009), however my results showed deviations from this with most of the variation occurring between generations, and to a limited extent between host plant and psyllid density treatments. Differences in 1:1 sex ratio were generally much larger between the four bioassay experiments than within each of them, where they were much lower in Experiment 1 than in Experiment 2 and 3. Sex determination in
insects is genetic but it can also be influenced by environmental factors, including temperature, nutrition, density, humidity, and light (Korpelainen 1990). Results from Experiments 1, 2 and 3 would suggest that environmental variation drove the observed sex differences, however the bioassays for F2 generation in Experiment 1 and the F1 generation in Experiment 2 was carried out concurrently in the same room and a large difference between the two occurred. There was a 20-30% difference between the sex ratio in each of these two bioassays despite both having nearly identical environmental conditions. The fact that neither experiment found a significant difference in the treatments tested and that the sex ratios within each bioassay were stable suggests that sex determination of offspring, and thus sex ratio, is more strongly determined by parental genetics than environmental factors in this psyllid system.

**Developmental time**

Developmental time is a critical fitness component in insects that determines generational length (Kingsolver and Huey 2008). Shorter developmental times in biocontrol agents allows for multiple generations per year and the increased potential to control target pests. I found developmental times to be significantly higher for eggs of F1 psyllids than for eggs of F0 psyllids in Experiment 2. The natal history of parental psyllids and the plant treatment they were exposed to appeared to have no effect on their developmental times in these bioassays. This would suggest that the offspring of F0 psyllids were set to develop faster than the offspring of F1 psyllids. However, the results from the Experiment 3 did not support this conclusion.

Experiment 3 was the only bioassay to directly compare developmental times of the offspring of F0 psyllids with F2 psyllids reared on knotweed plants concurrently within the same room. In this case, no differences were found between the offspring of F0 and F2 psyllids suggesting that developmental times were tightly linked to the environmental conditions they were reared under as opposed to maternal effects on the parent generation. This conclusion is supported by longer developmental times in offspring in Experiment 4, which had lower mean room temperatures and significantly longer development times than Experiments 1, 2 and 3.
Conclusions, recommendations, and future directions

The experimental conditions tested here in the bioassay experiments were expected to elicit plastic phenotypic developmental responses in psyllid offspring. The evidence gained here suggests that to some extent this is true, but it is more related to the rearing conditions of polyethylene bags than to the manipulated experimental treatments. My prediction that offspring will exhibit greater fitness when exposed to the same conditions as their parents had mixed support. Offspring fitness showed little change over the host plant treatments regardless of the natal environment of their parent, but continuous rearing in polyethylene bags showed a stable increase in oviposition and body size compared to colony psyllids.

Lower temperatures in the bioassay rooms may have contributed to the differences in body size observed here, but in reality, only accounted for a small percentage of that variation. Size differentials may also have been caused by varying psyllid densities between colony rearing and polyethylene bags, but if such differences occurred, they were not captured by the density bioassay here. Whether body size increases in the absence of bagged rearing remains unknown. To answer this question, a generation of psyllids could be reared in polyethylene bags in the same room as the Plexiglas colony cages, and in that way control for temperature. If psyllid body size increases under these conditions, as was seen in the F1 generation on bagged plants, then adult psyllids could be pooled into Plexiglas cages typically used in colony rearing and their offspring examined for smaller body size. In this way, the stability of changes in body size could be examined, and then studies set up to determine whether these shifts persist in the field.

Results from the bioassay experiments suggest that psyllids are less sensitive to changes in host plant quality than originally thought. Psyllids and their offspring repeatedly showed no difference in performance or fitness when exposed to a range of plant treatments originally thought to be limiting for population establishment in the field; e.g. the experiments here seem to suggest that it does not matter whether psyllids are provided with damaged foliage, mature foliage or plants just sprouting from the ground. This is good news for the effectiveness of A. itadori as a biocontrol agent against invasive knotweed in that it could be released on vulnerable, newly-emerging knotweed or knotweed that has experienced some form of mechanical control where it might have a greater impact and probability of establishing. Unfortunately, there still remains the question as to whether the plants used in the bioassays accurately represented plants
growing under field conditions, especially in terms of plant hardness. Furthering testing of plants collected in the field could be done to assess psyllid mortality and determine whether plant hardness is truly a limiting factor for field establishment.

Oviposition in *A. itadori* appears to be linked not only to body size, but also female age. Older females had greater rates of oviposition and thus psyllids seem to have a pre-ovipositional period not previously considered in releases. Determining the length of this pre-ovipositional period and the window of peak oviposition will aid considerably in helping populations establish once released. Work examining *A. itadori* fecundity with age is ongoing at AAFC Lethbridge.

Both right forewing and thorax widths were found to be good approximations of psyllid fecundity. These measures could be used to quickly estimate potential fecundity of *A. itadora* populations; of the two, forewing width was the easiest to measure accurately and would be recommended for application in the field.

Psyllids appear to be highly sensitive to changes in temperature and declines as small as 2°C resulted in large changes in developmental time. Temperature is also a significant factor in determining adult size. Thus, finding an optimal rearing temperature in order to maximize body size could be very beneficial when trying to maximize adult survival and oviposition in field releases.

Finally, the research conducted here should help develop a better framework for considering issues around biocontrol establishment in classical biological control programs. Through the lens of adaptive phenotypic plasticity, it is clear that desirable fitness traits such as body size and oviposition can be increased in *A. itadori* by altering rearing conditions. The individual pair bioassay approach used here has allowed us to examine individual variation and performance in psyllids under a variety conditions, something rarely done with biocontrol agents. These bioassays have shed light on the importance of psyllid age and its resiliency to various host plant qualities, and this will help inform future release program decisions.
References


Appendix A

The composition of Cornell Soilless Mix used as plant growth medium for all experiments:

- 2 parts - Horticultural grade sphagnum peat moss  
  - horticultural grade
- 4 parts - Vermiculite, medium horticultural grade
- 2 parts - Heat treated montmorillonite clay mineral (e.g. Turface MVP)
- 2 parts - Fertilizer mix composed of:
  - 1000g Calcium Carbonate powder, “O” grade, CAS # 471-34-1
    - Minimum of 37% calcium
    - Maximum of 1% magnesium
  - 1500g Osmocote 18-6-12
  - 1200g Monocalcium-dicalcium phosphate
    - Phosphorous not less than 21.00%
    - Calcium not more than 22.00%, not less than 18.00%
    - Fluoride not more than 0.21%
  - 15g Iron Chelates
    - Iron (Fe actual) chelated 13.2%,
    - Chelating agent ethylenediaminetetraacetate, 68%
  - 7g Zinc Chelates
    - Zinc (Zn actual) chelated 14%,
    - Chelating agent ethylenediaminetetraacetate, 68%