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Efficacy and host specificity compared between two populations of the psyllid *Aphalara itadori*, candidates for biological control of invasive knotweeds in North America

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HIGHLIGHTS

- Two populations of the psyllid *Aphalara itadori* are effective at reducing knotweed growth and biomass.
- The two populations differ in their performance among different knotweed species.
- Development of *A. itadori* occurred infrequently on several non-target plant species.
- The psyllid exhibited non-preference and an inability to persist on non-target plants.

GRAPHICAL ABSTRACT

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ABSTRACT

Invasive knotweeds are large perennial herbs in the Polygonaceae in the genus *Fallopia* that are native to Asia and invasive in North America. They include *Fallopia japonica* (Japanese knotweed), *F. sachalinensis* (giant knotweed), and a hybrid species *F.* x *bohemica* (Bohemian knotweed). Widespread throughout the continent and difficult to control by mechanical or chemical methods, these plants are good targets for classical biological control. We examined the suitability of two populations of the psyllid *Aphalara itadori* from Japan as biological control agents by comparing their impact on the target weeds and assessing their fundamental host ranges. Both populations were capable of halting knotweed plant growth and reducing both above and below ground biomass by more than 50% in just 50 days. Moreover, the psyllids caused mortality of several of the plants during this period. The two populations differed markedly in their reproductive potential on the different knotweed species. The Kyushu psyllid performed best on *F. japonica* and *F. bohemica* and the Hokkaido psyllid performed best on *F. sachalinensis*. Both were found to be specialized to knotweeds, with only very low occurrence of development on a small number of related non-target plant species. For the few non-target plant species that supported development, choice tests and multi-generational tests were used to further evaluate the likelihood of non-target host use. We conclude that *A. itadori* would be both effective and low risk as a biological control agent for invasive knotweeds and that both the Kyushu and Hokkaido populations may be needed to effectively control the entire knotweed species complex.

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1. Introduction

Invasive knotweeds are a complex of three closely related species in the family Polygonaceae that were introduced to North America from Japan during the late 19th century (Barney 2006). They include *Fallopia sachalinensis* (F. Schmidt) Ronse Decraene (giant knotweed), *F. japonica* (Houtt.) Ronse Decr. (Japanese knotweed), and the hybrid between these two, *F.* x *bohemica* (Chrtek & Chrtková) J. P. Bailey (Bohemian or hybrid knotweed). These large herbaceous perennials have spread throughout much of North America with the greatest infestations in the Pacific Northwest, the northeastern United States, and eastern Canada. Knotweeds are present in at least 41 U.S. states and eight Canadian provinces and are classified as noxious in eight states (USDA NRCS, 2011). Knotweeds are also invasive in much of Europe (Bailey, 2005) and parts of New Zealand (Williams and Hayes, 2007) and Australia (Sheppard et al., 2006). While capable of growing in diverse habitats, these knotweeds have become especially problematic along the banks and floodplains of rivers and streams, where they crowd out native plants and potentially affect stream nutrients and food webs (Beerling and Dawah ,1993; Maerz et al., 2005; Gerber et al., 2008; Urgenson et al., 2009; McIver and Grevstad , 2010). Several states and provinces have active control programs against knotweeds. However, the large scale of the knotweed invasion in North America, the inaccessibility of some of the infestations, and the difficulty with which the plants are killed, all suggest that the complete eradication of knotweeds is not feasible. Classical biological control has the potential to provide widespread and sustained reduction in knotweed abundance at a very low cost.

A potential agent for biological control of knotweeds is the psyllid, *Aphalaria itadori* Shinji which was previously described by Shaw et al., (2009). All stages of the knotweed psyllid feed by inserting sucking mouthparts into the phloem cells of the leaves and stems and removing sap. Adult female psyllids lay up to 600–700 eggs on the plant surface during their lifetime (Shaw et al., 2009). Eggs hatch after about 12 days and the nymphs pass through five instars. A full generation requires 33 days at 23 °C. While feeding, nymphs excrete crystallized honeydew (forming lerp) that is conspicuous as white strings or flakes on the plant surfaces. Adult *A. itadori* are winged and can fly. However, whether there is a distinct flight season and how far they can fly are unknown. The psyllids overwinter as adults. In Japan, they have been found wintering in the bark of *Pinus densiflora* Zieb and Zucc and *Cryptomeria japonica* D. Don (Miyatake, 1973, 2001; Baba and Miyatake, 1982). In the introduced range they are expected to use conifers for winter shelter as is the case for other *Aphalara* species (Hodkinson, 2009).

The native range of *A. itadori* includes Japan, Korea, and the Kurile and Sakhalin Islands (Burckhardt and Lauterer, 1997). In our surveys in Japan, it was found from sea level to 2150 meters above sea level (Shaw et al., 2009). *A. itadori* is relatively uncommon in Japan despite the abundance of its host plants. It was not found in the majority of the sites we visited in a 17 day survey in Japan in 2007. The psyllid is attacked by at least one parasitic wasp in Japan (possibly *Tamarixia* sp) that was found in a late stage nymph (Shaw et al., 2009).

Aphalara itadori is reported as being host specific to *F. japonica* and *F. sachalinensis* (Burckhardt and Lauterer, 1997). There are at least 17 species in the genus *Aphalara* occurring primarily in Eurasia (Burckhardt and Lauterer, 1997). As a group, the genus *Aphalara* is restricted to hosts within the Polygonaceae including *Rumex, Persicaria, Polygonum* and *Fallopia,* with most *Aphalara* species restricted to just one or a few closely related plant species (Burckhardt and Lauterer, 1997).

Here we present the results of impact and host-specificity testing of two populations of the knotweed-feeding psyllid *Aphalara itadori* Shinji, which are candidates for release into the United States and Canada. One population was collected from *F. japonica* on the island of Kyushu in the south of Japan (Shaw et al., 2009). This population was recently released as a biological control agent for knotweed in the United Kingdom (Djeddour and Shaw, 2011; Shaw et al., 2011). A second population was collected from *F. sachalinensis* on the Island of Hokkaido in northern Japan in 2007. We compared the impacts of these two populations on different knotweed species and measured their ability and propensity to oviposit and develop on 70 different North American plant species using single choice, multiple choice and generational tests where appropriate.

2. Materials and methods

2.1. Source of populations

The Kyushu (southern) population of *A. itadori* was collected in Kumamoto prefecture between the elevations of 747 m and 838 m on the Island of Kyushu in 2004 (Shaw et al., 2009). A colony was maintained in quarantine since this time by CABI in the United Kingdom. Starter colonies of this population were transferred to Insect-Microbial Containment Facility (IMCF) at AAFC-Lethbridge in 2008 and subsequently to Oregon State University Quarantine Facility in Corvallis, OR in 2010 for use in host range testing experiments. The Hokkaido population was collected from three sites, all in the vicinity of Lake Toya on Hokkaido in July 2007. These collections were pooled and then divided, with half of the insects going to the IMCF at AAFC-Lethbridge, Alberta, Canada and the other half to Oregon State University in Corvallis Oregon, USA. Upon arrival at the facilities, and over the next several generations, the insects were carefully examined for signs of parasites or other species that may have been collected accidentally. None were found.

The two psyllids do not appear to differ morphologically. DNA sequence variation between the two populations in the mitochondrial CO1 region was found to be about 1%, well within the expected range of variation within a species (E. Maw, Agriculture and Agri-food Canada, unpublished data).

2.2. Impacts on knotweed

In order to estimate the potential effectiveness of the two psyllid populations as biological control agents, impact experiments were carried out on *F. sachalinensis* and *F.* x *bohemica*. Rhizomes were collected from eight knotweed stands in western Oregon or southwest Washington. Pure *F. japonica* was not locally available and was not included in this experiment. The rhizomes were placed into trays of water until they sprouted. They were then grouped into blocks of three plants of the same species (confirmed through DNA analysis by J. Gaskin, USDA Agricultural Research Service), same collection site, and same initial rhizome/shoot size. The rhizomes were planted into pots of soil (3.79 liter volume) with 3 g of Osmocote brand fertilizer (N:P:K = 14:14:14) mixed in. The initial rhizome sizes varied across blocks from 6 to 13 cm. When the plant shoots had grown to between 10 and 16 cm tall, they were placed into fine mesh sleeve cages that were designed to fit tightly around the plastic pot and loosely around the plant.

The three treatments were applied randomly to the plants within each block. The three treatments were: 10 pairs of the Hokkaido psyllid, 10 pairs of the Kyushu psyllid, or no psyllids (control). After 50 days, F_1 adult psyllids were counted and the above and below-ground biomass was harvested, dried, and weighed. The experiment included seven replicate blocks of *F.* x *bohemica* and five

replicate blocks of *F. sachalinensis*. Differences between treatments in plant biomass and numbers of adults developing were assessed using separate analysis of variance for each plant species. The number of F1 adults was log-transformed prior to analysis. Tukey HSD post hoc tests were used to compare differences between treatments.

2.3. Test plant list

Our list of test plants used in the host specificity testing (Table 2) is based on a centrifugal phylogenetic approach (Wapshere, 1974) in which closely related taxa are emphasized more than distant taxa. All categories of native and economically important plants recommended by the Technical Advisory Group (TAG) on Biological Control of Weeds (USDA-APHIS, 1998) were included. The TAG is a panel representing 15 government agencies that reviews proposed weed biocontrol introductions prior to approval or rejection by the U.S. Department of Agriculture. A total of 70 plant species or varieties were tested, including several plant species that were added following the TAG review of a proposed test plant list. The test list considers the most recent molecular phylogenetic classification of the Polygonaceae by Sanchez et al. (2011) and we use nomenclature consistent with the Flora of North America North of Mexico vol. 5 (Freeman and Reveal, 2005). The test plants were selected from across North America and include three target weeds, six ornamental varieties of the target weed, 54 plants in the same family as the target weeds (Polygonaceae), and seven plants in families different from that of the target weed. The test list included ample coverage of plants within the same tribe. For more distant taxonomic groups within the family, selection of plants favored more common species, which occurred in the same habitats as the target (more likely to be encountered by the biological control agents), and which were morphologically similar to the target (e.g. larger and leafier species). All state, provincial, and federally listed threatened and endangered species in the Polygonaceae were either tested or represented using a closely related surrogate species.

The closest relatives of the target plants in North America are two native and three introduced *Fallopia* species and at least one introduced species in the closely allied genus *Muehlenbeckia* (Sanchez et al., 2009). *Fallopia cilinodis* (Michaux) Holub. (fringed bindweed) and *F. scandens* (Linnaeus) Holub (climbing buckwheat) are native, perennial, herbaceous vines (Freeman and Reveal, 2005). *F. cilinodis* occurs in dry woods, thickets, and clearings throughout much of the northeastern and midwestern United States and eastern Canada. The range of *F. scandens* is similar but extends further south to the Gulf States. It occurs in low habitats including moist woods and thickets. *Fallopia baldschuanica* (Regal) Holub (Russian vine or silver lace vine) is a cultivated woody ornamental vine from Eurasia. It is widely distributed in garden plantings in the United

Table 1
Number of dead and live plants of *Fallopia sachalinensis* and *Fallopia x bohemica* after 50 days exposure to either the Hokkaido or Kyushu biotype of *Aphalara itadori*.

	Number of dead / live plants after 50 days exposure	
	Fallopia sachalinensis	*Fallopia x bohemica*
Hokkaido psyllid	4 / 2	1 / 7
Kyushu psyllid	0 / 6	1 / 7
Control	0 / 6	0 / 8

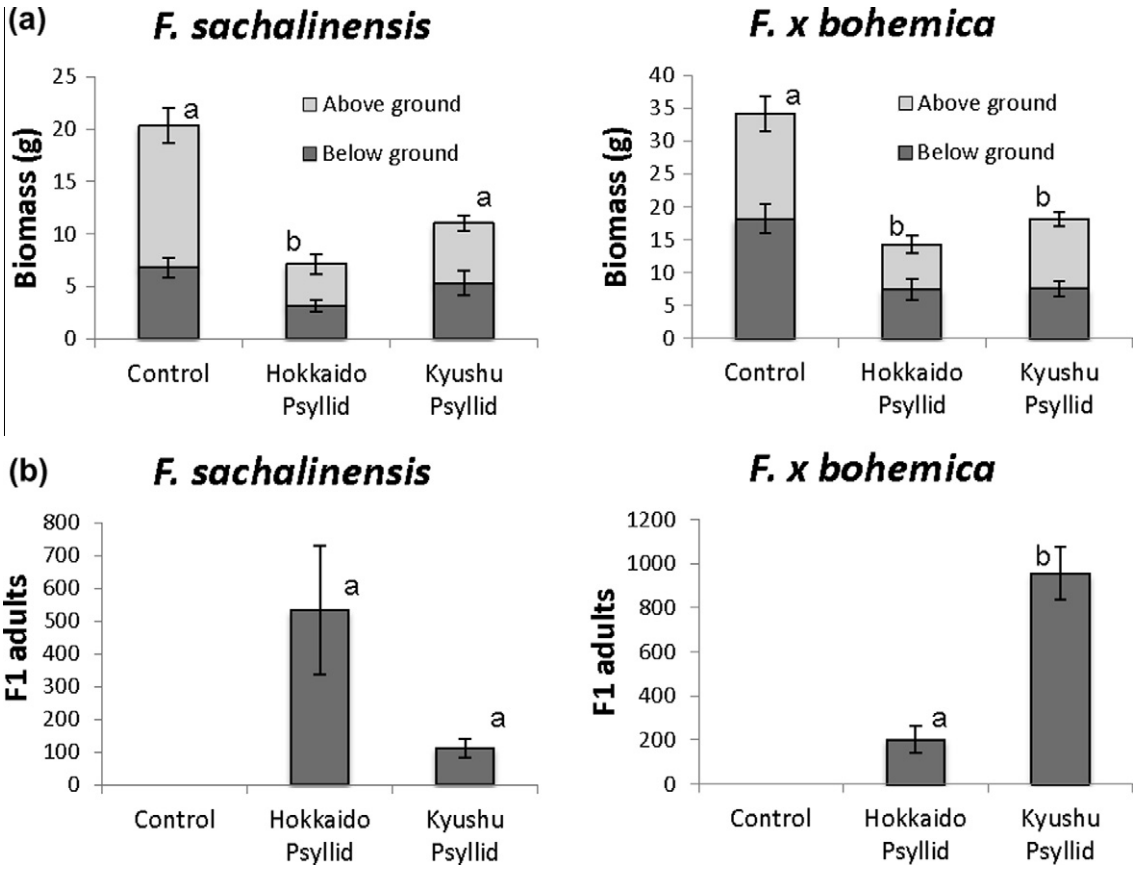

Fig. 1. (a) Final plant biomass and (b) numbers of F1 adults on *Fallopia sachalinensis* and *F.* x *bohemica* after plants were exposed to 20 pairs of Hokkaido or Kyushu biotypes of *Aphalara itadori* and their offspring for 50 days. Error bars represent one standard error. N = 5 for *F. sachalinensis*. N = 7 for *F.* x *bohemica*. Bars labeled with a different letter within each graph are significantly different from each other based on Tukey HSD post hoc tests.

Table 2
Mean numbers of *Aphalara itadori* eggs deposited and F1 adults that developed on each test plant species (± 1 S.E.) following exposure to adult psyllids of either the Hokkaido or Kyushu population. The test type used and location of the test are indicated for each plant species and psyllid biotype combination.

Taxon species		Hokkaido psyllid					Kyushu psyllid				
	Nat-ivity	Eggs	F1 Adults	N	Test type	Location	Eggs	F1 Adults	N	Test type	Location
Family Polygonaceae											
Subfamily Polygonoideae											
Tribe Polygoneae											
Target species											
Fallopia x bohemica OR	I	247.18 ± 34.64	11.60 ± 5.27	18	No-choice	Oregon	167.64 ± 33.90	86.46 ± 15.10	7	No-choice	Oregon
Fallopia x bohemica U.K.[a]	I						169.42 ± 41.45	NA	12	Multi	UK
Fallopia sachalinensis OR	I	160.31 ± 9.27	76.83 ± 5.83	107, 87[b]	No-choice	Oregon	113.04 ± 20.74	73.96 ± 31.70	6	No-choice	Oregon
Fallopia sachalinensis U.K.[a]	I						30.39 ± 6.96	NA	18	Multi	UK
Fallopia japonica OR	I	124.67 ± 19.87	0.95 ± 0.57	7	No-choice	Oregon	161.85 ± 22.82	73.33 ± 18.84	12	No-choice	Oregon
Fallopia japonica U.K.[a]	I						207.07 ± 15.99	NA	75	No choice	UK
Varieties of target species											
Fallopia japonica var. variegata	I	183.00 ± 21.06	126.00 ± 28.16	9	No-choice	Oregon	125.00 ± 38.65	81.00 ± 32.48	6	No-choice	UK
Fallopia japonica var. 'compacta'	I	120.50 ± 16.50	20.83 ± 3.38	6	No-choice	Oregon	36.75 ± 9.92	NA	6	Multi	UK
Fallopia japonica var. 'spectabile'	I	125.00 ± 9.61	67.17 ± 16.55	6	No-choice	Oregon					
Fallopia japonica var. 'crimson'	I	61.33 ± 7.56	12.00 ± 5.50	6	No-choice	Oregon	361.17 ± 113.30	143.67 ± 50.00	6	Multi	UK
Fallopia japonica var. 'freckles'	I	121.50 ± 15.50	7.50 ± 0.50	2	No-choice	Oregon					
Fallopia japonica var. 'tricolor'	I	146.00 ± 28.26	1.17 ± 0.98	6	No-choice	Oregon					
Non-target species											
Fallopia cilinodis	N	39.31 ± 14.13	6.98 ± 4.65	8	No-choice	Oregon	19.67 ± 8.95	7.50 ± 7.10	6	No-choice	UK
Fallopia baldshuanica	I	61.02 ± 17.81	0.14 ± 0.14	7	No-choice	Oregon	6.67 ± 1.98	0.00	15	Multi[c]	UK
Fallopia scandens	N	61.00 ± 17.34	0.00	6	No-choice	Oregon	17.50 ± 4.68	0.00	6	No-choice	UK
Fallopia convolvulus	I	60.10 ± 27.03	0.00	6	No-choice	Oregon	7.09 ± 3.08	0.00	6	Multi[c]	UK
Fallopia dumetorum	I	29.33 ± 6.90	0.00	6	No-choice	Oregon	7.75 ± 2.89	0.00	12	Multi[c]	UK
Muehlenbeckia axillaris	I	12.00 ± 1.77	5.17 ± 1.51	6	No-choice	Oregon	34.83 ± 18.57	3.67 ± 1.73	6	No-choice	UK
Polygonum douglasii	N	0.00	0.00	6	No-choice	Oregon	5.17 ± 2.97	0.67 ± 0.42	6	No-choice	UK
Polygonum aviculare	N	5.14 ± 2.51	0.00	7	No-choice	Oregon	0.33 ± 0.33	0.00	6	No-choice	UK
Polygonum achoreum	N	1.67 ± 1.67	0.00	6	No-choice	Oregon	3.18 ± 2.17	0.09 ± 0.09	11	No-choice	UK
Polygonum ramosissimum	N	0.00	0.00	5	No-choice	Oregon	0.00	0.00	6	Multi	Canada
Polygonum paronychia	N	7.00 ± 2.64	0.00	6	No-choice	Oregon	0.17 ± 0.17	0.00	6	No-choice	UK
Polygonum shastense	N	1.83 ± 1.47	0.00	6	No-choice	Oregon	6.50 ± 5.25	0.00	4	No-choice	Oregon and UK
Polygonum maritimum	N	2.83 ± 2.83	0.00	6	No-choice	Oregon	0.00	0.00	6	No-choice	UK
Polygonella robusta	N	2.00 ± 2.00	0.00	6	No-choice	Oregon	1.38 ± 0.66	0.00	6	No-choice	Oregon
Polygonella articulata	N	4.00 ± 4.00	0.00	4	No-choice	Oregon	0.00	0.00	6	Multi	UK
Tribe Rumiceae											
Rheum rabarbarum	I	68.75 ± 13.82	0.00	6	No-choice	Oregon	15.17 ± 3.64	0.00	6	No-choice	Oregon
Rheum palmatum	I	14.17 ± 5.54	0.00	6	No-choice	Oregon	17.33 ± 7.00	0.00	6	Multi	UK
Oxyria digyna	N	29.76 ± 10.28	0.00	6	No-choice	Oregon	3.58 ± 3.05	0.00	12	Multi	UK
Rumex acetosa	N	11.67 ± 6.18	0.00	6	No-choice	Oregon	0.00	0.00	12	Multi	UK
Rumex acetosella	N	0.50 ± 0.34	0.00	6	No-choice	Oregon	0.00	0.00	6	Multi	UK
Rumex arcticus	N	0.00	0.00	6	No-choice	Oregon	0.17 ± 0.17	0.00	6	No-choice	UK
Rumex britannica	N	0.00	0.00	6	No-choice	Oregon	0.00	0.00	6	Multi	UK
Rumex fuegenis	N	0.00	0.00	6	No-choice	Oregon	0.00	0.00	6	Multi	UK
Rumex occidentalis	N	3.74 ± 2.13	0.00	6	No-choice	Oregon	0.00	0.00	6	Multi	UK
Rumex orthoneurus	N	1.83 ± 1.47	0.00	6	No-choice	Oregon	0.21 ± 0.21	0.00	6	No-choice	Oregon
Rumex sanguinius	I	0.83 ± 0.83	0.00	6	No-choice	Oregon	0.00	0.00	6	No-choice	UK
Rumex scutatus	I	2.00 ± 1.48	0.00	6	No-choice	Oregon	0.00	0.00	6	Multi	UK
Rumex triangulivalvis	N	0.00	0.00	6	No-choice	Oregon	0.00	0.00	6	Multi	UK
Tribe Fagopyreae											
Fagopyrum esculentum	I	79.25 ± 21.91	3.18 ± 2.17	8	No-choice	Oregon	4.13 ± 1.77	0.13 ± 0.13	8	No-choice	UK
Fagopyrum tataricum	I	20.63 ± 5.29	0.00	6	No-choice	Oregon	1.22 ± 1.10	0.00	9	No-choice	UK

Tribe Persicarieae											
Aconogonon phytolaccaefolium	N	0.67 ± 0.67	0.00	6	No-choice	Oregon	0.67 ± 0.67	0.00	6	No-choice	UK
Persicaria affinis	I	0.00	0.00	6	No-choice	Oregon	0.00	0.00	6	Multi	UK
Persicaria amplexicaulis	I	3.67 ± 2.45	0.00	6	No-choice	Oregon	0.00	0.00	6	No-choice	UK
Persicaria hydropiperoides	N	1.33 ± 1.33	0.00	6	No-choice	Oregon	0.00	0.00	6	Multi	UK
Persicaria lapathifolia	N	0.00	0.00	6	No-choice	Oregon	0.00	0.00	6	Multi	UK
Persicaria microcephala	I	2.83 ± 1.72	0.00	6	No-choice	Oregon	0.00	0.00	6	No-choice	
Persicaria orientalis	I	0.00	0.00	6	No-choice	Oregon	2.43 ± 2.43	0.00	7	No-choice	UK
Persicaria pensylvanica	N	3.31 ± 1.86	0.00	6	No-choice	Oregon	0.00	0.00	6	Multi	UK
Persicaria sagittata	N	0.00	0.00	7	No-choice	Oregon	0.00	0.00	6	Multi	UK
Persicaria virginiana	N	0.00	0.00	6	No-choice	Oregon	0.00	0.00	6	Multi	UK
Persicaria wallichii	I	3.17 ± 1.94	0.00	6	No-choice	Oregon	0.83 ± 0.48	0.00	6	No-choice	Oregon
Bistorta vivipara	N	0.00	0.00	6	No-choice	Oregon	0.00	0.00	6	Multi	UK
Bistorta bistortoides	N	0.00	0.00	6	No-choice	Oregon	0.00	0.00	6	Multi	UK
Subfamily Eriogonoideae											
Tribe Brunnichieae											
Antigonon leptopus	N	19.13 ± 4.09	0.00	12	No-choice	Oregon	10.33 ± 3.09	0.00	6	No-choice	UK
Brunnichia ovata OR	N	0.83 ± 0.83	0.00	6	No-choice	Oregon	9.33 ± 4.59	0.00	6	No-choice	OR
Brunnichia ovata U.K.	N						9.33 ± 4.10	0.33 ± 0.20	6	No-choice	UK
Tribe Coccolobiae											
Coccoloba uvifera	N	0.00	0.00	6	No-choice	Oregon	3.83 ± 1.94	0.00	6	No-choice	UK
Tribe Eriogoneae											
Chorizanthe membranacea	N	12.00 ± 2.44	0.00	6	No-choice	Oregon	0.50 ± 0.50	0.00	6	No-choice	UK
Eriogonum parishii	N	0.00	0.00	6	No-choice	Oregon	0.00	0.00	6	No-choice	Oregon
Eriogonum cernuum	N	0.00	0.00	6	Multi	Canada	0.00	0.00	6	Multi	Canada
Eriogonum elatum	N	0.33 ± 0.33	0.00	6	No-choice	Oregon	0.00	0.00	6	No-choice	UK
Eriogonum nudum	N	0.00	0.00	6	No-choice	Oregon	1.00 ± 1.00	0.00	6	No-choice	UK
Eriogonum pyrolifolium	N	0.00	0.00	6	No-choice	Oregon	2.00 ± 2.00	0.00	6	No-choice	UK
Eriogonum umbellatum	N	0.00	0.00	6	No-choice	Oregon	0.00	0.00	6	Multi	UK
Oxytheca dendroidea	N	0.00	0.00	6	No-choice	Oregon	0.00	0.00	6	No-choice	Oregon
Family Plumbaginaceae											
Armeria maritima	N	0.00	0.00	6	No-choice	Oregon	0.00	0.00	6	No-choice	UK
Limonium carolinianum	N	0.00	0.00	6	No-choice	Oregon	0.00	0.00	6	No-choice	UK
Family Brassicaceae											
Brassica oleracea	I	0.00	0.00	6	No-choice	Oregon	0.00	0.00	6	No-choice	UK
Family Caryophyllaceae											
Dianthus gratianopolitanus	I	0.00	0.00	6	No-choice	Oregon	0.00	0.00	6	No-choice	UK
Family Ericaceae											
Vaccinium macrocarpon	N	0.00	0.00	6	No-choice	Oregon	0.00	0.00	6	No-choice	UK
Family Poaceae											
Zea mays	I	0.00	0.00	6	No-choice	Oregon	0.00	0.00	6	Multi	UK
Family Pinaceae											
Pseudotsuga mensiezii	N	0.00	0.00	6	No-choice	Oregon	0.00	0.00	6	No-choice	UK

[a] North American knotweed plants tested in the U.K.
[b] $N = 107$ for oviposition, $N = 87$ for development to F1 adults.
[c] Multi-choice tests of *F. baldshuanica, F.dumetorum, and F. convolvulus* did not include *F. japonica* in the arrays.

Table 3
Number of F1, F2, and F3 generation adults resulting in repeated attempts to rear *Aphalara itadori* on non-target plant species. Populations were initiated with the specified number of adults or with eggs (in parentheses) that were deposited on plants in oviposition choice tests.

Psyllid biotype	Non-target plant	Trial #	Initial adults or (eggs)	F1 adults	F2 adults	F3 adults
Hokkaido	*F. cilinodis*	1	30	3	0	0
		2	20	1	0	0
		3	(239)	5	0	0
		4	30	1	0	0
	M. axillaris	1	20	11	0	0
		2	30	0	0	0
	F. esculentum	1	(88)	5	2	0
		2	(39)	0	0	0
		3	(34)	5	2	0
		4	20	0	0	0
		5	(36)	1	0	0
Kyushu	*F. cilinodis*	1	30	0	0	0
		2	30	1	0	0
	M. axillaris	1	(41)	3	13	56
	F. esculentum	1	20	0	0	0
		2	20	0	0	0
		3	20	0	0	0
		4	60	0	0	0

States, occasionally escaping cultivation. *Fallopia dumetorum* (L.) Holub (copse bindweed) and *F. convolvulus* (L.) Á. Löve (black bindweed) are introduced weedy plants without ornamental value. The former is similar to the native *F. scandens* except for its annual habit (Freeman and Reveal, 2005). It occurs primarily in the eastern half of the United States and Canada. The latter, also an annual, occurs throughout temperate North America and can be an aggressive crop weed. *Muehlenbeckia axillaris* (Hook.f.) Endl. (creeping wirevine) is an introduced ornamental plant occasionally used as a ground cover in U.S.D.A. hardiness zones 5–10. Recent molecular evidence places the genus *Muehlenbeckia* in the same clade with the vining *Fallopia* spp. (Sanchez et al., 2009).

2.4. Host specificity testing

Host specificity testing for the North American biocontrol program was carried out primarily at the Oregon State University Quarantine Facility and at the CABI quarantine facility in the United Kingdom. Most of the test plants were tested in a no-choice design, where insects were caged onto individual plants. This provides the most conservative test of specificity and identifies the fundamental host range (plants on which the insect can complete its life cycle) (Schaffner, 2001). However, some of the early testing of the Kyushu psyllid involved exposing insects, in multiple-choice tests, to two or three plant species at once. Two non-target species that had not survived several attempts at shipping for testing to either Oregon or the UK were tested in Canada, in a multiple-choice design.

2.4.1. No-choice tests

All of the host specificity trials for Hokkaido (northern) population and over half of the trials carried out for the Kyushu (southern) population were of the no-choice type, in which the insects were caged onto individual test plants. Individual potted plants grown in a greenhouse in 3.79 liter pots served as the experimental unit. The size of the test plants varied, but they were matched with knotweed control plants that were of similar size, except in cases where test plant species were very small at maturity. A fine mesh sleeve cage was fit tightly around the rim of the pot and loosely over the plant. For each replicate, five pairs of *A. itadori* were placed into each cage for five days. At the end of five days, the adults were removed and the plants were searched for eggs under a magnifying lens. The plants were kept watered and fertilized for six to eight weeks, sufficient time to allow any F1 adults to emerge. The presence or absence of developing nymphs was noted during the development period. This helped to verify that any adults found at the end of the experiment actually developed on the plant.

Groups of plant species were tested in blocks, each block containing one of each of several test plant species plus one target plant to serve as a positive control. Initial experiments revealed that Hokkaido population of *A. itadori* performed best on *F. sachalinensis*, while the Kyushu population performed best on *F. japonica*. *F. sachalinensis* was therefore used as the positive control for tests with the Hokkaido psyllid and *F. japonica* for tests with the Kyushu psyllid. In some cases, all six replicate blocks were tested simultaneously, but at other times the blocks were replicated through time as necessary based on the availability of test plants of the right stage. If the insects did not reproduce on the positive control plant (this happened only once), then the entire block was discarded and the experiment repeated. Each plant species was tested at least six times with a few exceptions (see Table 2). Statistical comparisons between the two psyllid populations were made for the number of eggs deposited and the numbers of F1 adults developing using the two-sample Student T-test. The data were log-transformed prior to analysis. These tests were limited to several key plant species on which development occurred and for which the testing was done using identical methods (no-choice tests). In addition, one-way analysis of variance was used to test differences in the number of adults developing among the three knotweed species.

2.4.2. Multiple-choice tests with Kyushu psyllid

For the Kyushu psyllid, 30 test plant species were tested in a multiple-choice test rather than a no-choice test. Three plants of each of two non-target plant species were interspersed with three target plants (nine plants total) in a cage with 30 psyllids (see Shaw et al., 2009). After seven days of exposure the adults were removed and the eggs were counted. Any non-target plants that received eggs were isolated from the target weed controls and maintained long enough to determine the number of F1 adults that developed.

Differences in the number of plants, psyllids, and exposure days used in the two approaches to host range testing (no-choice and multiple-choice) mean that the data from these two sets of tests are not directly comparable. Plants in the multi-choice tests performed on the Kyushu psyllid had just under half of the exposure time to female psyllids as did the plants in the no-choice tests. (No-Choice: 5 females per 1 plant for 5 days = 25 female days per plant; versus Multi-Choice: 15 females per 9 plants for 7 days = 17.5 or 11.7 female days per plant).

2.5. Oviposition choice tests

To further evaluate the host-range of *A. itadori*, oviposition choice tests were carried out for three non-target plant species identified in no-choice tests as marginal hosts for one or the other of the psyllid populations. The plant species tested this way included *F. cilinodis*, *M. axillaris*, and *Fagopyrum esculentum* Moench (buckwheat). Hereafter *Fagopyrum* is abbreviated *Fg.* to distinguish it from *Fallopia.*). In these tests, three of the non-target plant species were interspersed with three *F. sachalinensis* (for Hokkaido psyllid) or *F. japonica* plants (for Kyushu psyllid) of similar size in a cage measuring 61 × 91 × 61 cm. Twenty psyllids (10 pairs) were released from a vial that was placed in the center of the cage. After five days, the

number of eggs on each plant was counted. The test was repeated three times for each of the three focal non-target plant species.

The significance of oviposition preference was tested using a one sample *T*-test that compared the natural log of the odds ratio of total eggs on each plant species within each cage (ln(Eggs on nontarget/Eggs on target) to the expected value of zero if there were no preference.

2.6. Multiple generation tests

On the same three marginal host plant species subjected to choice tests above, we tested the ability of *A. itadori* populations to persist for multiple generations. In these tests, cages were set up with 2–3 pots of the focal non-target plant. In some trials, the populations were initiated with plants that received eggs in the choice tests. In other cases, 20, 30 or 60 adult psyllids were placed into the cage with the plants (as reported in Table 3). Fresh plants were added to the cage as needed when the original plants began to die back. The plants were searched for the presence of psyllids after a period of time equal to that required for one, two, and three generations.

3. Results

3.1. Impacts on knotweed

Both populations of *A. itadori* significantly reduced the growth of *F. sachalinensis* and *F. x bohemica* resulting in more than a 50% reduction in biomass after 50 days exposure as compared to controls (Fig. 1a). Interestingly, reductions in biomass occurred even if the psyllid population did not reproduce well on the plant (Fig. 1b). Reduced growth of the plant and damage to the meristems appeared to occur as a result of feeding by early instar nymphs before most of the nymph mortality occurred.

Patterns of reproductive success of the two populations on the two hosts were opposite of each other. On *F. x bohemica*, five times more of the Kyushu psyllid developed into adults than the Hokkaido psyllid (ANOVA: $F_{1,7} = 13.24$, $p = 0.008$). On *F. sachalinensis*, five times more of the Hokkaido psyllid developed than the Kyushu psyllid.This latter difference was non-significant owing to high variance in the numbers of Hokkaido adults developing per plant (range from 0 to 992) (ANOVA: $F_{1,4} = 0.170$, $p = 0.70$). Low numbers of F1 adults corresponded with high plant damage levels suggesting that resource limitation affected the ability of nymphs to successfully develop, particularly for the Hokkaido psyllid on *F. sachalinensis*. Overall, the mean numbers of F1 adults presented (Fig. 1b) are likely to be underestimates of reproductive potential of *A. itadori* in situations where plant resources are not limited.

Several of the experimental plants died toward the end of the exposure period. A majority of these cases (four out of six) were *F. sachalinensis* exposed to the Hokkaido psyllid (Table 1).

3.2. No-choice tests

Both psyllid populations exhibited a high degree of specialization to the knotweed species with very little development occurring on other plant species (Table 2). The two populations differed notably in their rates of development on different knotweeds reflecting the results of the impact experiment above. They also differed slightly in their use of non-target plants. The specific outcomes for each population follow.

3.2.1. Hokkaido psyllid

Within the knotweeds, the Hokkaido psyllid performed best on *Fallopia sachalinensis* with a mean of 77 F1 adults developing per plant. This population also did well on certain ornamental varieties of *F. japonica* (especially var. '*variegata*', var. '*spectabile*', and var. '*compacta*') (Table 2). We found it had very low nymphal survival on wild collected *F. japonica* with a mean of just under one developing adult per plant. On *F.* x *bohemica*, just under 12 adults developed per plant on average. Post hoc tests (Tukey HSD) indicated that successful development by the Hokkaido psyllid on *F. sachalinensis* was significantly higher than on the other two knotweed species, but that the difference in development on *F. x bohemica* and *F. japonica* was not significant.

Oviposition by the Hokkaido psyllid occurred on many of the non-target test plants in the no-choice tests, but at much reduced rates compared to *F. sachalinensis* controls (Table 2). Development occurred at very low rates on four non-target test plants: *F. baldschuanica, F. cilinodis*, *M. axillaris*, and *Fg. esculentum*. Development of a single individual occurred on *F. baldschuanica.* On the other three plants, the number of individuals developing to adult was in the range of 4–10% of the number developing on *F. sachalinensis*. Development was delayed on the non-targets species. On *Fg. esculentum*, it took 52.5 ± 2.4 d (at approximately 23 °C) for all nymphs to complete development (mean ± S.E. for $N = 4$ observed cohorts). *F. cilinodis*, it took 47.7 ± 2.9 d ($N = 3$) and on *M. axillaris*, it took 46.0 ± 1.2 d ($N = 3$). This compares to 43.4 ± 0.40 d on *F. sachalinensis* controls.

3.2.2. Kyushu psyllid

In the no-choice tests, the Kyushu population oviposited and developed well on all three target weed species (means of 73–86 F1 adults per plant) (Table 2), but with high variability among individual plants, especially *F. sachalinensis*. The Kyushu population also performed well on the two ornamental cultivars that were tested (*F. japonica* var. *crimson* and *F. japonica* var. *variegata*). Patterns of oviposition among non-target plants were similar to those of the Hokkaido psyllid, except that the number of eggs laid on non-target plants was often lower for the Kyushu psyllid (71% of non-zero cases). Part of this disparity is associated with the lower overall exposure times for some of the plant species to Kyushu females in the multiple choice tests versus no-choice tests. For some of the key species, *F. cilinodis*, *F. scandens*, *M. axillaris*, and *Fg. esculentum* (buckwheat), both populations were tested using the same no-choice methods (though in different quarantine facilities). On these plants, differences in oviposition rates and adult development were not significant, with one exception. With the same exposure time on buckwheat, the Kyushu psyllid laid significantly fewer eggs than did the Hokkaido psyllid (two sample *t*-test: $t_{15} = 5.41$; $p < 0.005$).

Like the Hokkaido psyllid, low rates of development occurred on *F. cilinodis* (mean and SE of 7.50 ± 7.10 F1 adults per plant) and *M. axillaris* (3.67 ± 1.63 adults per plant). In addition, extremely low rates of development were detected on *Polygonum douglasii* Greene (total of four adults from six plants), *Polygonum achoreum* S.F. Blake (total of one adult on 11 plants), *Fg. esculentum* (total of one adult on eight plants), and *Brunnichia ovata* (Walter) Shinners (total of two adults on 12 plants). Development was slow on these non-target plants. The times required for all nymphs to develop (at approximately 23 °C) were 50.5 ± 1.7 d for *Fg. esculentum* (mean ± S.E. for $N = 4$ observed cohorts), 60.0 ± 5 d for *F. cilinodis* ($N = 3$), 48.0 ± 8 d for *M. axillaris* ($N = 4$), 60 ± 0 d for *B. ovata* ($N = 2$), 62.0 ± 1.0 d for *P. douglasii* ($N = 2$), and 70 d for *P. achoreum* ($N = 1$). These compare to 42 d for complete cohort development on *F. japonica* (Shaw et al., 2009).

3.3. Oviposition choice tests

When offered a choice, both populations of *A. itadori* exhibited a strong bias toward oviposition on the target plant vs. non-target plants (Fig. 2). *Aphalara itadori* females from Hokkaido laid 96% of their eggs on knotweed controls versus 4% on *M. axillaris*; 98%

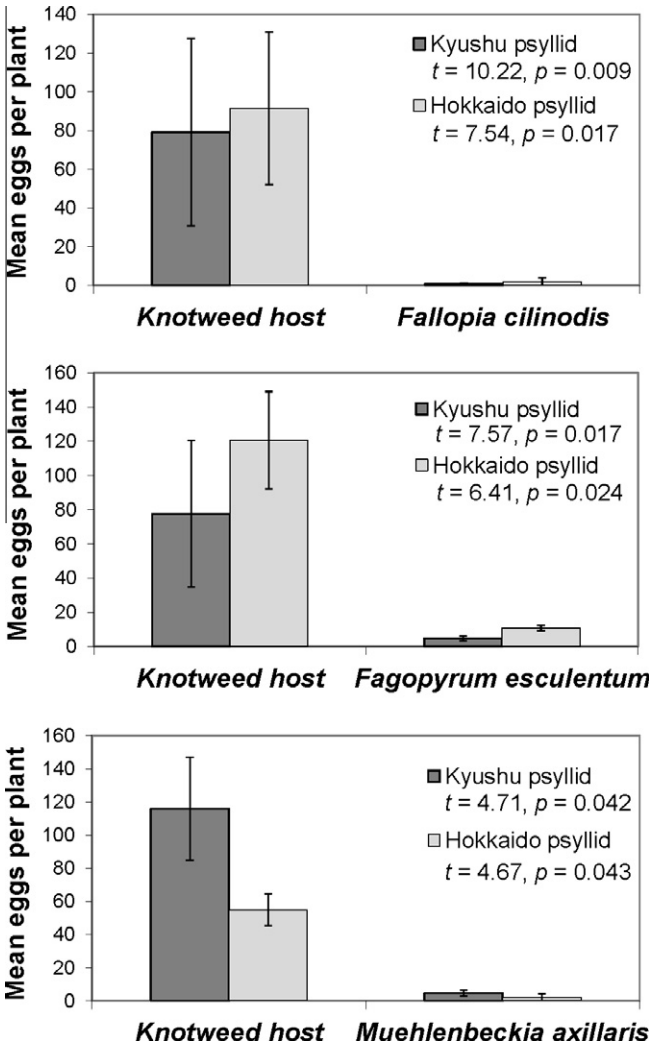

Fig. 2. Number of eggs laid on target vs. non-target plant in choice tests. The knotweed species used in the tests was *Fallopia japonica* for the Kyushu psyllid and *F. sachalinensis* for the Hokkaido psyllid. Each bar is the grand mean eggs per plant for 3 plants in each of 3 replicate cages. Error bars represent one standard error. T-test statistics and significance values for the difference in the logarithm of the odds ratio (eggs on knotweed / eggs on non-target) from the null hypothesis of zero are provided in the legend for each psyllid source.

on knotweed vs. 2% on *F. cilinodis*; and 92% on knotweed vs. 8% on *Fg. esculentum*. Results for the Kyushu psyllid were similar (Fig. 2) with 96% on knotweed vs. 4% on *M. axillaris*; 98% on knotweed vs. 2% on *F. cilinodis*; and 94% on knotweed vs. 6% on *F. esculentum*.

3.4. Multiple generation tests

Both the Kyushu and Hokkaido populations were incapable of sustaining themselves on either *Fallopia cilinodis* or *Fg. esculentum* (Table 3). In these tests, reproduction often failed in the very first generation. Where F1 adults developed, they either did not reproduce a second time, or there were declining numbers for the F2 generation. Only one combination, the Kyushu psyllid on *M. axillaris*, resulted in an expanding population. Numbers went from 41 initial eggs to 3 F1adults to 13 F2 adults to 56 F3 adults. At this point the plants senesced and the psyllids also died off.

4. Discussion

Both the Kyushu and the Hokkaido populations of the knotweed psyllid *Aphalara itadori* were very effective at reducing the above- and below-ground biomass of knotweed in a greenhouse setting. Feeding by psyllid nymphs slowed the growth of plants by damaging meristem tissue and caused necrosis of the leaves. Several plants died during a 50-day exposure period with the greatest mortality found for the Hokkaido psyllid feeding on *F. sachalinensis*. Also, potted knotweed plants (all 3 species) routinely died in our psyllid rearing colonies. In the field, knotweed plants are much larger than our potted greenhouse plants and have extensive energy stores in their roots. *A. itadori* should be an effective biocontrol agent provided that it is able to build up to and sustain high population densities. However, it could take multiple years to reach these densities and impact the plants.

The two populations differed notably in their reproductive success on different knotweed species, which will likely influence the population level effectiveness in the field. The Hokkaido psyllid clearly performed best on *F. sachalinensis* in both the no-choice tests and the impact experiment. The Kyushu psyllid performed well on all three knotweed species in the no-choice test, but showed reduced performance on *F. sachalinensis* in the impact experiment. (The different outcomes on *F. sachalinensis*, including ovipositon rates between the UK and US testing (Table 2), may be related to the particular genotypes used in the experiments.) The two psyllids also differed in their performance on ornamental varieties of knotweed. Based on the differences in performance on different host plants, we propose that the two populations could be considered distinct biotypes or, more specifically, distinct host races (Diehl and Bush 1984). Both host races are likely to be needed to fully control the varied knotweed populations in North America. If *A. itadori* is approved for release, the appropriate host race should be selected for each release location based on the knotweed species present.

Throughout North America, local knotweed populations vary greatly in the amount of genetic diversity they contain, from one extreme of a single clone spreading vegetatively throughout a river system, to single species populations with multiple genotypes, to diverse mixes of species and introgressed hybrid genotypes (Gammon et al., 2007). A few dominant genotypes (spread clonally) are vastly more common than others (Gaskin and Grevstad unpublished). Thus it would be valuable to compare the effectiveness of the two psyllid host races on specific targeted knotweed genotypes in advance of their release. Where there is a mixture of knotweed species, but only one of the two host races is released, there is the possibility for the resistant knotweed genotypes to increase in frequency in a situation of self-defeating biocontrol (Garcia-Rossi et al., 2003). Such a situation has been documented in only one weed biocontrol system (as reviewed by Hufbauer and Roderick, 2005) in a case involving a pathogen introduced for rush skeletonweed (*Chondrilla juncea* L.) in Australia (Burdon et al. 1981).

Further experimentation is also needed to determine if impact and host specificity are altered when the two *A. itadori* host races interbreed, which will inevitably occur in the field if both are released in proximity. In another biocontrol program, Hoffmann et al. (2002) showed that the performance of two biotypes of the cochineal scale *Dactylopius opuntiae*, each specializing on a different species of *Opuntia*, was altered in the F1 and F2 generation crosses and this may have diminished the agent's effectiveness in South Africa. In preliminary tests, we crossed the two knotweed psyllid lines and successfully reared offspring on clones of all three knotweed species, but have not yet completed tests with F2 crosses (Bourchier, unpublished).

Beyond the knotweeds, the two psyllid populations were found to have similarly narrow fundamental host ranges. For the vast majority of non-target plants tested, there was either no oviposition or low oviposition without development to the adult stage. Development did occur at low rates on two close relatives of

knotweed, *M. axillaris* and *Fallopia cilinodis*, and at extremely low rates on *Fg. esculentum* (both biotypes), *Fallopia baldschuanica* (Hokkaido only), *Polygonum douglasii* (Kyushu only), *Polygonum achoreum* (Kyushu only), and *Brunnichia ovata* (Kyushu only). Development was always slower on these non-target plants than on the knotweeds, which would further limit the viability of these populations in the field. Differences between the two psyllids in their use of non-targets were minor. While the Kyushu psyllid developed on more species, the low numbers of adults developing were not significantly different from the zero values obtained for the Hokkaido psyllid. One significant difference was that the Hokkaido psyllid, tested in Oregon, laid more eggs on *F. esculentum* than the Kyushu psyllid, tested in the United Kingdom. However, we cannot rule out the confounding influence of different quarantine facilities and different times.

Given the possibility for development to occur on some of the non-targets, additional tests help to determine the likelihood that these plants would be used in the field as part of their realized host range (sensu Schaffner 2001). In general, the fundamental host range of phytophagous insects found in laboratory studies is broader than the realized host range in the field where environmental cues and influences tend to further limit the number of hosts used (Balciunas et al. 1996; Marohasy 1998; Baars et al., 2000; DeClerck-Floate and Schwarzlander, 2002; Briese 2005; Pratt et al. 2009). When adult psyllids were offered a choice, both biotypes laid many more eggs on the target plants versus the non-target plants. In repeated multiple generation tests, *A. itadori* was unable to sustain populations on the non-target plants with one exception being the Kyushu population on the introduced ornamental *M. axillaris*. Given the expanding size of the caged population from 20 to 56 over three generations, we feel that there is the possibility that this plant could be used as a host in the field. *M. axillaris* is reported as naturalized outside of cultivation only in Hawaii (US Plants Database) and sold commercially in the United States as a ground cover (USDA hardiness zones 8–10) and filler for container plantings. Two congeners *M. complexa* and *M. hastatula* (both also introduced) are reported from one and two counties in California (US Plants Database) where they are locally invasive (Pollak 2008; Baldwin et al. 2012). Neither was found to be commercially available, suggesting a lack of importance of these two plants in the nursery trade. In working with *M. axillaris*, we found that it was much more vulnerable to generalist horticultural pests such aphids, scale insects, and spider mites than it was to *A. itadori*. Thus colonization of *M. axillaris* by *A. itadori*, if it occurs, is unlikely to cause an added pest burden to the nursery trade.

The native plant *Fallopia cilinodis* and the economically important crop plant *Fg. esculentum* could not sustain ongoing populations of either psyllid so it is unlikely that these plants would attract or be harmed by the psyllid in the field. However, where these plants grow in close proximity to knotweeds, some transient feeding by stray psyllids could occur. It should be noted that buckwheat is a common crop throughout the native range of *A. itadori*, yet *A. itadori* is not recorded as a pest of buckwheat in its native range (Japanese Society of Applied Entomology, 1987). Field choice tests at knotweed biocontrol release sites in the United Kingdom are currently underway to further aid in predicting field specificity of *A. itadori*.

The fact that some individuals of *Aphalara itadori* were capable of developing on several non-target plant species raises a question of whether the agent population could evolve an increased ability to use these hosts over time (Secord and Kareiva 1996). In fact true host shifts, involving change in genetic frequency allowing increased performance on a new host, have not been convincingly documented in any weed biocontrol agent (van Klinken and Edwards 2002; Hufbauer and Roderick 2005). Moreover, in a review of the field host specificity of 117 introduced weed biocontrol agents in the United States and Carribean, none were found to use hosts beyond those predicted from the pre-release host specificity tests (Pemberton 2000) suggesting that the narrow host ranges of introduced specialist insects are stable. In the case of *A. itadori*, the near zero fitness of individuals that attempt to use non-target plants might instead provide selection for behavioral avoidance of these plants and maintenance of specificity to knotweeds where fitness is much higher.

As with any classical biocontrol introduction, the introduction of *A. itadori* into North America would not be 100% risk-free. The level of development occurring on non-target plants falls within the range of agents that have been previously approved and released without incident of harm to non-target plants in the field, including the melaleuca psyllid *Boreioglycaspis melaleucae* (Center et al. 2007) and others (Blossey et al. 2001; Paynter et al. 2004; Breiter and Seastedt 2007). The small risk of introduction of *Aphalara itadori* should be weighed in relation to the known detrimental effects for multiple species of allowing knotweeds continue to spread and degrade fragile riparian habitats.

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