

**A Pest Risk Analysis for *Aphalara itadori* for
the European Union (EU) Member States in
North Western Europe**

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A Pest Risk Analysis of *Aphalara itadori* for the European Union (EU) Member States in North Western Europe Compiled Using the CAPRA Software Developed by the European and Mediterranean Plant Protection Organization (EPPO) (EPPO Standard PM 5/3(5)).

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The original PRA for the United Kingdom (UK) was produced by Richard Shaw, Alex Brook and René Eschen. This PRA for Northwestern Europe is based upon the original PRA for the UK and updated by Kate Constantine, Suzy Wood, Marion Seier and Richard Shaw, with supporting documentation produced and compiled by Alex Brook.

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Executive summary

A Pest Risk Assessment (PRA) for the psyllid *Aphalara itadori* (Shinji), as a biological control agent for Japanese knotweed (*Fallopia japonica*), was compiled previously for the United Kingdom (UK) and submitted to the relevant UK authority DEFRA in 2009. Based on this PRA, a peer review and a public consultation, the psyllid was approved for release in the UK in 2010. This current PRA was requested by The Netherlands and is based on the PRA prepared for the UK, incorporating additional data of relevance to other North Western European Union Member States (including The Netherlands). The PRA was completed using the PRATIQUE Computer Assisted Pest Risk Analysis (CAPRA) developed by the European and Mediterranean Plant Protection Organisation (EPPO). The baseline data in the PRA were collected and compiled by CABI and are supported by up-to-date references.

- *Fallopia japonica* is one of the top 100 invasive species in the world according to IUCN listing. It is a highly invasive non-native perennial species introduced into Europe in the late 19th century as a prized ornamental. Since its introduction from Japan, where it is native, *F. japonica* has spread throughout invaded countries, outcompeting native flora and fauna and affecting human activities and the economy. Chemical and manual controls are difficult and costly to implement. The total annual cost of Japanese knotweed to the British economy is estimated at £166 million (Williams *et al.*, 2010). The cost to the EU due to invasive species has been estimated to be at least €12 billion every year.
- Since 2000, CABI has researched the potential for classical biological control of *F. japonica*. This management approach utilises co-evolved, host-specific, natural enemies of the target weed, collected from its native range, for sustainable, permanent control of the weed in its invasive range. The psyllid *Aphalara itadori* under evaluation was collected on *F. japonica* in the Mount Aso region of Kyushu (Kumamoto Prefecture), Japan (“Kyushu strain”) and subsequently imported into CABI’s quarantine for evaluation.
- *Aphalara itadori* is specialized to Japanese knotweed and its closely related congener species and varieties in Japan. It completes its whole lifecycle on the following species in Japan: *Fallopia japonica*, *Fallopia japonica* var. *compacta*, *Fallopia sachalinensis*. The following plant species can also be included as hosts, after studies have shown them capable of receiving eggs able to develop fully to adults: *Fallopia x bohemica* and *Fallopia conollyana*. All of these plants can be considered to be within the fundamental host range of *A. itadori*.
- The nymphs are the main feeding, and thus damaging stage of the psyllid. Since nymphs are largely sessile, host selection is determined by the adult’s oviposition preferences, which appears to limit the choice for completion of the lifecycle to five varieties of *Fallopia*.
- Due to their sap-sucking activities, the nymphs are capable of killing potted plants of Japanese knotweed if present in high numbers. Manipulative studies in the CABI, UK quarantine laboratory revealed that *A. itadori* can reduce the rate of growth of *F.*

japonica var. *japonica* and significantly increase leaf count under relatively low nymph loads (White, 2007). Such proliferation of very small leaves at the growing points of the plant is detrimental to its performance and leads to a net reduction in photosynthetic area. In the field in Japan, *A. itadori* nymphs have been observed causing significant damage to mature plants of Japanese knotweed, stunting growth, limiting leaf expansion and reducing flowering (personal observations, R. Shaw).

- The psyllid is tolerant of a wide range of temperatures which includes those normally experienced in The Netherlands during the growing season of the plant. Thus the psyllid will be suitable for release in The Netherlands and other North Western European countries.
- Although the overwintering habit of the psyllid is not well known, recent research in the UK has found that living psyllids can be recovered from trunk sections of *Pinus sylvestris* and *Quercus robur*. They were also found to overwinter on *F. japonica*. While it is unknown whether this takes place on dead canes, in leaf litter or soil, the findings suggest that it is not essential for winter hosts to be alive, but rather that the microhabitat provided by mature tree trunks is important for overwintering success (Clewley and Wright, 2014).
- The psyllid has been tested against a comprehensive test plant list including 73 species selected for UK evaluation. In addition to these, a further 9 species have been tested for North America, plus retesting of 4 species already assessed for the UK but using plants of North American origin (Grevstad *et al.*, 2013). Ten additional non-target species were evaluated on behalf of The Netherlands within UK facilities, plus retesting of 3 species previously assessed but using plants of Dutch origin. The selected test plant species included native, ornamental, economically important and related introduced species.
- *A. itadori* is not recorded as a vector of any plant pathogens. Furthermore, the complete absence of pathogenic agents on its normal food plant, Japanese knotweed, in The Netherlands means it is highly unlikely to transmit a pathogen.
- It is envisaged that following the release of the psyllid, *F. japonica* populations will, over time, diminish in area and density allowing native plant species to recolonize invaded areas.
- A new regulation on invasive alien species has been adopted by the Council of the European Union on 29 September 2014. The new legislation “Regulation (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species” has now been published in the Official Journal of the European Union. The regulation seeks to address the problem of invasive alien species in a comprehensive manner so as to protect native biodiversity and ecosystem services, as well as to minimize and mitigate the human health or economic impacts that these species can have. The legislation foresees three types of interventions; prevention, early warning and rapid response, and management. A list of invasive alien species of Union concern will be drawn up with Member States using risk

assessments and scientific evidence. (See: http://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1415116378291&uri=OJ%3AJOL_2014_317_R_0003).

- In The Netherlands the Nature Conservation Act and specifically the Flora and Fauna Act protects native biodiversity from invasive, potentially harmful, exotic species such as Japanese knotweed.
(See: <http://www.government.nl/issues/nature-and-biodiversity/legislation-on-nature-conservation-in-the-netherlands>).
- In conclusion, this PRA has identified no risks to native biodiversity or to any economically significant plant species, from the release of the psyllid in the PRA area. Potential negative impacts on the economy and society, related to possible reduction of income for weed control specialists and for traders of the invasive ornamental *Fallopia japonica* var. *compacta*, are very low, while the financial benefits that could be gained from successful control of this invasive species by biological control are high.

Guideline on Pest Risk Analysis
Decision-support scheme for quarantine pests Version N°5

Pest Risk Analysis for *Aphalara itadori* in North Western European Union (EU) Member States requested and funded by The Netherlands Food and Consumer Product Safety Authority.

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Stage 1: Initiation

1.01 - Give the reason for performing the PRA

Other reason

1.01b - If other reason, specify

Request for assessing the risk associated with a potential release of the "beneficial pest", the psyllid *Aphalara itadori*, as a biological control agent for the non-native invasive plant species *Fallopia japonica* (Japanese Knotweed) in The Netherlands. The request is made on behalf of the Bureau for Risk Assessment and Research Programming (BuRO) of The Netherlands Food and Consumer Product Safety Authority (NVWA). The PRA-area should include not only The Netherlands but, as far as feasible, other North Western (NW) EU-countries.

1.01c – Enter the name of the pathway

Deliberate introduction

1.02a - Name of the pest

Aphalara itadori (Shinji). Common names include *Itadori madarakijirami* (Japanese for “Japanese knotweed speckled psyllid”) and the English translation Japanese knotweed psyllid.

1.02b - Indicate the type of the pest

Arthropod

1.02c – N/A

1.02d - Indicate the taxonomic position

Aphalara itadori [Insecta: Hemiptera : Psyllidae]

1.03 - Clearly define the PRA area

The PRA area incorporates NW European Union (EU) Member States: The Netherlands (who requested this PRA) and other EU countries, namely Austria, Belgium, Czech Republic, Denmark, France, Germany, Poland and Switzerland.

1.04 - Does a relevant earlier PRA exist?

Yes

A previous PRA was compiled with the United Kingdom (UK) as the PRA area and submitted to the relevant authority (DEFRA) in 2009 (available upon request from CABI). Based on the PRA, peer review and a public consultation the psyllid *Aphalara itadori* was approved for release in the UK 2010. This current PRA is based on the PRA for the UK incorporating additional data of relevance for The Netherlands and the remaining NW EU Member States as the PRA area.

1.05 - Is the earlier PRA still entirely valid, or only partly valid (out of date, applied in different circumstances, for a similar but distinct pest, for another area with similar

conditions)? Explain your judgement (edit in the part justification)

Not entirely valid

The PRA is still entirely valid for the original PRA area, the United Kingdom, but as such is applicable in slightly different circumstances for a different PRA area. This current PRA is based on the original PRA, but modified to take into consideration the different geographic position of NW Europe with different climatic and environmental conditions and economic and social characteristics. There have been some scientific developments thanks to research funded by various sponsors including The Netherlands and UK since the original PRA and these are incorporated in this PRA.

1.06 - Specify all host plant species (for pests directly affecting plants) or suitable habitats (for non-parasitic plants). Indicate the ones which are present in the PRA area.

Host is defined in the Dictionary of Entomology (Gordh & Headrick, 2000) as “an organism which supplies nutrients or protection essential for the development of another organism”. *Aphalara itadori* is a specialist on Japanese knotweed and its closely related congener species and varieties in Japan. It completes its whole lifecycle on the following species in the wild in Japan:

1. Japanese knotweed, *Fallopia japonica* (Houtt.) Ronse Decraene var. *japonica* [Polygonaceae], syn. *Polygonum cuspidatum*, syn. *Reynoutria japonica*. Widespread plant in Central and Southern Japan. The native range of *Fallopia japonica* includes East Asia to Japan, Korea, Taiwan, and northern China. *Fallopia japonica* is invasive in most European countries.

2. Compact knotweed – *Fallopia japonica* var. *compacta* (Hook.f.) J.P.Bailey, an invasive hybrid of *F. japonica*, is found on the upper slopes of Japanese mountains, and is a dwarf form with red flowers and small leaves with a wavy margin. Unlike *F. japonica*, which spreads clonally throughout Europe, compact knotweed has both functional female and male plants (Duistermaat *et al.*, 2012). In the PRA area, GBIF¹ holds 13 records of its distribution in the wild in Belgium, 1 in Germany and 1 in Sweden. Although there are no records available to indicate its presence in the wild in The Netherlands (GBIF; DAISIE²; NOBANIS³; nederlandsesoorten.nl⁴; soortenbank.nl⁵), *F. japonica* var. *compacta* is sold in the Dutch horticultural trade; the variegated variety ‘Milk Boy’ is available to purchase as a garden plant. This species is reported to be of limited distribution dependent on rare cultivation and subsequent escape in the Czech Republic (Mandák *et al.*, 2004). In Sweden it was noted in one province introduced probably from garden refuse (Lindstrom, 2002).

3. Giant knotweed – *Fallopia sachalinensis* (F.Schmidt ex Maxim.) Ronse Decraene, is

¹ Global Biodiversity Information Facility (<http://www.gbif.org/>)

² Delivering Alien Invasive Species Inventories for Europe (<http://www.europe-aliens.org/>)

³ The European Network on Invasive Alien Species (<http://www.nobanis.org/About.asp>)

⁴ Overzicht Van De Nederlandse Biodiversiteit (<http://www.nederlandsesoorten.nl/>)

⁵ SoortenBank.nl (<http://www.soortenbank.nl/>)

found in the Northern part of Japan from Sakhalin Island southwards through Hokkaido to Honshu, also an invasive plant across most of Europe but not yet as much of a problem as Japanese knotweed.

Data to support the prediction of the potential host-range of *A. itadori* in the UK (applicable to NW Europe) as the PRA area can be found in Shaw *et al.* (2009) (Appendix 4). Based on extensive laboratory host range oviposition studies the following species can also be included as hosts, as they have been shown capable of receiving eggs that were able to fully develop to adult. It is not certain that these exact varieties exist in the field in Japan, where the taxonomy is far more complicated than in the UK and Europe as a whole.

4. Bohemian knotweed – *Fallopia x bohemica* (Chrték and Chrtková) J.P. Bailey (hybrid of *F. japonica* & *F. sachalinensis*). Widespread invasive plant in the UK, Europe and North America and possible pollen source for *F. japonica* var. *japonica*. This hybrid is competitive and spreading at a fast rate in Central Europe.

5. Conolly's knotweed – *Fallopia conollyana* Bailey is the hybrid of *F. japonica* and Russian vine *F. baldschuanica*. It is known from only one location in the UK (Bailey, 2001), and there are no records of its presence in the NW EU PRA area including The Netherlands (Duistermaat *et al.*, 2012; GBIF; DAISIE; NOBANIS; nederlandsesoorten.nl; soortenbank.nl).

Therefore, the target *F. japonica*, the two other knotweed species *Fallopia x bohemica* and *Fallopia sachalinensis*, plus the other two species tested for the UK, *Fallopia japonica* var. *compacta* and *Fallopia conollyana*, can be considered to belong to the fundamental host range of *A. itadori*. Experimental work shows that they are the only species that were regularly selected for oviposition as well as being capable of supporting the complete development of the psyllid. Extensive host range testing has been carried out for *A. itadori* and it has been found that all eggs laid on any other non-target plants were incapable of developing to adults (see section 1.17).

Nymph transfer tests on some of the species that received eggs (*F. japonica* var. *japonica* and thirteen non-target species) showed that only two species: *Fallopia conollyana* and *Meuhlenbeckia complexa* supported development from 1st instar nymphs to adults in 48% and 7% of cases respectively (See section 1.17, section 6.02 and Appendix 4). However this was an artificial environment and would be unlikely to occur in the field where host selection is determined by the adult's oviposition preferences, and which, as nymphs are largely sessile, limits the choice for completion of the lifecycle to five species of *Fallopia*. Indeed during the initial UK 4-year project period in Japan (2003-2007), *A. itadori* was never found on any host plant other than Japanese knotweeds despite the fact that other members of the Polygonaceae were present and regularly examined in detail.

In addition, the potential role of evergreen trees as shelter plants for overwintering adults is detailed in section 3.2.

1.07 - Specify the pest distribution for a pest initiated PRA, or the distribution of the pests identified in 2b for pathway initiated PRA

The distribution of *A. itadori* is recorded as Japan, Korea, Russia, Sakhalin, Kurile Islands (Kwon 1983; Gegechkori & Loginova, 1990). The psyllid considered by this PRA is from the Mt. Aso region of Kyushu (Kumamoto Prefecture), the "Kyushu strain" so as to distinguish it from the "Hokkaido strain" being held by American and Canadian researchers. The "Kyushu strain" has also been released in the UK and is undergoing evaluation in Switzerland, the US and Canada for potential release in order to control invasive knotweeds.

Stage 2: Pest Risk Assessment Section A: Pest categorization

Identity of the pest (or potential pest)

1.08a – do you want to go to the main Pest Risk Assessment or continue with the Pest Categorization?

- continue with pest categorization

The psyllid *Aphalara itadori* needs to be classed as a beneficial pest, to be deliberately introduced as a biocontrol agent for the invasive *Fallopia japonica* in The Netherlands. As such the insect presents a risk, albeit deliberate and intended, to this plant species, thus a full PRA needs to be conducted.

1.08 - Does the name you have given for the organism correspond to a single taxonomic entity which can be adequately distinguished from other entities of the same rank?

Yes

Aphalara itadori can be distinguished from other members of the same genus and has been described by Shinji (1938) and Burkhardt & Lauterer (1997).

1.09 – N/A

1.10 - Is the organism in its area of current distribution a known pest (or vector of a pest) of plants or plant products?

Yes. *A. itadori* is a pest of Japanese and other related knotweeds ('Itadori' in Japanese) as its name suggests, which is the reason that it is seen as a potential beneficial biological control agent for controlling invasive knotweeds in Europe.

The organism is not recorded as a pest of agricultural or horticultural crops or forestry in Japan (JSAEZ, 2006). *A. itadori* is not recorded as a vector of any plant pathogen. The Central Science Laboratory (CSL) team in the UK (now FERA - Food and Environment Research Agency) found that there were no records of *A. itadori* acting as a vector of plant pathogens in its current area of distribution (see 6.14). However, in Japan, *A. itadori* could be implicated in the spread of the pathogen, *Puccinia polygoni-amphibii* var. *tovariae* Arthur, a highly damaging rust on Japanese knotweed which is not present in the EU. In

addition, the psyllid could also play a role in the lifecycle of the knotweed leaf-spot pathogen (*Mycosphaerella polygoni-cuspidati* Hara), native to Japan and absent in the EU, as a vector in the cross-fertilisation or mating by exchange of spermatia.

Psyllids are known vectors of plant pathogens such as bacteria, in the case of citrus greening disease (Halbert & Manjunath, 2004), and phytoplasmas e.g. apple proliferation disease (Tedeschi *et al.*, 2002). However, even if *A. itadori* is capable of vectoring a pathogen, the complete absence of candidate agents on its normal food plant, Japanese knotweed, in the EU means that even if it does use a native or commercial pine species as an overwintering host/shelter plant, it is highly unlikely for it to transmit a pathogen (on the presumption that it will not feed). Research published in 2010 (Reeder *et al.*, 2010) identified a phytoplasma in a knotweed sample from Berkshire, UK but it is in the “16Srii” group and therefore not in a group reported to be transmitted by psyllids (which are only known to transmit group 10 phytoplasmas). Compared to aphids, whiteflies and thrips, psyllids transmit fewer pathogens and do not have specific virus genera such as the thrip-transmitted tospoviruses for example, which they alone transmit. However, there are some psyllid-transmitted bacterial diseases that occur in some perennial and annual crops (Munyaneza, 2010). It is unlikely that *Aphalara* adults would use other host plants for food in the summer in the presence of the normal host Japanese knotweed but if they do it will most likely be plants in the same family, existing sympatrically. Such adults are unlikely to create a new pressure from vectored pathogens since other oligophagous or polyphagous sucking insects such as *Aphalara polygoni* already feed on, and between, members of the Polygonaceae. Thus, whilst there is a potential risk that *A. itadori* could transmit pathogens found in the EU to non-target plants; the risk is believed to be extremely low.

1.11 - Does the organism have intrinsic attributes that indicate that it could cause significant harm to plants?

Yes

The sap-sucking activities of the psyllid nymphs are capable of killing potted plants of *Fallopia japonica* under high loads. It is the nymphal stage that causes the most significant damage with adult feeding damage only evident by sporadic honeydew production. Laboratory experiments showed that adult feeding had no significant impact on the height, leaf count and leaf area of potted *F. japonica* plants and that adults do not appear to inflict any perceptible damage in the absence of nymphs (Birkin, 2008, supported by Wineriter *et al.*, 2003). A similar situation was revealed in studies of another psyllid, *Boreiglycaspis melaleucae*, released for the biological control of *Melaleuca quinquenervia* (Purcell *et al.*, 1997), and a study by Wineriter *et al.* (2003) which confirmed that adult feeding caused no short-term damage to the target plant.

As detailed under section 1.06 in this PRA, *A. itadori* can be very damaging to its preferred hosts (*F. japonica*, *F. x bohemica* and *F. sachalinensis*) as well as inflicting less significant damage to Conolly's Knotweed (*F. conollyana*) and Compact Knotweed (*F. japonica* var. *compacta*). The development of, and thus the feeding damage inflicted by, the psyllid nymphs on other closely related non-target species is severely compromised. Manipulative studies in the CABI UK quarantine laboratory revealed that *A. itadori* can reduce the rate of growth of *F. japonica* var. *japonica* and significantly increase leaf count under relatively low nymph loads (White, 2007). Such proliferation of very small leaves at

the growing points of the plant is detrimental to its performance and leads to a net reduction in photosynthetic area. In the field in Japan, *A. itadori* nymphs have been observed causing significant damage to mature plants of Japanese knotweed, stunting growth, limiting leaf expansion and reducing flowering (personal observations, R. Shaw).

1.12 - Does the pest occur in the PRA area?

No.

The psyllid has been intentionally released in England and Wales (not included in the PRA area considered here) but is not yet considered ordinarily resident. It is not currently present in the wild in any other EU country. It has been assessed for its suitability for release in The Netherlands at facilities of CABI, Bakeham Lane, Egham, Surrey, UK.

Outside the PRA area, *A. itadori* is also currently being assessed under quarantine conditions for its suitability for Switzerland at the CABI facilities in Delémont, Switzerland. In addition, host range testing and further studies have culminated in an application for release of the Kyushu strain in North America.

1.13 – N/A

1.14 - Does at least one host-plant species (for pests directly affecting plants) or one suitable habitat (for non-parasitic plants) occur in the PRA area (outdoors, in protected cultivation or both)?

Yes

Principal hosts:

A. itadori's main host, Japanese knotweed (*F. japonica*), which is believed to be clonal in the PRA area (Hollingsworth & Bailey, 2000) and the experimentally-susceptible hybrid *F. x bohémica* (Bailey *et al.* 1996), are widespread invasive weeds throughout the EU with their ranges continuing to expand. *F. x bohémica* is probably more widespread than the distribution maps suggest (Appendix 2) since it is relatively hard to distinguish from *F. japonica*. *F. sachalinensis*, a natural host in Japan, is also fairly widespread across Europe. The invasive knotweed species are found in riparian habitats and derelict land as well as along roadsides, lay-bys and railway embankments. The distribution of *F. conollyana*, an experimentally-susceptible host is much more limited than the other principal *Fallopia* hosts and there is no record of its presence in The Netherlands or the neighbouring EU countries. *F. japonica* var. *compacta*, a natural host in Japan is planted as an ornamental in gardens in the PRA area.

The overwintering habit of the psyllid is not well known and its shelter plants, in the absence of the primary knotweed host, which dies back at the first frost, are presumed to be evergreen trees (See section 3.2).

1.15a - Is transmission by a vector the only means by which the pest can spread naturally?

No.

No vector is needed. This is a free-living organism.

1.16 - Does the known area of current distribution of the pest include ecoclimatic conditions comparable with those of the PRA area or sufficiently similar for the pest to survive and thrive (consider also protected conditions)?

Yes.

Climate comparisons indicate a discrepancy between the native (Japan) and the introduced range (UK) of *A. itadori* (Myint *et al.*, 2012), with a wider range of temperatures experienced in Japan. This discrepancy in climate is also apparent between Japan and NW EU. However Japan's volcanic nature provides higher altitude sites which are comparable to NW EU, even on Kyushu Island which can be considered sub-tropical. Populations of *A. itadori* can be found on Kyushu Island from sea level to the top of Mount Aso (over 1,500m. above sea level) so it can be assumed to be very tolerant of climatic extremes. The culture under consideration was collected from the Mt. Aso region of Japan's Kumamoto Prefecture at relatively high altitudes. Studies of its thermal tolerance and degree-day requirements showed that no development took place at 10°C and very little at 12°C (see section 3.03 and Appendix 3).

Birkin (2008) reports that adult psyllids had a high tolerance for low temperatures, taking 4h for mortality to exceed 50% at -10°C. As a rule of thumb, species with a 10°C minimum threshold for reproduction have been considered to be marginal for the UK and this would likely be the same for NW EU (Baker & Bailey, 1979; Baker, 2002). An assessment of the climatic conditions in the PRA area is presented in Appendix 3. It is considered unlikely that the lowest extremes of temperature experienced where invasive knotweeds grow in NW EU will be a limiting factor to establishment. It should be noted that different population biotypes may be required if releases are planned over a wider climatic region, as has been considered in North America (Grevstad *et al.*, 2013), and may be of relevance for European countries with greater variations in temperatures.

In addition, for *A. itadori* to overwinter successfully in NW EU the climatic conditions must fall within the fundamental tolerances of the species and there must be suitable overwintering sites available. As their host plants dieback in late autumn, many psyllids overwinter in temperate regions as adults on shelter plants, often conifers (Hodkinson, 2009). *Pinus densiflora* Sieb. & Zucc. and *Cryptomeria japonica* (Thun. ex L. f.) D. Don are reported to be the principle winter hosts of *A. itadori* in their native range (Baba & Miyatake, 1982; Miyatake, 1973; Miyatake, 2001). Both these species have a sparse, low density distribution in Europe. However recent research has demonstrated psyllids overwintering on trunk sections of the close relative, Scot's pine, *Pinus sylvestris* L., which is common, and *Quercus robur* as well as overwintering on or near *F. japonica* (whether on dead canes, in leaf litter or soil is unknown). (See section 3.02, Appendix 2 and Clewley & Wright, 2014).

1.17 - With specific reference to the plant(s) or habitats which occur(s) in the PRA area, and the damage or loss caused by the pest in its area of current distribution, could the pest by itself, or acting as a vector, cause significant damage or loss to plants or other negative economic impacts (on the environment, on society, on export markets) through the effect on plant health in the PRA area?

Yes, *A. itadori* will intentionally damage invasive knotweeds and thereby have positive economic and ecological impacts; it will not pose a risk to other non-target plant species.

It is intended that *A. itadori* inflicts significant damage on invasive knotweed species. It is expected that a successful biological control agent would provide significant control and a reduction in current control effort and costs, not least through reduced use of chemicals. For example, the use of a psyllid, *Heteropsylla spinulosa* against *Mimosa invisa* in Papua New Guinea led to reductions in the cost of chemical control (Kuniata & Korowi, 2004). If successful, the release of *A. itadori* will provide positive rather than negative economic impacts. Recent research has confirmed that invasive knotweeds have a detrimental impact on biodiversity (Dassonville *et al.*, 2007, 2008; Gerber *et al.*, 2008; Topp *et al.*, 2008; Murrell *et al.*, 2011) which underlines the need to control these plants. There should also be a cost saving on knotweed management, and an improvement in the built environment, as the presence of invasive knotweed species places a burden on society due to the cost of control and also on urban developers (Pimentel, 2002; Rotherham, 2010). If successful in control of invasive knotweeds the psyllid may cause a decrease in the profits of some weed control companies who earn income from Japanese knotweed management.

The choice of host and therefore the potential damage is determined by the oviposition behaviour of the adult psyllids since the damaging nymph stages are mainly sessile. Adult oviposition was unaffected by cage conditions in quarantine, thus extensive multiple choice host range studies were carried out during the UK phase of the project. These have shown that, of the 87 test plant species assessed only 18 plant species received any eggs with the highest number being laid on *F. japonica* var. *japonica*. In total the location of over 146,855 eggs were recorded on individual plants and 98.5% of these were laid on species in the invasive knotweed group, namely *Fallopia japonica*, *F. x bohemica* and *F. sachalinensis*. Only 1.5% were laid on non-target species or varieties of other ornamental knotweed, *F. conollyana* and *F. japonica* var. *compacta* along with the invasive Russian vine, *F. baldshuanica*, accounting for another 1,422 eggs (0.97%). In additional tests conducted for invasive knotweeds present in The Netherlands (relevant to NW EU) adult development only occurred on the invasive knotweed species (*F. japonica*, *F. x bohemica* and *F. sachalinensis*) with minimal eggs laid in multiple choice tests on two non-target plant species (*Chenopodium bonus-henricus* and *Beta vulgaris* spp. *maritima*). It is essential to highlight that *A. itadori* only developed through all damaging nymph stages into adult on *Fallopia* species: *F. japonica*, *F. japonica* USA (Ithaca), *F. x bohemica*, *F. sachalinensis*, *F. conollyana* and *F. japonica* var. *compacta* (not *F. baldshuanica*). Although other plant species received eggs crucially limited or no further development (i.e. hatching or later nymph instars) occurred.

The development of nymph instars was not directly observed for fear of disturbing/damaging them. However, the presence of wax and the maximum development was recorded on the following non-knotweed species during the UK studies and highlights the inability of these species to support development of *A. itadori* through to adulthood:

Muehlenbeckia complexa – 3rd instar, dead by 27 days
Rheum palmatum – 1st instar, dead by 10 days
Fagopyrum esculentum – 2nd instar, dead by 11 days
Fallopia dumetorum – 2nd instar, dead by 13 days

Fallopia baldshuanica – 1st instar, dead by 14 days

In these instances, damage through nymph feeding would have been minimal, firstly because none developed to the larger more damaging instars, and secondly because the psyllids were not able to complete their lifecycle on these plants, and thus a viable population would not occur.

For details see excerpt in Table 2 below from Shaw *et al.*, 2009 amended to include respective NL non-target species (complete tables in Appendix 4 and 5).

The non-target species on which egg development occurred include *Fallopia japonica* var. *compacta* which is an ornamental plant not commonly sold and *F. conollyana* which is a non-native ornamental species restricted to gardens, possibly a garden escapee in some areas.

Excerpt from Table 2, Appendix 4:

Results of multiple choice oviposition studies carried out with adult *A. itadori* using various test plants (including additional plant species tested for The Netherlands) that received eggs. Development success was scored according to the relative number of adults that emerged (good = >50%, moderate = 10-50%, poor = <10%). For detailed information on these plant species see Appendix 1 for status and Appendix 2 for distribution maps in the EU.

Test plant	No replicates	Eggs laid	Eggs laid/rep	Development
<i>Fallopia japonica</i>	324	140,517	433.7	Good
<i>Fallopia Japonica</i> USA (<i>Ithaca</i>)	3	1,523	507.7	Good
<i>Fallopia x bohémica</i>	15	2,033	135.5	Good
<i>Fallopia conollyana</i>	15	866	57.7	Poor
<i>Fallopia japonica</i> var. <i>compacta</i>	12	441	36.8	Poor
<i>Fallopia sachalinensis</i>	18	547	30.4	Moderate
<i>Muehlenbeckia complexa</i>	7	132	18.9	-
<i>Rheum palmatum</i>	12	208	17.3	-
<i>Fagopyrum esculentum</i>	14	237	16.9	-
<i>Fallopia dumetorum</i>	12	93	7.8	-
<i>Fallopia convolvulus</i> ex. USA	11	78	7.1	-

<i>Fallopia baldshuanica</i>	15	100	6.7	-
<i>Oxyria digyna</i>	12	43	3.6	-
<i>Persicaria polystachyum</i>	6	15	2.5	-
<i>Rheum Glaskins</i>	9	8	0.9	-
<i>Fallopia convolvulus</i>	14	11	0.8	-
<i>Rumex hydrolaphum</i>	6	2	0.3	-
<i>Fagopyrum dibotrys</i>	9	1	0.1	-
Additional species:				
<i>Chenopodium bonus-henricus</i>	6	2	0.3	-
<i>Beta vulgaris spp. maritima</i>	6	1	0.2	-

(Further detail of the test results is covered in Section 6.02).

It is important to note that the host range testing contributing to this PRA has a Northern European country focus. Although based on current knowledge impact is unlikely to occur on any non-target species, it is recommended that the test plant is extended if release of the psyllid were to be considered for the rest of the continent, in particular the southern EU countries. For example, searches have revealed 2 species on the IUCN red list that have not been tested namely:

Persicaria salicifolia

Red list category and criteria: Least concern

Range Description:	This species occurs from Mediterranean Europe south to South Africa and east to the Arabian Peninsula. It is difficult to establish its precise range due to taxonomic confusion but it seems likely that it does not extend into the Indian Sub-continent or tropical Asia.
Conservation Actions:	The species is protected in the French region Languedoc-Rousillon. There are no other conservation measures in place or needed.

[See: <http://www.iucnredlist.org/details/164278/0>].

Polygonum romanum

Red list category and criteria: Least concern

Range Description:	This species is endemic to France, Spain and Italy. In France, it is found in Languedoc and Roussillon (France). In Spain, it is present in Girona and the Balearic Islands. It occurs in central and south Italy, in Sardinia, in seven regions in total.
Conservation	In France, the species is under regional protection in Languedoc and some populations are included in protected areas.

Actions:

It is recommended to monitor the existing sites as well as the population dynamics and to raise public awareness. Search for new sites and historic sites in Spain, the Balearic Islands and France is suggested.

[See: <http://www.iucnredlist.org/details/163966/0>].

This pest could present a phytosanitary risk to the PRA area.**1.18 - Summarize the main elements leading to this conclusion.**

The psyllid *A. itadori* will pose a phytosanitary risk to the invasive *F. japonica* and its varieties and hybrids, which is intended. As such the psyllid needs to be considered as a beneficial pest. Any potential economic and/or social benefits derived from this plant species will be impacted upon.

Even though oviposition studies showed that other species of *Fallopia* can support development from eggs to adults these are unlikely to be targeted.

Eradication of the target host plant species is rarely the outcome of classical biological control. If a situation occurred where there was a high level of psyllids with an insufficient level of the invasive knotweed host, which is very unlikely, theoretically spill-over effects could occur on the most closely related non-target species present in the area. However adult psyllids inflict minimal damage and development to adult has only been demonstrated on the invasive knotweed species as well as *F. conollyana* (a hybrid with limited distribution) and *F. japonica* var. *compacta* (an uncommon ornamental species). Any such instances are therefore likely to be transient and not support establishment of a viable population (see section 6.02).

Stage 2: Pest Risk Assessment Section B: Probability of entry of a pest

2.01a - Describe the relevant pathways and make a note of any obvious pathways that are impossible and record the reasons. Explain your judgement

Pathway 1 - Intentional release

Intentional release is the main (only relevant) pathway for the psyllid *A. itadori* (Kyushu strain) as a biological control agent from research facilities into the wild.

It is theoretically possible for establishment to be achieved with a single release of a large number of biocontrol agents. If initial establishment of the insect fails then repeated releases would be anticipated.

Subsequent spread aided by humans is likely to be intentional within the PRA area and possibly beyond.

Pathway 2 - Public introduction

It is theoretically possible that people with a knotweed problem, and knowledge of the psyllid having been released in the UK, intentionally and illegally introduce the psyllid either from the UK, or potentially even from Japan.

Pathway 3 - Accidental introduction

Through air currents or as a contaminant on travellers clothing or possessions.

2.01b - List the relevant pathways that will be considered for entry and/or management. Some pathways may not be considered in detail in the entry section due to lack of data but will be considered in the management part.

- Intentional release
- Illegal introduction
- Accidental arrival

Pathway 1: **Intentional release**

2.02 – Select the pathway to run the entry section:

Intentional release

2.03 - How likely is the pest to be associated with the pathway at the point(s) of origin taking into account the biology of the pest?

Very likely

Level of uncertainty: low

The psyllid would be supplied as a clean culture from the UK and deliberately introduced and released under licence with the aim of establishment.

2.04 - How likely is the pest to be associated with the pathway at the point(s) of origin taking into account *current management conditions*?

Moderately likely

Level of uncertainty: low

This question is not phrased appropriately for a biocontrol agent as this would be a deliberate introduction and release.

2.05 - Consider the volume of movement along the pathway (for periods when the pest is likely to be associated with it): how likely is it that this volume will support entry?

Very likely

Level of uncertainty: low

As the process constitutes a deliberate release of the psyllid into the wild in the PRA area with the intention of establishment the volume of movement is intended to ensure entry of the psyllid into the PRA area.

2.06 - Consider the frequency of movement along the pathway (for periods when the pest is likely to be associated with it): how likely is it that this frequency will support entry?

Very likely

Level of uncertainty: low

Releases of the psyllid into the PRA area will be made according to an agreed protocol in which the number of release sites and number of individuals of the psyllid reared and introduced to these sites will be specified. The protocol will aim to facilitate entry of the psyllid into the PRA area, thus the frequency of movement would need to support this.

2.07 - How likely is the pest to survive during transport or storage?

Very likely

Level of uncertainty: low

Survival of the psyllid is intended and will be ensured as it would be a deliberate introduction into the wild in the PRA area.

2.08 - How likely is the pest to multiply/increase in prevalence during transport or storage?

Very unlikely

Level of uncertainty: low

The development time from egg to adult in constant conditions (23°C) is 32.2 to 37 days ± 0.5 (n=42) (Appendix 4). Transported psyllids may mate and lay eggs during transit but will not develop further before arriving at their destination. If, for example, the psyllids are transported from the culture held in the UK on plant material and lay eggs, this material would be disposed of upon arrival and adults moved and cultured on target plant material from the host country before field release (depending on the agreed release strategy).

2.09 - Under current inspection procedures how likely is the pest to enter the PRA area undetected?

Likely

Level of uncertainty: low

This is assuming arrival of the psyllid and that this question refers to detection.

2.10 - How likely is the pest to be able to transfer from the pathway to a suitable host or habitat ?

Very likely

Level of uncertainty: low

The transfer of the psyllid onto its host invasive knotweeds is intended and suitable habitats will be selected to ensure this. As invasive knotweeds have the tendency to form dense stands it is expected that the psyllid will move throughout the population.

2.11 - The probability of entry for the pathway should be described

Very likely

Level of uncertainty: low

The probability is high as deliberate introduction will ensure entry of the psyllid into the wild in the PRA area.

2.11b – Tools (Genie entry)

2.12 – Do other pathways need to be considered?

No

2.13a – In the table below, you can change the order of pathways to reflect the outcome of the evaluation of entry for each pathway (in particular pathways presenting the main risk of entry should appear first).

2.13b - Describe the overall probability of entry taking into account the risk presented by different pathways and estimate the overall likelihood of entry into the PRA area for this pest (comment on the key issues that lead to this conclusion).

Very likely

Level of uncertainty: low

The entry of the Kyushu strain of the psyllid *A. itadori* into the PRA area is intended and will be ensured as it is a deliberate introduction of a biocontrol agent.

A potential arrival of the psyllid from the UK where the insect has been released as a biocontrol agent into the PRA area cannot be entirely ruled out.

An illegal introduction of *A. itadori*, either from the UK or from Japan, is less likely as this would require entomological background knowledge, and any establishment of the insect following such an introduction is questionable as this would require a "critical mass" of several hundred individuals.

Stage 2: Pest Risk Assessment Section B: Probability of establishment

In a first step, assessors should select the ecological factors that influence the potential for establishment.

Seven factors may influence the limits to the area of potential establishment and the suitability for establishment within this area:

- 1 - Host plants and suitable habitats
- 2 - Alternate hosts and other essential species
- 3 - Climatic suitability
- 4 - Other abiotic factors
- 5 - Competition and natural enemies
- 6 - The managed environment
- 7 - Protected cultivation

No.	Factor	Is the factor likely to have an influence on the limits to the area of potential establishment?	Is the factor likely to have an influence on the suitability of the area of potential establishment?	Justification
1	Host plants and suitable habitats (see note for Q3.01)	Yes (see 3.01)	Yes (see 3.09)	
2	Alternate hosts and other essential species	Yes (see 3.02)	Yes (see 3.10)	
3	Climatic suitability	Yes (see 3.03)	Yes (see 3.11)	
4	Other abiotic factors	Yes (see 3.04)	Yes (see 3.12)	
5	Competition and natural enemies	Yes (see 3.05)	Yes (see 3.13)	
6	The managed environment	No	No	The managed environment (agriculture, horticulture, forestry, managed gardens) provides no hosts. Management of Japanese knotweed in the form of chemical or manual control probably will not

				<p>favour establishment on a local level but is not carried out on a large enough scale to impact on the likelihood of establishment of <i>A. itadori</i>. As the psyllid would be intentionally released with the aim of establishment, none of the release sites selected would be subject to any chemical or manual management of Japanese Knotweed.</p>
7	Protected cultivation	No	No	<p><i>Aphalara itadori</i> is not a pest of crop species and has never been recorded in protected cultivation.</p>

Host plants and suitable habitats

3.01 - Identify and describe the area where the host plants or suitable habitats are present in the PRA area outside protected cultivation.

Psyllid feeding and development has been shown to be almost completely restricted to Japanese knotweed varieties and its hybrids and as such there are virtually no other suitable species or habitats available other than the very many knotweed infestations in the PRA area.

Japanese knotweed and its hybrids are very widely distributed in the PRA area (Appendix 3). Suitable habitats for the psyllid will be predominantly characterised by the presence of the invasive knotweeds as host species. Geographic regions in the PRA area not supporting the growth of *Fallopia japonica* are the higher altitude regions of NW EU member states as well as the more Mediterranean regions where the climate is too hot and dry - for instance, *F. japonica* does not appear on the DAISIE distribution map in southern Spain, southern Italy, Greece or Cyprus.

The species that are considered within the host range of the psyllid includes the target *F. japonica*, the two other invasive knotweeds *F. x bohemica* and *F. sachalinensis* as well as *F. japonica* var. *compacta* (Hook. f.) and the hybrid *F. conollyana*. The invasive knotweed species *F. japonica* and *F. x bohemica* are widely distributed in the PRA area and spreading. *F. sachalinensis* is present but less frequent. *F. japonica* var. *compacta* is an uncommon horticultural curiosity and there is only a single record of the hybrid *F. conollyana* in Europe (UK). This suggests these species are not likely to be present in areas where the intended target and other invasive knotweeds are present. It is only these non-target species which supported development from eggs to adult.

In terms of habitat, whilst there is no evidence to suggest that overwintering of the psyllid is reliant on a particular host species, it is believed that evergreen trees, such as Japanese pine, *Pinus densiflora* [Pinaceae] and Japanese cedar, *Cryptomeria japonica* [Taxodiaceae] act as overwintering or shelter plants in Japan in the absence of the primary knotweed host, which dies back at the first frost. These two evergreen tree species are both quite rare across Europe, however (as covered in section 3.02) it has been shown that the more common Scot's pine, *Pinus sylvestris* and *Quercus robur* can serve as overwintering hosts. In addition some psyllids have demonstrated overwintering when left on *F. japonica* suggesting *A. itadori* will not be limited by overwintering habitats (Appendix 3; Gary & Wright, 2014).

Alternate hosts and other essential species

3.02 - Does all the area identified in 3.01 have alternate hosts or other essential species if these are required to complete the pest's life cycle?

Yes (please provide justification)

Evergreen trees may provide the overwintering host/shelter for adult psyllids in the absence of knotweed, between the first frost and its emergence in spring, though it may be possible for overwintering to take place on leaf litter and even graminaceous species. In the summer, the intended target and preferred host for the psyllid includes both *F. japonica* and *F. x bohemica* which are widely distributed in the proposed PRA area. During winter surveys carried out by Japanese collaborators, adults of *A. itadori* proved to be too difficult to find on potential evergreen hosts, even where the summer psyllid population was found to be high. The survival of one individual from a population intentionally sleeved on Japanese cedar has been recorded in Fukuoka (Kyushu) over the winter period (N. Takahashi, personal communication, 2005).

Currently there is no evidence to suggest that overwintering of the psyllid is reliant on a particular host species, or with the exception of those *Fallopia* species listed 1.06, that it

causes any noticeable damage to other species in the absence of its normal food plant, *Fallopia japonica*. Hodkinson (1974) indicated in a review of general psyllid biology that it is not known whether overwintering adults feed on shelter plants, though a consideration of their moisture requirements would suggest they do. Ossiannilsson, (1992) stated that "most of our [Scandinavian] psyllids hibernate in the adult stage, a few on their host-plants (e.g. *Cacopsylla pyricola*-the pear psyllid) but most species on "shelter plants", usually conifers, or in crevices in bark or other protected sites". Ossiannilsson goes on to say "whether psyllids hibernating on conifers do actually feed on them, as was supported by Reuter (1909), is still unknown".

During experiments conducted in the initial UK phase of the project, adults of *A. itadori* were kept alive for 8 weeks at 5°C on a piece of *Pinus radiata* (radiata or Monterey pine) bark collected from the ground, compared to 12 days on a plastic plant with moisture at 23°C, which suggests that the psyllid does not need a specific host to obtain the moisture required for survival. Hodkinson (2009) reports that attempts to use radiolabels failed to provide definitive evidence for winter feeding by *Cacopsylla melanoneura*, although the maintenance of body condition and levels of hydration suggests that some feeding must have taken place (Jackson *et al.*, 1990). The pear psyllid mentioned above was found to be able to derive moisture from a wide variety of plant species during diapause in cut leaf studies, though survival was no better than with water alone and around half as long as when provided with pear leaves (Kaloostian, 1970). Another psyllid species that has been studied in depth is the carrot psyllid, *Trioza apicalis*, which is a serious crop pest. *T. apicalis* was collected equally from Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*), juniper (*Juniperus communis*), silver fir (*Abies spp.*) and dwarf mountain pine (*Pinus mugo*) (Kristoffersen & Anderbrant, 2005). The same authors later indicated that Norway spruce was the preferred conifer over Scots pine and juniper in Southern Sweden.

The overwintering habit of *A. itadori* is not well known and its shelter plants, in the absence of the primary knotweed host, which dies back at the first frost, are presumed to be evergreen trees. Baba & Miyatake (1982) and Miyatake (1973; 2001) recorded *A. itadori* as overwintering on Japanese pine, *Pinus densiflora* [Pinaceae] and Japanese cedar, *Cryptomeria japonica* [Taxodiaceae] in Japan. Miyatake (1973) states "it is unknown whether adults suck their shelter plants such as *Pinus* spp. and *Cryptomeria* spp." (translated from Japanese). *C. japonica* is a monotypic genus of conifer in the cypress family, Cupressaceae, formerly belonging to the family Taxodiaceae and is endemic to Japan. It is a medium or tall evergreen tree sometimes grown in forestry plots. More widely found in parks and large gardens, though seldom in towns, the dwarf cultivar finds favour in gardens, although this type of tree is sometimes included in conifer collections. *P. densiflora* originates from the hills and low mountains of Japan and has a very limited distribution in Europe, it is reported as unlikely to be a worthwhile timber tree (Bean, 1981).

However, Dolling (1991) reports that *Aphalara* species feed on various dock, bistort and knotgrass species, adults are often encountered sheltering on evergreens from late summer through to spring. However, several other *Aphalara* species have been shown to overwinter on both conifers and leaf litter (Lauterer 1976, 1982, 1991). Furthermore, several species of psyllids, including *Aphalara* spp., which normally overwinter on conifers can be successfully overwintered on grass tussocks (Heslop-Harrison, 1937).

Following the information presented in section 1.14 and in an review of the PRA for the UK the then Central Science Laboratory team (now FERA, the Food and Environment Agency of DEFRA) looked into the overwintering behaviour of other *Aphalara* spp. in the UK and discovered an old reference listing 24 psyllids from Scotland, including *Aphalara exilis*, abundant on juniper, pine and spruce in Perthshire and Inverness-shire in winter (Harrison, 1936). This appears to be the first time hibernating adults of *A. exilis* have been found on conifers. Furthermore, *A. polygoni* has been recorded on larch and spruce in The Clevelands (Harrison, 1915) and the same author reported beating many female *A. exilis* from conifers near Kielder in UK, in spring (Harrison, 1947). There was no mention of any associated damage. We can find no record of a psyllid causing any significant damage to overwintering hosts.

Essential recent research has furthered knowledge on *A. itadori* overwintering habits. Clewley and Wright (2014) found living psyllids recovered from trunk sections of Scot's pine, *Pinus sylvestris* and *Quercus robur* suggesting winter hosts do not need to be living (both widespread in Europe, Appendix 2). Some psyllids also overwintered when left on *F. japonica*, whether on dead canes, in leaf litter or soil is unclear but suggests suitable sites are not limited to tree trunks although it is hypothesised that the microhabitat of mature tree trunks, with cracks and crevices, was an important factor of a suitable overwintering site. The study confirms *A. itadori* will not be restricted to just a few rare conifers and it has a larger overwintering host range. One concern over the release of *A. itadori* was the chance of creating a new pathway of disease transmission to a winter host but as *A. itadori* can overwinter successfully on non-living hosts this suggests host feeding is not essential and further supports that the risk of disease vectoring from the release of this species is very low (Shaw *et al.*, 2009; Clewley & Wright, 2014).

Climatic suitability

3.03 - Does all the area identified as being suitable for establishment in previous question(s) have a suitable climate for establishment?

Yes (please provide justification)

This is dealt with in detail in Appendix 3. Though Kyushu Island as a whole has a more sub-tropical climate than Europe, increased elevation gives similar temperatures at similar times of year, although it is acknowledged that humidity is likely to be very different. The psyllid has been found on Japanese knotweed, on the upper slopes of Mt. Aso, over 1,500 metres above sea level. In general the winters in its native range are harsher than those normally experienced in the PRA area with much more snowfall and the summers can be very hot and dry, so climatic extremes are likely to be greater in the psyllids native range. It is not known whether the psyllid actually requires these extremes but populations exist from the coast to mountaintops in Japan, where conditions contrast markedly. It is thought that *A. itadori* could establish successfully across most of the PRA area, as much as its host plant has already done. The extensive test plant list can be found in Appendix 1 and includes Alpine species of relevance found within the PRA area.

The lower thresholds indicated by studies are in the range demonstrated by Hodkinson (2009) for tropical/sub-tropical species though temperate species are tolerant of 2-6 °C. It

should be assumed that the psyllid would establish successfully and widely.

It has been found that *A. itadori* had significantly higher intrinsic rates of natural increase, survival rates and fecundity rates at 20 and 25 °C than at 15 and 30 °C, at these latter temperatures egg-to-adult survival was also significantly lower, but survival was higher at 15 °C than at 30 °C, indeed adults held at 30 °C began dying 3 days after emergence (Myint *et al.*, 2012). This suggests that *A. itadori* would survive and establish in the PRA area.

Temperature controls the development of an organism throughout its life cycle- effecting the time of development, fecundity, oviposition and mortality. The upper and lower development threshold of an organism determines when the development of an organism stops. Following the method of Olsen *et al.*, (2003), the lower development threshold of *A. itadori* was determined under controlled temperature quarantine conditions. Development times of individual psyllids were recorded under 7 constant temperatures (10 °C, 12 °C, 17 °C, 21 °C, 23 °C and 28 °C). Figure 4 in Appendix 3 shows the relationship between the development rates (1/day) at 7 temperatures. There is a lower development threshold at 8 °C, however there was no development at 10 °C and very limited development at 12 °C. Given the poor survivorship at 12 °C , the insect's response is likely non-linear at low temperatures, however additional experimentation is required to fit this more explicit model.

Degree days are the total amount of heat required, above the development threshold, for an organism to develop from one stage to another in its life cycle. Calculations gave a figure of 462.5 degree days as the number required for the psyllid to develop from egg to adult. As an example, when this degree day figure is applied to the Netherlands it is possible to envisage the possible distribution of the psyllid based on temperature. Across most of The Netherlands the psyllid could establish one, possibly two generations, and in the east, south-east and south-west two generations could be possible per year (Appendix 3, Figure 5). This degree day figure should be applied to the remaining PRA countries.

Other abiotic factors

3.04 - Does all the area identified as being suitable for establishment in previous questions have other suitable abiotic factors for establishment?

Yes (please provide justification)

The psyllid is only likely to be limited by the availability of its intended host plant and ambient temperature, humidity and overwintering hosts may also play a role.

The climate in the majority of PRA areas is not expected to restrict *A. itadori*, except in higher altitude areas, where the host plant is rarely found growing.

When culturing *A. itadori* in a controlled environment humidity ranged from 50-85%, which is likely to have allowed higher levels of development unlikely to occur in the field (Shaw *et al.*, 2009).

A. itadori's tolerance to pollution (chemical or particulate), which may be higher in parts of the PRA area than much of its more mountainous range in Japan, is unknown. *A. itadori* does however thrive on the slopes of volcanic fumaroles where hydrogen sulphide gas

escapes are a frequent occurrence.

Limiting factors for the establishment of the psyllid are primarily host plant presence thus abiotic factors such as soil type, salinity and water saturation of the soil would also indirectly impact on the psyllid.

Competition and natural enemies

3.05 - Is all the area identified as being suitable for establishment in previous questions likely to remain unchanged despite the presence of competitors and natural enemies?

Yes (please provide justification)

The host plants, invasive knotweeds, support almost no phytophagous arthropods in its invasive range in the PRA area so competition should not be an issue. *Chaetocnema concinna* (Marsham) has been found to feed on *F. japonica* but this species is a generalist on polygonaceae and chenopodiaceae with a broad host range. Field observations have also revealed Auchenorrhyncha and the aphid *Aphis fabae*, both generalist species, feeding on *F. japonica*.

Recent field studies in the UK (2014) observed some casual opportunistic feeding on *Aphalara itadori* (eggs) by generalist predators, however this was minimal. The main opportunistic feeders were Anthocoridae and Coccinellidae. Araneae used the structure provided by the plant to form webs rather than feeding on the psyllid. To date there has been no impact on any native natural enemies.

The issue of specialist natural enemies is dealt with in the report by A. Polaszec (Natural History Museum) which is presented in Appendix 6. It would seem that the likelihood of significant attack by native parasitoids in the PRA area on *A. itadori* is low. In its native range, it is expected that *A. itadori* has a large number of specialist natural enemies which can be expected to have evolved alongside the psyllid. However, only one unidentified eulophid parasitoid has been reared out during the biocontrol project in Japan.

There is very little known about parasitoids of *Aphalara* spp. but there are 14 species of *Aphalara* listed for Europe in Fauna Europaea (<http://www.faunaeur.org/index.php>) namely: *A. affinis* (Zetterstedt 1828), *A. avicularis* Ossiannilsson 1981, *A. borealis* Heslop-Harrison 1949, *A. calthae* (Linnaeus 1761), *A. crispicola* Ossiannilsson 1987, *A. exilis* (Weber & Mohr 1804), *A. freji* Burckhardt & Lauterer 1997, *A. grandicula* (Gegechkori 1981), *A. longicaudata* Wagner & Franz 1961, *A. maculipennis* Low 1886, *A. nigrimaculosa* Gegechkori 1981, *A. polygoni* Foerster 1848, *A. sauteri* Burckhardt 1983 and *A. ulicis* Foerster 1848. Only *A. calthae* (Linnaeus 1761) is present in The Netherlands..

According to Noyes (2007), no known parasitoids of *A. itadori* are listed, and out of a total of 90 records of Psyllidae parasitoids, only two are associated with *Aphalara* species. Both apparently concern North American species, and both are likely to be *Psyllaepagus* species (Encyrtidae), although one is recorded as "*Encyrtus aphalarae*". Of the 90 records of chalcidoid parasitoids of Psyllidae, more than 60 concern *Psyllaepagus* spp. With

regards to the UK there is some probability that an indigenous British *Pyllaephagus* species, of which there are currently two, could use *A. itadori* as a potential host. However, of these two species, one (*P. lusitanicus* Mercet) is known only from the scale family Asterolecaniidae, and the other (*P. pilosus* Noyes) is known only from *Ctenarytaina eucalypti* – a psyllid living on eucalypts. It is therefore very unlikely that *A. itadori* will be attacked by any known British chalcid parasitoid (Appendix 6).

Japanese knotweed plants possess extra-floral nectaries (EFN) and such structures have been shown to attract predators and parasitoids (Bouchery *et al.*, 1975; Pemberton & JangHoon, 1996). This is also the case with Japanese knotweed in Japan (Kawano *et al.*, 1999) though no evidence of this association has been seen in NW EU, not least because there are virtually no potential prey organisms to be found there on Japanese knotweed.

Attempts to induce EFN activity through psyllid feeding in the laboratory were not reliable. In Japan, high populations of the psyllid have been observed despite the presence of ants in the local area so it can be concluded that they should not prevent establishment. It is not known whether ants present in the PRA area would be predators or tenders of the psyllid. Observations in the field in the UK found *Lasius niger* utilising the EFN's with no interaction with *A. itadori* that were present. Indeed the psyllids were ignored by the ants when they came into physical contact.

Studies conducted during the UK phase of the biocontrol project using generalist predators including *Syrphus ribesii* L., *Chrysoperla carnea* (Stevens), *Adalia bipunctata* (L.) and *Orius majusculus* (Reuter) have shown that these species can include the psyllid in their diet in a no-choice situation but in choice studies there was an apparent preference for their normal (aphid) prey (See Appendix 7). None of the experiments revealed any preference of these generalist predators for any stage of the psyllid over aphid prey. This suggests, at least in the short term, that *A. itadori* will not become their preferred food source providing their normal prey are available.

The managed environment

Protected Cultivation

3.08 - By combining the cumulative responses to previous questions, identify the part of the PRA area where the presence of host plants or suitable habitats and other factors favour the establishment of the pest.

With the invasive knotweeds (i.e. *Fallopia japonica*, *F. x bohemica* and *F. sachalinensis*) widespread throughout the PRA area the psyllid *A. itadori* is expected to establish throughout the PRA area, except in higher altitude areas.

After host presence, the most critical factor impacting on the psyllid's establishment is climate and here in particular ambient temperature. Temperature will exert and impact on the development of the insect. In most parts of the PRA area the temperature requirements of the psyllid will be met and thus it is likely that temperature will not be a limiting factor for the psyllids establishment and spread. Humidity is also likely to be a

factor in the establishment of *A. itadori*, therefore the selection of suitable release sites is important. Invasive knotweeds often grow in riparian habitats and so providing favourable conditions should not be a limiting factor.

The presence of certain evergreen trees, acting as overwintering or shelter plants for the insect in the absence of the primary knotweed hosts, when these die back after the first frost is another factor that needs to be considered. Alternative species have been studied and found to be suitable for overwintering and are widely distributed across the PRA area. In addition, psyllids have been found on leaf litter and the dead canes of the host plant suggesting they do not require a live overwintering host but rather a microhabitat conducive for overwintering.

Other abiotic factors are of lesser importance as they would impact indirectly on the psyllid through affecting the occurrence of its knotweed hosts.

Host plants and suitable habitats

3.09 - How likely is the distribution of hosts or suitable habitats in the area of potential establishment to favour establishment?

Very likely

Level of uncertainty: low

The psyllid *Aphalara itadori* is closely associated with its host *F. japonica* through its high host specificity. The only other susceptible hosts are the hybrid *F. x bohemica* and *F. sachalinensis*. Therefore, the establishment of the psyllid is crucially linked to the presence of these invasive knotweeds. While *F. conollyana* and *F. japonica* var. *compacta* can support the development of the psyllid to some extent, these species will not be crucial to the establishment of the psyllid.

Regarding the suitability of habitats, of greatest importance will be the temperature regime. A detailed assessment of the thermal tolerance of *A. itadori* is presented in Appendix 3. This suggests *A. itadori* would establish successfully across most of the PRA area (Appendix 3; Birkin, 2008).

Alternate hosts and other essential species

3.10 - How likely is the distribution of alternate hosts or other species critical to the pest's life cycle in the area of potential establishment to favour establishment?

Very unlikely

Level of uncertainty: low

In the summer, the intended target and preferred host for the psyllid is Japanese knotweed. Host range studies have shown that with the exception of two other invasive knotweeds present in the PRA area - a rarely planted horticultural species and an experimentally susceptible species not occurring widely in the PRA area, no other plants can sustain development of the psyllid from egg to adult. It is very likely that any non-target feeding by nymphs and adults would be incidental. Overwintering hosts/shelter for

adult psyllids in the absence of knotweed, between the first frost and its emergence in spring, are not expected to be a limiting factor. Recent research has found successful overwintering on widespread species in the PRA area as well as associated with knotweed stands, suggesting it is possible for overwintering to take place on leaf litter and even graminaceous species (see section 1.14).

Climatic suitability

3.11 - Based on the area of potential establishment already identified, how similar are the climatic conditions that would affect pest establishment to those in the current area of distribution?

Moderately similar

Level of uncertainty: medium

This is dealt with in detail in Section 3.03, Appendix 3.

The EU has less extreme variation in climate compared to that of Japan. The mean annual temperature range across the selected European countries ranges from 7°C to 12.4°C . Figure 2 in Appendix 3 shows the mean monthly temperatures for ten European countries compared to Kumamoto, Japan (the origin of the Kyushu psyllid biotype).

As an example, The Netherlands has a less extreme variation in temperature compared to Japan. It has a temperate maritime climate influenced by the North Sea and Atlantic Ocean, with cool summers and moderate winters. Daytime temperatures varies from 2-6°C in the winter and 17-20°C in the summer. Since the country is small there is little variation from region to region, although the marine influences are less inland. The Koeppen-Geiger classification classifies the climate of the Netherlands as a warm temperate humid climate with the warmest month lower than 22°C over average and four or more months above 10°C over average. Japan's topography is also very different to that of The Netherlands, almost 75% of Japan is mountainous. The steep undulating landscape adds to climatic variation throughout Japan. Temperature decreases with altitude or increases with descent, at a rate of 0.0065°C per metre (0.65 °C /100m - the Standard Atmosphere Temperature Gradient SATG). (Figure 3, Appendix 3 shows the mean annual temperatures for The Netherlands and Kyushu (Mt. Aso)).

Overall it is thought that *A. itadori* would establish successfully in The Netherlands and probably across most of the rest of the PRA area, as much as its host plant has already, with the exclusion of areas dominated by lower extremes of climates.

Other abiotic factors

3.12 - Based on the area suitable for establishment already identified, how similar are other abiotic factors that would affect pest establishment to those in the current area of distribution?

Not similar

Level of uncertainty: medium

Chemical pollution and particulate deposition might influence the establishment of the psyllid but nothing is known about this. Given the presence of the psyllid on volcanic slopes in Japan, it can be assumed that the insect is to some extent adapted to lower air quality.

Competition and natural enemies

3.13 - Based on the area suitable for establishment already identified, how likely is it that establishment will occur despite competition from existing species, and/or despite natural enemies already present?

Likely

Level of uncertainty: low

As outlined under 3.05 competition should not affect the establishment of the psyllid as Japanese knotweed in its invasive range supports almost no phytophagous arthropods.

The Kyushu strain of the psyllid has been reared and released in the UK as a clean culture and will as such be imported into the PRA area for release without any of its natural enemies associated with it in its centre of origin.

Studies conducted during the UK phase of the project using different predators showed that commonly their natural prey is preferred over the psyllid (see 3.05 and Appendix 7). It has also been outlined under 3.05, that the likelihood of a significant attack of *A. itadori* by parasitoids native in the PRA area is low.

The managed environment

Protected Cultivation

3.17 - How likely are the reproductive strategy of the pest and the duration of its life cycle to aid establishment?

Very likely

Level of uncertainty: low

Aphalara is a multivoltine species and reproduces very rapidly with fertile adult females producing a mean \pm SE of 637 eggs \pm 121.96 (range = 117 - 1,343, n=11) over a production period of 37.5 days \pm 5.85 days (n = 11) and live up to 75 days (at 23°C). Development from egg to adult takes 32.2 days \pm 0.5 (n = 42) at 23°C. At least one generation is possible across most of the PRA area and in some areas it is likely that two generations would be possible according to the assessment presented in Appendix 3 and Appendix 4.

3.18 - Is the pest highly adaptable?

No, moderately adaptable or less

Level of uncertainty: medium

A. itadori has been found at sea level in Kagoshima and on the upper slopes of Mt. Also in Kyushu Island so it is very tolerant of climatic extremes. The only evidence of subspecies or varieties comes from host range studies of a population collected from the far north of Honshu and Hokkaido which revealed a reversed preference between *F. sachalinensis* and *F. japonica*. The northern “strain” has been confirmed as *A. itadori* by US scientists. Adaptability in the selection of new hosts appears very limited.

3.19 - How widely has the pest established in new areas outside its original area of distribution? (specify the instances, if possible; note that if the original area is not known, answer the question only based on the countries/continents where it is known to occur)

Not widely

Level of uncertainty: low

The pest has been introduced into licensed quarantine facilities in the UK, North America and Switzerland. Following extensive research, a PRA, peer review, a public consultation and ministerial approval the psyllid has been released into the wild in the UK (restricted to 8 release sites) as a biological control agent against Japanese knotweed and is currently still undergoing a monitoring programme which will come to an end in 2014.

The results from this monitoring confirm that there have been no negative impacts on native vegetation or invertebrate community composition since release of the psyllid. Some overwintering has been observed, however populations have yet to establish. In 2014 a field cage study was conducted in the UK with the aim to record impact on Japanese knotweed and any effects on the receiving environment in semi-natural conditions, this study is ongoing and no final results are available to date.

3.20 - The overall probability of establishment should be described.

High

Level of uncertainty: High

Based on the lab and native range field studies, the likelihood of establishment is certainly high. However, attempts to establish the psyllid in the UK have been unsuccessful so far. The UK release strategy was understandably conservative: sites were isolated, and selected to allow monitoring for any non-target side effects, not to be favourable for establishment; psyllid numbers for release were also restricted. Therefore, to date no conclusive data is available on establishment of the psyllid. Results from UK field cage studies currently being conducted should provide some indicators to assist the psyllid’s establishment.

The host plant is widely distributed in the PRA area and the climatic conditions are thought to be favourable for psyllid establishment; as the identified critical factors, establishment of the psyllid is likely and indeed is intended.

3.20b – Tools (Genie Establishment)

Stage 2: Pest Risk Assessment Section

Stage 2: Pest Risk Assessment Section B: Conclusion of introduction

c1 - Conclusion on the probability of introduction.

The probability of introduction is high. Introduction of the Kyushu strain of *A. itadori* into the PRA area is intended as this will be a deliberate licensed introduction.

Stage 2: Pest Risk Assessment Section B: Probability of spread

Spread is defined as the expansion of the geographical distribution of a pest within an area. Spread potential is an important element in determining how quickly impact is expressed and how readily a pest can be contained. In the case of intentionally imported plants, the assessment of spread concerns spread from the intended habitat or the intended use to an unintended habitat, where the pest may establish. Further spread may then occur to other unintended habitats. The nature and extent of the intended habitat and the nature and amount of the intended use in that habitat will also influence the probability of spread. Some pests may not have injurious effects on plants immediately after they establish, and in particular may only spread after a certain time. In assessing the probability of spread, this should be considered, based on evidence of such behaviour.

In the PRATIQUE project, spread modules have been investigated. To decide whether it is appropriate to try to use these modules, please follow this link "[quantitative spread module](#)" (only available when online)

4.01 - What is the most likely rate of spread by natural means (in the PRA area)?

Moderate rate of spread

Level of uncertainty: medium

The rate of spread will initially be largely dependent on the release strategy, including the number of individuals used as the seed population, the density of host plant stands and their proximity to other host plant populations. The psyllid is capable of reproducing at a rate of 1-2 generations per year in The Netherlands, according to the average number of degree days in the region (Appendix 3). It is only the adult stage that is fully mobile (with nymphs being largely sessile) and adults are not strong flyers, relying more on jumping/flying to local plants. Nonetheless, they are likely to be carried by air currents. In Florida, the *Melaleuca* psyllid, *Boreioglycaspis melaleucae* dispersed 2.2-10.0 km/year, with the slower rates in dense, continuous *Melaleuca* stands and faster rates in fragmented stands (Center *et al.*, 2006). It is notable that several psyllid species overwinter on *Pinus* in northern England at a distance of around 13km from the nearest host plant (Hodkinson, 1972) so dispersal across land can be medium range. In all cases the long-range natural spread will be reliant on wind assistance.

4.02 - What is the most likely rate of spread by human assistance (in the PRA area)?

Moderate rate of spread

Level of uncertainty: medium/high

The rate of spread depends on the future number of intentional releases throughout the PRA area. High numbers of the psyllid (*A. itadori*) would be required to promote establishment if approval was granted.

Accidental spread of the psyllid *A. itadori* by humans would be low. Any transportation by humans will almost certainly be intentional not least because the growing of its host plant in the wild is either prohibited by law, requires permission, or is generally not accepted.

If the biocontrol agent is perceived as a solution to invasive knotweed populations by the general public, it is likely that demand for the psyllid will be high and its intentional redistribution will take place repeatedly.

4.03 - Describe the overall rate of spread

Moderate rate of spread

Level of uncertainty: medium

A. itadori is a biological control agent that has been selected based on its ability to establish and spread as well as on its high level of host specificity. It is likely to spread throughout the PRA area albeit natural spread might be slow, depending on wind assisted transport and the level of connectivity between patches of the host plant.

The arrival and rapid spread of the *Eleagnus* psyllid *Cacopsylla fulguralis* in Europe (including The Netherlands and France) (Malumphy & Halstead, 2003) suggests that spread could be very rapid for *A. itadori* but this case is likely to have been aided by the commercial movement of the desirable host plants. This has also been the case with the New Zealand *Pittosporum* psyllid *Trioza vitreoradiata*, which became established in Cornwall (Martin & Malumphy, 1995) and is now found in Ireland (O'Connor *et al.*, 2004).

Spread of the psyllid in the PRA area might increase if many intentional releases were carried out, as has been practiced by Australia in a number of their weed biocontrol programmes.

4.04 - What is your best estimate of the time needed for the pest to reach its maximum extent in the PRA area?

This would be highly dependent on the release strategy in the initial stages of the first releases and wind patterns etc. but could be decades.

Level of uncertainty: high

4.05 - Based on your responses to questions 4.01, 4.02, and 4.04 while taking into account any current presence of the pest, what proportion of the area of potential establishment do you expect to have been invaded by the organism after 5 years?

This would depend on the release strategy and conditions attached as much as on the ability of the psyllid to spread. Further discussions with the relevant authorities in the PRA area are required to answer this question fully.

Level of uncertainty: high

Stage 2: Pest Risk Assessment Section B: Eradication, containment of the pest and transient populations

5.01 - Based on its biological characteristics, how likely is it that the pest could survive eradication programmes in the area of potential establishment?

Very likely

Level of uncertainty: medium

Eradication programmes are not anticipated, but in the early stages following release it might be possible to eradicate the psyllid using commercially-available chemical pesticides. There is no published information available on the psyllid's susceptibility to pesticides though it is likely that adults will be vulnerable to products commonly used to control pest psyllids in the PRA area. Limited (unpublished) studies showed that systemic pesticides were capable of eradicating mixed populations in the laboratory whilst contact pesticides were not 100% effective. Eggs are relatively resilient and are often laid under the papery sheaths surrounding the nodes of the host plants giving them some degree of protection to topical pesticide application. At high numbers, nymphs conglomerate and produce a protective and highly hydrophobic waxy flocculence or lerp that could provide protection against contact pesticides. The size and architecture of the host knotweed might also limit the ability of operators to apply pesticides, as is the case with herbicide treatment. However, non-target native species or crops that might be identified as endangered during the post-monitoring phase could be sprayed in the usual way.

Outbreaks and occurrences of non-native psyllids such as the Acacia psyllid, *Acizzia uncatoides* (Ferris & Kylvær) and the olive sucker, *Euphyllura olivina* Costa, do occasionally occur in the UK and are readily controlled using insecticides such as deltamethrin (CSL comments on an earlier version of this document). Control in a 'home and garden' situation may prove more of a challenge, although suitably trained personnel could be recruited to apply professional products in domestic gardens, if required (application of pesticides is not recommended or usually permitted in riparian habitats).

5.02 - Based on its biological characteristics, how likely is it that the pest will not be contained in case of an outbreak within the PRA area ?

Very likely

Level of uncertainty: low

The psyllid has a very efficient reproductive strategy, although natural dispersal might be slow due to the limited mobility of the agent. There are no significant topographical

barriers between the PRA area and its neighbouring EU countries which would impede the spread of the psyllid beyond the designated PRA area once the insect has established and started to spread from the original release sites. Furthermore, spread of the psyllid will be aided and enhanced by air currents.

The natural host Japanese Knotweed, as well as other invasive knotweeds, are abundant in the PRA area and there are continuous host populations which will provide the psyllid with continuous corridors for reproduction and spread. Releases would probably be required in other EU countries to encourage establishment of a population.

Kristoffersen & Anderbrant (2005) indicated that the carrot psyllid, *Trioza apicalis* could cover distances of up to one kilometre and that there was a trend to spread in the direction of the prevailing wind. However, the same authors later found the wind direction seemed to be irrelevant but that most psyllids were collected within 250m of the crop (Kristoffersen & Anderbrant, 2007).

It is also highly likely that demand for the psyllid as an organism which will significantly damage invasive knotweeds, might encourage members of the public to spread this agent to other knotweed-affected countries in Europe.

5.03 - Are transient populations likely to occur in the PRA area through natural migration or entry through man's activities (including intentional release into the environment) or spread from established populations?

Yes

Level of uncertainty: low

Yes, the psyllid would be introduced intentionally as a “beneficial organism” and from such releases natural migration is likely to occur. Transient populations may occur in the PRA area, especially if *F. japonica* and/or other invasive knotweeds are subject to localised control and eradication programmes.

Stage 2: Pest Risk Assessment Section B: Assessment of potential economic consequences

6.01 - How great a negative effect does the pest have on crop yield and/or quality of cultivated plants or on control costs within its current area of distribution?

Minimal

Level of uncertainty: low

None. There are no crop hosts that are affected by *A. itadori* in its current area of distribution (JSAEZ, 2006). It is not thought to feed on or damage the overwintering hosts on which the psyllid has been shown to use as a shelter during winter in Japan and in tests in the UK (Baba & Miyatake, 1982; Miyatake, 1973; Miyatake, 2001; Clewley & Wright, 2014).

6.02 - How great a negative effect is the pest likely to have on crop yield and/or quality of cultivated plants in the PRA area without any control measures?

Minimal

Level of uncertainty: low

Aphalara spp. are, in the main, restricted to the Polygonaceae (see Burkhardt & Lauterer, 1997) and not recognised as crop pests. The key reference book to crop pests in Japan does not record any *Aphalara* sp. as a pest (JSAEZ, 2006). However, the congeneric, *A. polygona*, has once been recorded in an unpublished report as damaging buckwheat (*Fagopyrum esculentum*) in Hokkaido, Japan in 1983 (N. Takahashi, personal communication, March 2008).

Referring to the host range tests conducted for the UK (Shaw *et al.*, 2009, Appendix 4) only the target species, the invasive knotweeds and two horticultural curiosities (*F. conollyana* and *F. japonica* var. *compacta*) supported development of *A. itadori* from egg through to adult (see section 1.17). There was an extreme difference in oviposition rates for the target species (and the two horticultural curiosities) and the non-target species that received eggs, with the highest mean egg count ($\pm 1SE$) per species recorded for *R. palmatum*, with 17.3 ± 7 eggs/plant, (n=12), *F. esculentum* (buckwheat) with 16.9 ± 6.7 eggs/plant (n=14) and wire plant, *M. complexa*, which received 14.7 ± 5 eggs/plant (n=7). Compared to the rate of 433.7 ± 28.3 eggs per plant (n = 324) for the target *F. japonica* this constitutes a highly significant difference (Appendix 4, Table 2).

Forced nymph transfer studies carried out at artificially high humidity revealed that in addition to the target plants, four species were able to support nymphal development at 28 days (*F. conollyana*, *F. dumetorum*, *R. hydrolapathum*, *M. complexa*). Development beyond third instar occurred for *F. conollyana*, *F. dumetorum*, *F. baldshuanica* and *M. complexa*. However only *F. conollyana* and *M. complexa* supported development through to adult in this study. The percentage survival on these two plant species was 48.3% and 6.7%, respectively, compared to >66% for *F. japonica* (Appendix 4).

In contrast to the results of the multiple choice oviposition/development study where none of the 103 eggs laid on *M. complexa* were able to develop to adult, this nymph transfer experiment revealed that *A. itadori* can indeed develop on this species, although only 4 out of 60 (6.7%) individuals achieved this. *Muehlenbeckia complexa* has a limited distribution mainly occurring in the UK and Spain (GBIF). It is reported to be naturalized on coasts of W. Europe i.e. France, but not reported in The Netherlands (See test plant justification in Appendix 1 and European distribution maps in Appendix 2).

Overall, development of *A. itadori* on non-target species is severely compromised due to adult oviposition preference; these species are rarely targeted for oviposition by mated female psyllids, and failure of eggs hatching successfully and/or nymphs to develop through to adult stage. As nymphs are the main feeding and thus damaging stage of the psyllid (see section 1.11) any non-target impact/damage is likely to be minimal, indeed there is a very low probability that these species will be damaged by the psyllid. As stated under 1.06, adult survival on the most closely related species to be found in Europe was also severely compromised with limited survival on *F. dumetorum*, a species on which

eggs failed to develop.

Adult survival studies over a 12–day period (Appendix 4, Figure 3) on the target species and four of the most closely related species present in the UK show that survival was severely compromised on species other than *F. japonica* var. *japonica*, which suggests that adult feeding is highly restricted too. *F. dumetorum* did support survival of ca. one-quarter of the number of adults that survived on *F. japonica* var. *japonica* over the period. This suggests that some adult feeding had taken place on this species. However, *F. dumetorum* did not support the development of eggs to adults in the oviposition studies, or nymphs to adults in the nymph transfer studies.

In the unlikely scenario that a high number of psyllids was present combined with an insufficient number of the natural host i.e. invasive knotweeds, spill-over effects could theoretically occur. Such effects where adults may also be found on the most closely-related non-target species present in the area would be only temporary. Adult psyllids firstly inflict generally little damage. Secondly, with the exception of the plant species belonging to the invasive knotweeds as well as *F. conollyana* and *F. japonica* var. *compacta*, eggs have been shown to be incapable of developing to adult, thus preventing a population to be sustained on these non-targets (unless nymphs were to be artificially transferred). If very high populations of adult psyllids were to build up in an area where the exotic ornamental and often invasive *M. complexa* had been planted, there is a possibility of some damage, but sustainable populations are unlikely to establish on this species.

Further information on the importance of Buckwheat, *Fagopyrum esculentum* in NW EU is now addressed. Buckwheat belongs to the Polygonaceae family and is included in the test plant list. It is an economically important crop species in many countries throughout the world. In Europe, formerly it was extensively cultivated, but this is now restricted to a few countries, although the plant has become naturalised in disturbed land throughout much of Europe. The total production across Europe in 2011 was 1,352,875 tonnes (<http://faostat.fao.org>). Buckwheat is a minor crop in Austria, Belgium, Denmark, Germany, Switzerland and The Netherlands but an important crop in the three remaining countries considered in this PRA (Czech Republic, France and Poland):

Country	Production (tonnes)
Europe (Total)	1,352,875
Eastern Europe	1,223,164
Northern Europe	35,800
Southern Europe	2,511
Western Europe	91,400

Buckwheat production in Europe (FAO statistics for 2011)

Europe	Production (tonnes)
Austria	0
Belgium	0
Czech Republic	2,076
Denmark	0

France	91,400
Germany	0
Poland	92,985
Switzerland	0
The Netherlands	0

Buckwheat production in PRA area countries (FAO statistics for 2011)

In North America *Fagopyrum esculentum* is an important crop species, and initial host range tests found eggs laid on *F. esculentum* (16.9 ± 6.7 eggs/plant ($n=7$)) (Shaw *et al.*, 2009, Appendix 4). Further studies have been conducted in both North America and the UK. When offered a choice between *F. japonica* and *F. esculentum*, *A. itadori* strongly preferred to oviposit on the target plant (94% on knotweed vs. 6% on buckwheat for Kyushu psyllid) (pers. comm., R. Bouchier). Field oviposition choice tests conducted in the UK found that from a small number of eggs laid on buckwheat none developed into adults, confirming buckwheat is a poor host for *A. itadori*.

6.03 - How great a negative effect is the pest likely to have on yield and/or quality of cultivated plants in the PRA area without any additional control measures?

Minimal

Level of uncertainty: low

As outlined under 6.02 *Aphalara itadori* poses a minimal threat to cultivated plants. Thus, no additional control measures would be required.

6.04 - How great a negative effect is the pest likely to have on yield and/or quality of cultivated plants in the PRA area when all potential measures legally available to the producer are applied, without phytosanitary measures?

Minimal

Level of uncertainty: low

See answers 6.01 to 6.03.

6.05 - How great an increase in production costs (including control costs) is likely to be caused by the pest in the PRA area in the absence of phytosanitary measures?

Minimal

Level of uncertainty: low

If establishment of *A. itadori* is successful, control costs for Japanese knotweed and other invasive knotweeds should actually decrease.

6.06 - Based on the total market, i.e. the size of the domestic market plus any export market, for the plants and plant product(s) at risk, what will be the likely impact of a

loss in export markets, e.g. as a result of trading partners imposing export bans from the PRA area?

Minimal

Level of uncertainty: low

The only plants that have been shown to suffer damage due to attack by the psyllid in Japan and in our experimental studies are invasive knotweeds and two other *Fallopia* species. It is therefore predicted that there are no plants or plant-based commodities that are exported from the PRA area that would be affected by *A. itadori*. If knotweed becomes an important crop plant or biofuel in the future then this may change. Owing to its rapid rate of growth, it has been considered as an energy source (Bernik & Zver, 2006) although in early studies it was not found to be economically viable (Callahan *et al.*, 1984).

6.07 - To what extent will direct impacts be borne by producers?

Minimal extent

Level of uncertainty: low

As outlined under 6.06, *Fallopia japonica* and other invasive knotweeds do not currently constitute a commodity either for the domestic or the export market. Thus this question is not applicable.

If they should become plant species of commercial value in the future then this might change.

6.08.0A - Do you consider that the question on the environmental impact caused by the pest within its current area of invasion can be answered?

No, but there is some evidence that the environmental impact may be significant in the PRA area.

To date (although population numbers remain low) no negative impacts of *A. itadori* have been found in its introduced range in the UK. Extensive host range testing has been conducted in order to ensure no negative effects of the psyllid on the environment (See 6.09.01).

The psyllid *A. itadori* has been selected as a promising biological control agent for *F. japonica* due to the damage and regulative effects exerted on its host populations as observed in field studies and surveys in its native range in Japan, as well as during detailed research conducted under quarantine conditions prior to its release in the UK. The previous deliberate introduction of the psyllid into the UK and the proposed deliberate introduction into the PRA area is anticipated to fulfil the same regulative function. The environmental damage caused by the target weed, *F. japonica* is very high indeed and any reduction in its performance and dominance through the influence of the psyllid can only be beneficial.

A reduction in the range and dominance of *Fallopia japonica* and other invasive knotweeds in affected habitats is anticipated as a result of the activities of the psyllid. This should take place slowly and allow native species to re-establish. However, in some

riparian habitats other weeds such as Himalayan Balsam (*Impatiens grandulifera*) and/or giant hogweed (*Heracleum mantegazzianum*) may become replacement weeds. These species should be easier to control than Japanese knotweed but are not desirable replacements by any means. In invaded urban or derelict sites there is a danger that the abundance of weedy species might increase following control of invasive knotweeds. Depending on initial experiences concerning the impact of the insect on *F. japonica* and depending on specific characteristics of sites/areas invaded by this weed it might be advisable to put a land-management plan in place in order to prepare for potential scenarios associated with a decrease in invasive knotweed populations.

6.08 - How important is the environmental impact caused by the pest within its current area of invasion?

No Importance

Level of uncertainty: low

There have been no negative impacts recorded across the release sites when compared to controls in the 5 year release and monitoring programme in the UK.

There are no records of environmental damage being caused by the agent in Japan. It is an ordinary member of the local fauna.

6.09.01 - What is the risk that the host range of the pest includes native plants in the PRA area?

Low risk

Level of uncertainty: low

Comprehensive host-range testing conducted under quarantine conditions in the UK prior to release of the psyllid in the UK in 2010 ensured that the insect is host specific to invasive knotweeds. Additional testing has been conducted for the PRA area assessing the susceptibility of Dutch biotypes of the invasive knotweeds (i.e. *F. japonica*, *F. sachalinensis* and *F. x bohemica*) as well as representative plant species of the Dutch flora. Assessments showed that all of the Dutch invasive knotweed species supported the development of the psyllid from egg to adult. Furthermore, none of the additionally tested non-target species with relevance to the PRA area proved to come under attack from the psyllid (Appendix 5).

Thus, apart from the invasive knotweeds and two *Fallopia* species, *Fallopia japonica* var. *compacta* (an ornamental species) and *Fallopia conollyana* (a hybrid only present in one location in the UK), the insect is not expected to impact on non-target species in the PRA area. If high populations of adult psyllids occurred in an area where the exotic ornamental and often invasive *M. complexa* had been planted, there is a possibility of damage from overspill of nymphs, but again sustainable populations are unlikely (damage was only demonstrated in nymph transfer tests). Since nymphs are the main feeding and thus damaging stage of *A. itadori* and they are mainly sessile the psyllid can be expected to cause no non-target environmental damage in the PRA area post-release.

However, a potential alternative host for adult psyllids unable to find their preferred

knotweed host is copse bindweed, *F. dumetorum*, which is fairly common in parts of The Netherlands and EU countries. It was listed as Vulnerable in the IUCN (2001) Red List for the UK but currently is not considered at threat, with stable or increasing populations. Experimental work indicates that *A. itadori* is unlikely to be able to complete its lifecycle on this species. In one study this plant received very few eggs in the presence of the knotweed host (an average of 8 per plant versus 434 for knotweed) (Appendix 4). These eggs did not develop beyond 2nd instar (i.e. none of these developed to adults). In a separate study, when 1st instar nymphs were transferred to *F. dumetorum* under elevated humidity, development to 5th instar was possible in 10% of cases (so some feeding activity by the nymphs would be possible) but again no adults were produced.

It should also be noted that a biological control agent should not totally eradicate its host. Normally the host and the agent will reach an equilibrium which is resource limited. Therefore, it is likely that *A. itadori* will be able to find its preferred host plant somewhere, although what the final equilibrium level will equate to (in terms of knotweed density), is largely unknown.

The Kyushu strain of the psyllid has been maintained in quarantine over many generations and freed of any of its natural enemies (i.e. specific parasitoids) before its approved release in the UK in 2010. It can therefore be expected that the impact on invasive knotweeds in the release area would be higher than in its native range. Introduction of the psyllid into the PRA area would be facilitated by the culture currently maintained in the UK, thus the same would apply in this circumstance.

6.09.02 - What is the level of damage likely to be caused by the organism on its major native host plants in the PRA area? (If possible, this question should be answered by taking account the impacts on its major host plants in the PRA area. If the effects on the host plants in the PRA area are not well known, then the answer should be based on damage levels in other areas, but with a higher level of uncertainty).

Low level

Level of uncertainty: low

Studies have demonstrated that *A. itadori* does not complete its lifecycle on any representative native plants tested. The psyllid's host range is restricted to *F. japonica*, *F. sachalinensis* and *F. x bohemica*, which are introduced exotic invaders in the PRA area, and two minor *Fallopia* spp. The damage to the invasive knotweed species, however, is envisaged to be high.

Impact on ecosystem patterns and processes

6.09.03 - What is the ecological importance of the host plants in the PRA area?

Low importance

Level of uncertainty: low

Being exotic invasives the main ecological importance of *Fallopia japonica*, *F. x bohemica* and *F. sachalinensis* manifest themselves through their severe impacts on native biodiversity in the PRA area. Its early emergence and vigorous growth lead to out-shading of other vegetation (Sukopp & Sukopp, 1988), and a general reduction in species diversity has been found (Palmer, 1990; Scott & Mars, 1984). A European study (Gerber *et al.*, 2008) comparing knotweed-invaded habitats to native habitats showed a reduction in plant species number and in the abundance and richness of invertebrates. Invertebrate biomass was nearly halved in invaded habitats, suggesting serious knock-on ecosystem effects for species whose diets rely largely on arthropods. This is supported by Maerz *et al.* (2005). A similar study in Belgium comparing invertebrate populations in *F. japonica* invaded versus uninvaded sites found the total number of individuals of above and below ground fauna was lower in invaded plots (Nijs *et al.*, 2012). Concerning the impact on ecosystem properties, invasion by *Fallopia japonica* was shown to increase topsoil mineral nutrient concentrations in invaded sites as compared to uninvaded site (Dassonville *et al.* 2007, 2008). The impacts were recorded greatest in sites with low nutrient concentrations in uninvaded plots, suggesting that *F. japonica* may contribute to soil homogenization in invaded landscapes. *F. japonica* was also shown to slow down soil organic matter dynamics by reducing C mineralisation (Koutika *et al.*, 2007).

Wider habitat impacts include increasing the risk of flooding either by directly impeding water flow, but also by disrupting the integrity of flood defence structures.

On the other hand, certain vertebrate species may have established an association with Japanese knotweed, as for example Gilbert (1994) documents in the case of grass snakes (*Natrix natrix*) and otters (*Lutra lutra*), for which the plant can provide habitats. UK field studies have highlighted a number of generalist phytophagous insects that will feed opportunistically on Japanese knotweed (see Section 3.05).

However, it can be assumed that the replacement of knotweed by other native plant species following its expected reduction in dominance through the impact of the psyllid, would provide similar if not better resources for these species.

Again it is highlighted that a biological control agent should not totally eradicate its host and that normally the host and the agent will reach an equilibrium which is resource limited.

As pointed out under 6.08.0A it might be advisable to put a land-management plan in place in order to prepare for potential scenarios associated with a decrease in invasive knotweed populations.

Conservation impacts

6.09.04 - To what extent do the host plants occur in ecologically sensitive habitats (includes all officially protected nature conservation habitats)?

Medium extent

Level of uncertainty: low

The host plants *Fallopia japonica*, *F. x bohemica* and *F. sachalinensis* occur in a wide

range of habitats. They are often associated with sub-/urban habitats, including roadsides, derelict land, railway embankments and gardens, where they cause the most economic damage. However, they are also common in riparian habitats, and these can often be ecologically sensitive, some of which may be protected.

6.09.05 - What is the risk that the pest would harm rare or vulnerable species? (includes all species classified as rare, vulnerable or endangered in official national or regional lists within the PRA area)

Low risk

Level of uncertainty: low

In terms of direct effects, the risk posed by the psyllid to rare or harmful species is low. The psyllid has been shown to be host specific to *F. japonica* and other invasive knotweeds and consequently there is no risk to any non-target plant species in the PRA area. Equally, the psyllid will not out-compete other phytophagous insects as invasive knotweeds have very few phytophagous generalists associated with it in the PRA area (See 6.09.02).

Indirect effects of the psyllid *A. itadori* in the ecosystem are more difficult to predict. If a native parasitoid or predator switches its attentions to *A. itadori* in preference to its normal phytophagous host then populations of the normal host may increase and exert additional pressure on its own host. Alternatively, this switch might lead to a massive increase in the predator/parasitoid population leading to the opposite result, i.e. a suppression of its normal host. Such apparent competition is extremely hard to predict and indeed quantify and could be considered insignificant when compared to the negative impact and direct competition of the target Japanese knotweed were it to be allowed to continue its spread.

Impact of pesticides

6.09.06 - What is the risk that the presence of the pest would result in an increased and intensive use of pesticides?

Low risk

Level of uncertainty: low

The presence of the psyllid in the PRA area would impact positively on the use of herbicides to control invasive knotweeds, i.e. lower the necessity to spray invaded areas. Any attempts aiming to eradicate the psyllid after its initial release would have to involve insecticide treatment. However, eradication programmes are not anticipated.

6.09 - How important is the environmental impact likely to be in the PRA area?

Minimal

Level of uncertainty: medium

As the psyllid would be deliberately introduced into the PRA area to control populations of

the exotic invasive *Fallopia japonica* (and other invasive knotweeds) with the positive environmental impact expected to be high.

The psyllid is expected to impact negatively on its host thereby reducing invasive populations and contributing to restoring ecosystems in the PRA area. A reduction in the population of *F. japonica* and other invasive knotweeds should promote populations of native plant species and their associated fauna.

Control of *F. japonica* through the psyllid would be over time and would avoid more disruptive and detrimental control methods such as mechanical removal or chemical application.

6.10 - How important is social damage caused by the pest within its current area of distribution?

Minimal

Level of uncertainty: low

There are no records of social damage being caused by the agent in Japan where it is an ordinary member of the local fauna, nor in the UK where it has been released since 2010.

6.11 - How important is the social damage likely to be in the PRA area?

Minimal

Level of uncertainty: low

Rather than causing social damage, it is more likely that social damage caused by the invasive knotweed, which is often associated with dereliction and urban decay, will be reduced.

A small, but significant and growing industry of weed control “specialists” has grown along with the Japanese knotweed problem in the UK, although this specialist industry does not appear to be widely present in NW EU for invasive knotweed control..

If the psyllid biocontrol agent is highly successful in controlling knotweed, then these people may lose income related to this specialist activity. However, opportunities should exist for those able to ensure that control efforts, where required, do not conflict and even act in synergy with the agent.

If, as a result of the introduction of *A. itadori*, the target host plant ceases to be a serious riparian invader, any contracted work directly associated with the plant’s ability to exacerbate flood risk, i.e. flood recovery, would be reduced, but this would most likely be offset by reduced insurance claims and premiums.

6.12 - To what extent is the pest likely to disrupt existing biological or integrated systems for control of other pests?

Minimal

Level of uncertainty: low

There are no such integrated control measures in place in this system. *A. itadori* is closely associated with Japanese knotweed and as such would not disrupt other integrated systems; also it is not the preferred food for native predators (shown in Appendix 7). If control measures were implemented for the psyllid then this will probably have negative impacts on the environment through the use of chemical insecticides. Non-target effects of pesticides would be low given that the host plant does not support any native species of note.

6.13 - How great an increase in other costs resulting from introduction is likely to occur?

Minor

Level of uncertainty: medium

It is normally recommended that biocontrol releases be carried out alongside medium term monitoring programmes to capture any non-target impacts and to enable an assessment of the success of the agent's establishment, spread and level of control. Such monitoring programmes have a financial cost but could be incorporated into other national monitoring programmes already in place, or any regular voluntary biological monitoring activities (for example, including citizen science activities) and/or research work undertaken at universities.

There will also be a demand for information from the general public and this will require some resources (staff time and materials costs) for publicity. If there is a public consultation phase of the licensing process, this will also incur costs.

As this proposed release of a biocontrol agent is against a weed in a European Member State bordering other EU members, there may be some requirement for civil servant time to address future queries from those EU Member States wishing to develop their own biocontrol programmes or to benefit from this one.

6.14 - How great an increase in the economic impact of other pests is likely to occur if the pest can act as a vector or host for these pests or if genetic traits can be carried to other species, modifying their genetic nature?

Minimal

Level of uncertainty: low

The psyllid culture of the Kyushu strain, which is held in the UK and from which imports and releases would be made, has been cleaned of arthropod and fungal parasites and maintained free of pests for the past 10 years, and so it should not act as a host or vector of any pests from its native range. However, there are no economic pests that either specialise on the genus or exist on the host plant that could be vectored (see question 1.10).

During the earlier review of the PRA compiled for the application for release of the psyllid in the UK, the CSL (now FERA) team looked into the genus *Aphalara* as a vector of plant pathogens and discovered that buckwheat, *Fagopyrum esculentum*, was affected by a disease known as chlorotic leafspot in Russia and this was associated with the presence

of a rhabdovirus with the most common vector being *Aphalara exilis* (Alekseeva *et al.*, 1988). However, this record is uncertain as there are no recognised virus species affecting buckwheat in the VIDE database (Plant Viruses Online) and therefore it is unlikely that there are any plant viruses affecting buckwheat in the PRA area that could be vectored by *A. itadori*. As described in 6.02, buckwheat is not a major crop in The Netherlands. CSL found no other records of plant pathogens being vectored by *A. itadori* or other *Aphalara* species.

It is unlikely that *A. itadori* will hybridise with any native congeneric species (of which there are at least 14 in Europe) on the basis that such species do not readily interbreed. This minimises this risk. No attempts have been made to test the possibility of hybridisation so it is unknown whether it is possible.

6.15a - Describe the overall economic impact (*sensus stricto*)

Minor

Level of uncertainty: low

Most of the PRA area in which Japanese knotweed exists should be suitable for the establishment of *A. itadori*. Providing the species behaves as predicted and only attacks the target host plant and its hybrids, none of these areas are at economic risk other than some lost market for weed management companies specialising in knotweed. Traders of the ornamental *F. japonica* var. *compacta* may incur economic losses, but this risk is minimal as the market for this species appears to be limited.

There should be some reduction in current impact and control costs of knotweed leading to the reduced economic impact of the weed in the PRA area in riparian and built environments and development sites alike.

Loss of employment in knotweed control industries is unlikely as there are many more weed species to control and commercial control is likely to be required on building sites for many years to come. Nevertheless, it is likely that *A. itadori* will be used as part of an integrated control programme for Japanese knotweed and so there will still be employment for weed control firms that are able to adapt to this specialist approach.

6.15b - With reference to the area of potential establishment identified in Q3.08, identify the area which is at highest risk from economic, environmental and social impacts. Summarize the impact and indicate how these may change in future.

Minimal

Level of uncertainty: low

With the host *Fallopia japonica* and other invasive knotweeds spread across the whole of the PRA area, the whole area would be equally affected by the release of the psyllid *A. itadori* (Kyushu strain).

No negative environmental impact is to be expected from the introduction of the psyllid

into the PRA area, in fact the envisaged impact of the psyllid on invasive knotweeds would have a positive environmental impact by reducing invasive populations of these exotics. This positive environmental impact would be highest where invasive knotweeds have invaded sensitive ecosystems as is the case in many riparian situations. The impact of the psyllid on its host is anticipated to be gradual over time thus enabling the native vegetation to recolonize invaded habitats without the problems associated with disruptive and detrimental control methods such as mechanical removal or chemical application. However, there needs to be awareness that other exotic invasive plant species could make use of the resources made available after the reduction of *F. japonica* and such areas might be in need of habitat restoration plans and active work. This may include adding native seed or plants into the system or adjustment of the soil conditions. With continuous reduction of the size of invasive knotweed populations over time the positive environmental impact will maximise until those populations are suppressed below a damaging threshold and become integrated as part of the respective vegetation.

Economically, the negative impact of the psyllid in the PRA area is envisaged to be minimal. Rigorous host range testing showed that *A. itadori* only attacks the target host plant and its hybrids. Currently, none of these invasive knotweeds has any economic value, however, and at this point in time it is unlikely, should they become important crop or biofuel plants in the future then this may change. Any monitoring programmes associated with the release of the psyllid in the PRA area will need to be publically funded either at the civil service or the research level.

A positive economic impact in the PRA area is envisaged through a reduction in current impact and control costs of invasive knotweeds.

Contractors specialized in invasive knotweed control are likely to suffer some loss in their income if the psyllid successfully reduces invasive knotweed populations. However, it is likely that opportunities will exist/arise for those contractors able to capitalize on the presence of the psyllid by devising control efforts, where required, which do not conflict and even act in synergy with the agent. Furthermore, restoration practices should be undertaken following the control of invasive knotweeds and this would provide an alternative income for the industry. It is also possible that contracted work mitigating the effects of increasing flood risks by invasive knotweeds would diminish, however, there would be a positive effect on reduced insurance claims and premiums should the psyllid impact negatively on the abundance of these invasives.

Stage 2: Pest Risk Assessment Section B: Degree of uncertainty and Conclusion of the pest risk assessment

c2 - Degree of uncertainty: list sources of uncertainty

Sources of uncertainty include:

The probability of establishment and spread of the psyllid once released can only really be predicted to a low degree.

The degree of predation and parasitism of the psyllid is anticipated to be low but difficult to predict.

The overwintering behaviour of the psyllid has not been fully elucidated.

The impact on the environment: although these impacts should be positive by reducing the levels of invasive knotweeds in the PRA area - actually predicting the outcome remains a medium level of uncertainty. Secondary and tertiary effects on the ecosystem are difficult to predict.

The cost-benefit ratio of the introduction of the psyllid is difficult to assess and a cost-benefit analysis would need to be conducted to establish this. However, the cost of knotweed to the EU economy is very high so any reduction in this figure would be significant.

c3 - Conclusion of the pest risk assessment

The PRA has been written based on scientific evidence as a result of field observations, experimental research and initial field studies following the releases of the psyllid made in the UK under licence. For the PRA information from the wider scientific literature has also been drawn upon.

The psyllid *A. itadori* has undergone extensive host range testing, both prior to its release in the UK as well as during assessments conducted for North America and The Netherlands, to ensure that it is sufficiently host specific.

Based on the extensive research conducted with this potential biological control agent, the psyllid *A. itadori* ex. *Fallopia japonica* (Kyushu strain) should have a significant and deliberate impact on the non-native species *F. japonica* and its hybrids in the PRA area. Through the biological control of these invasive knotweeds with the psyllid, native vegetation and associated biological diversity should benefit through the reduction in the occurrence of these invasives in the PRA area. Negative impact on non-target species is assessed to be minor.

Specific points

Entry: Provided that release is authorised, the entry of *A. itadori* is certain.

Establishment and spread:

Establishment and therefore spread is highly dependent on the release strategy implemented throughout the PRA-area, particularly the seed population size used and the location of release sites. The psyllid has been shown to be capable of breeding in the climatic conditions available in the PRA areas which are infested by the host plant *F. japonica* (Appendix 3) and there is no shortage of the host plant across the PRA area.

The psyllid is unlikely to establish if releases take place outside the growing season of the host plant, and if native parasites (none identified) and predators eradicate it in the early stages of establishment. Establishment is an intended outcome of intentional release.

Host range testing:

Whilst every effort has been made to adequately test the host range of the psyllid using established techniques and an extensive test plant list there are no certainties in biological systems. Non-target risks are predictable (Pemberton, 2000) and classical biological control has a very good track record (McFadyen, 1998; Fowler *et al.*, 2000) but it is impossible to test all the plant species in the PRA area. Thus the centrifugal phylogenetic host range testing sequence applied in this case was developed to ensure a scientific approach to plant selection. The principal of this testing sequence comprises an initial assessment of a group of non-target plant species, taxonomically most closely related to the target weed and usually exhibiting similar morphological and biochemical characteristics. The scope of the testing is then gradually expanded to include more and more distantly related plant species until specificity is established. Established protocols include ornamental species as well as economically important crops (see Appendix 1 for a detailed explanation). It is also possible that the agent will behave differently in the field than the laboratory but it is probably more likely that an effective agent will be rejected by the testing process than an unsafe agent will be released.

Overwintering:

Despite the best efforts of the research teams in both the UK and Japan, the exact overwintering behaviour of the psyllid is not certain. It is likely that they shelter on evergreen trees once their knotweed host has died off but it is not yet certain which species, if any, would be used and whether the hibernating psyllids would actually feed (see 1.14). It was possible to keep a few adults alive for 8 weeks on sections of bark of *P. radiata* at 5°C in a cooled/illuminated incubator which suggests there is no need to feed overwinter, but survival was too low to draw firm conclusions. The UK's post-release monitoring programme, which has focussed on assessing any impact, direct or indirect, on these potential hosts, has not highlighted any impact. Studies have shown an ability for the psyllid to overwinter on trunk sections of *Pinus sylvestris* and *Quercus robur*, suggesting that winter hosts do not need to be living. Overwintering psyllids also survived when left on *F. japonica*, but it is unclear whether this was on dead canes, in the leaf litter or soil (Clewley & Wright, 2014).

Climate:

The climatic matching included in this PRA is preliminary and such work is inherently uncertain. Nonetheless, the worst-case scenario from a biocontrol perspective is that the psyllid won't establish or reproduce adequately to achieve weed control. From a PRA perspective however, it is wise to assume that according to current host plant distribution the psyllid will establish successfully and widely, and would only be excluded in areas where degree-day requirements for one psyllid generation are not met (i.e. <462.5) (Appendix 3). Future impacts due to climate change are difficult to predict.

Natural enemies:

The potential for predation or for parasitism to affect establishment is low but unknown.

During recent field studies in the UK, some casual opportunistic predation was observed, but careful monitoring did not reveal any resulting flux in predator numbers. Laboratory studies have shown that 4 generalist predators have a preference for aphid prey above any psyllid stage (Appendix 7). No known parasitoids of *A. itadori* exist.

Adaptability:

A. itadori appears to be highly adaptable as far as climate and habitats are concerned, with medium uncertainty for the laboratory culture. Its adaptability to new host plants is believed to be very low because the host range testing and observations on other Polygonaceae in the field revealed a level of specialism on its host plant (for feeding and development) that is not often shown by more polyphagous species.

Delimiting spread/human spread:

It is expected that the psyllid will spread rapidly within the PRA area but this has a medium level of uncertainty. Spread by human assistance is possible but has medium uncertainty. If the psyllid establishes and spreads within the PRA area further spread into neighbouring European countries is expected.

Impacts

Secondary effects:

Whilst it is possible to make predictions on what host plants a biocontrol agent will and won't attack, it is notoriously difficult to predict any secondary, tertiary etc. effects on the rest of the food chain. The likely lack of specialist, native natural enemies should mean that these risks are minimised but some indirect effect resulting from the presence of a new organism in a habitat can never be ruled out in any situation. Generalist predators have been observed to feed on sedentary psyllid eggs or nymphs (Appendix 7). No evidence of tending by native ant species as has been observed in UK field trials. The main effect of any significant unanticipated predation will be to limit the effectiveness of the biological control agent (see Ireson *et al.*, 2003; Pratt *et al.*, 2003).

It is unlikely (with a low level of uncertainty) that any native species, including vertebrate species, which have chosen to use Japanese knotweed as a habitat will experience any negative impacts from a reduction in knotweed abundance.

There is potential for other weed or other plant species to replace Japanese knotweed following successful control by *A. itadori*, but this is uncertain.

Vectoring:

The potential for *A. itadori* to act as a vector of plant pathogens in the PRA area has not been explicitly tested but remains highly improbable given that these relationships are normally highly specialised in psyllids and that there are no records of *A. itadori* acting as a plant pathogen vector, nor are there any obvious candidate agents.

Hybridisation:

The potential for hybridisation with other [native psyllid species] in the PRA area is not known, but it is unlikely on the basis that such species do not readily interbreed.

Costs versus benefits of introduction:

The costs and benefits arising from introduction have not been evaluated but a cost-benefit analysis would be useful and may be needed to support the application for release.

Overall conclusion

Overall, based on research evidence *A. itadori* should not be considered to be a problematic pest and therefore risk management should not be required. It is likely that the high potential benefits outweigh the very low risks associated with the introduction of such a specialist natural enemy as a biocontrol agent. *A. itadori* can be considered a “beneficial pest” and as such the pest risk is low.

Stage 3: Pest Risk Management

A decision has to be made to determine whether the risk from any pest/pathway combination is an acceptable risk. This decision will be based on the relationship between the level of risk identified in the pest risk assessment stage (i.e. the combination of the probability of introduction and the potential economic impact) and the importance/desirability of the trade that carries the risk of introduction of the pest.

7.01 - Is the risk identified in the Pest Risk Assessment stage for all pest/pathway combinations an acceptable risk?

Yes

Over 10 years of studies have been conducted on *A. itadori* in the native range and under quarantine, non-quarantine and field conditions in the UK to evaluate the safety of the insect. *A. itadori* has undergone extensive host range testing against both native, ornamental and economically important plant species to ensure that it will pose a minimum risk to the PRA area, NW EU.

Following the PRA compiled for the psyllid for the UK authorities, peer review and a public consultation the insect was given ministerial approval for release in the UK in 2010 judging all associated potential risks to be acceptable.

Further safety testing has been conducted for the The Netherlands for which this PRA was compiled, and the risk to neighbouring European countries has been taken into consideration.

APPENDIX 1

The test plant list

The determination of host range is a critical consideration in the development of a biological agent for weed control. On commencement of specificity testing of the agent *Aphalara itadori* (Kyushu biotype) in the UK, a comprehensive test plant list was drawn up following the centrifugal-phylogenetic system detailed below. In total 73 species were selected for UK testing. In addition to these, a further 9 species (plus 4 replicate species of local origin) have since been tested in North America (Grevstad *et al.*, 2013) and 10 species (plus 3 replicate species of local origin) on behalf of The Netherlands (The Netherlands Food and Consumer Product Safety Authority) within UK facilities. Listed below are details of the original UK test plant list, including justifications for their inclusion applicable to the EU, as well as details of the North American, Dutch and Swiss test plants.

UK Test Plant List

Introduction and approach

This comprehensive test plant list follows the centrifugal-phylogenetic system for specificity proposed in 1974 (Wapshere, 1974). This approach continues to serve as the basis of current host-range testing protocols as recognized by the IPPC Code of Conduct for the Import and Release of Exotic Biological Control Agents (ISPM No.3). Essentially an initial small group of taxonomically related plants, with similar morphological and biochemical characteristics to the target weed are tested, gradually expanding the scope to include more distantly related plants until specificity is established. Established protocols also include ornamental species as well as economically important plants and crops and those closely related plants found in neighboring countries.

Japanese knotweed (*Fallopia japonica*) belongs to the Class Dicotyledonae, Order Caryophyllales and Family Polygonaceae. The family Polygonaceae is composed of two sub families (1) Eriogonideae and (2) Polygonoideae with *Fallopia japonica* belonging to the latter. There are approximately 44 genera in the sub family Polygonoideae, but only

eight are found in the UK, *Fagopyrum*, *Persicaria*, *Polygonum*, *Fallopia* (includes *Fallopia japonica*), *Rumex*, *Rheum*, *Oxyria* and *Muehlenbeckia*.

In compiling the test plant list, initially, plant species were selected from within the Family Polygonaceae, sub-family's Polygonoideae and Eriogonideae. As it was unfeasible to include all native and ornamental species from the UK from this Family, careful consideration was given to the status of the species. Native species took priority, along with commonly available ornamentals and economically important genera - such as *Rheum*. After selecting 47 species in the Family Polygonaceae, following the centrifugal-phylogenetic system, selection moved out of the Family to select more distantly related species. This involved selecting representative species, with UK relevance, from the other Families in the Order Caryophyllales (Figure 1). Then species with similar morphology, similar biochemical characteristics and plants of economical importance were selected. Lastly, as a result of interactions with regulators for plant pathogens, a further three plant species were added to the proposed test plant list namely *Lemna minor*, *Larix decidua* and *Populus tremula*.

The taxonomy and phylogeny of the species selected was taken from Stace (2003) and Mabberly (1987). The status of each species was evaluated using Preston *et al.* (2002) and the JNCC Red Data List (Cheffings *et al.*, 2005). Ornamental species were selected using the Royal Horticultural Society's Plant Finder (2003). The complete test plant list can be found in Table 1 and a further introduction to each species can be found in the following section. In all, 73 species from 19 families were included in the test plant list, consisting of 41 species native to the UK, 10 introduced species, 2 native to Europe, 6 ornamentals and 14 economically important species. Some species are a combination of some of the above.

The evolution of the test plant list was ongoing throughout the project and as a result a number of species with a high degree of phylogenetic separation to the target weed were removed from the test plant list in 2006, namely, *Coccoloba uvifera*, *Drosera intermedia*, *Nepenthes fusca*, *Phytolacca americana*, *Bougainvillea* species, *Cactus* species, *Delosperma cooperi*, *Lewisia columbiana*, *Chenopodium giganteum*, *Celosia argente* var.

cristata, *Polygonatum verticillatum*, *P. multiflorum* and *Solanum melongena*. This allowed more time to concentrate on the more closely related species.

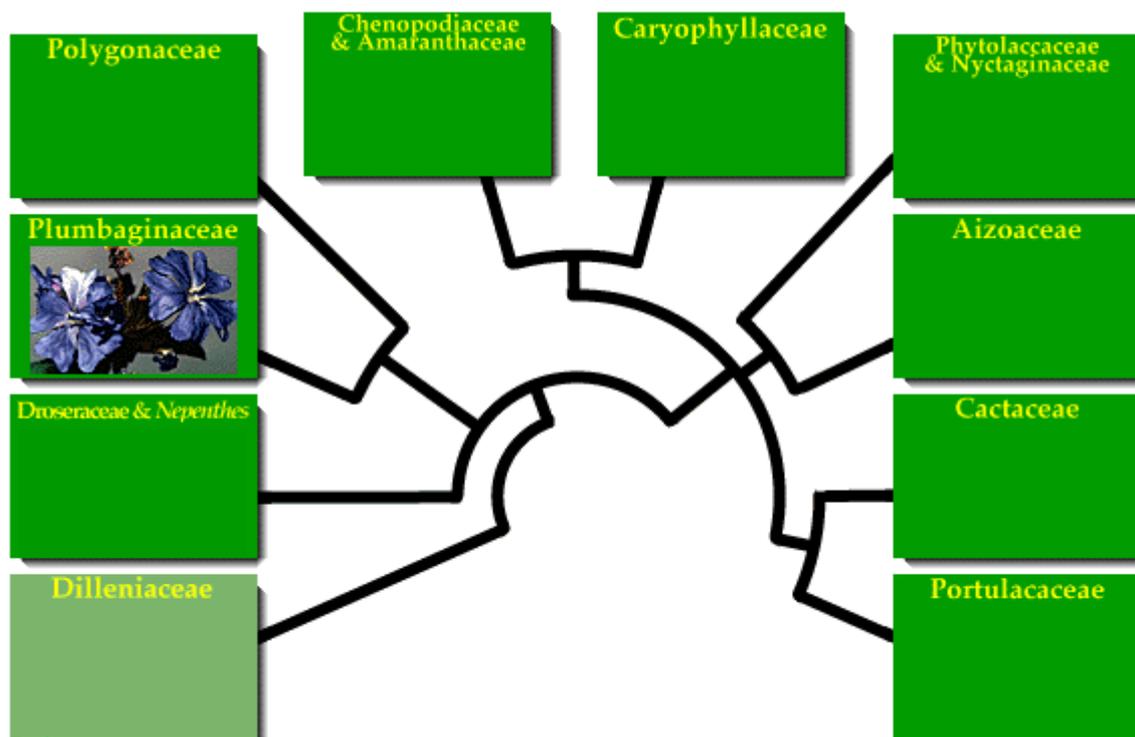


Figure 1 - A cladogram of the phylogenetic relationships between families in the Caryophyllids (Figure courtesy of University of Berkley)

In contrast, as a result of suggestions with regulators for plant pathogens, a further three plant species were added to the proposed test plant list namely *Lemna minor*, *Larix decidua* and *Populus tremula*. In addition it was decided to add *Frankenia laevis* to the test plant list due to its close phylogenetic relatedness to Polygonaceae.

Test plant suppliers included numerous garden centers and nurseries and national collections, especially from Rowden Gardens in Cornwall- the UK's national *Polygonum* collection. We sourced seed stock from throughout the UK and in some cases abroad where UK stock was limited. Kew's Millennium Seed Bank supplied us with many hard to find seeds from closely related plant species like *Polygonum maritimum* and *Fallopia dumetorum*.

Three plant species were very difficult to obtain, namely *Polygonum boreale*, *Persicaria mitis* and *Persicaria minor*. Information was eventually secured on their locations in the wild, and field collections of these species were made.

North American Test Plants

These species were selected using the same phylogenetic approach utilised for the UK test plant list, with recommendations from the Technical Advisory Group (TAG) on Biological Control of Weeds included (Grevstad *et al.*, 2013). The testing was primarily carried out at Oregon State University, USA and CABI in the UK, with a small number of species tested in Canada (Agriculture and Agrifood Canada).

Table 1 lists those species tested with the psyllid *A. itadori* (Kyushu biotype) in the UK and North America combined.

The Netherlands Test Plants

In 2013, CABI were contracted by the Team Invasive Aliens (TIE) of The Netherlands Food and Consumer Product Safety Authority (NFCPSA) to extend testing of the psyllid *A. itadori*. Along with local target plant specimens, an additional 10 species were selected for testing, including both native species and others of horticultural importance (Table 2).

Swiss Test Plants

Additional tests are currently underway in Switzerland (CABI) to test the susceptibility of local target knotweed species to *A. itadori*, as well as testing its specificity against 10 species that are closely related and native/economically important to the region (Table 3).

Table 1 – Combined UK & North America Test Plant List for *A. itadori* (Kyushu biotype)

<u>Species</u>	<u>Common name</u>
Order Caryophyllales	
Family Polygonaceae	
<i>Fallopia</i>	
<i>japonica</i> ex. UK	Japanese knotweed
<i>japonica</i> ex. NA	
var. ' <i>compacta</i> '	Varieties
var. <i>variegata</i>	
var. ' <i>crimson</i> '	
<i>x bohemica</i> ex. UK	Hybrid (<i>F. japonica</i> x <i>F. sachalinensis</i>)
<i>x bohemica</i> ex. NA	
<i>x conollyana</i>	Hybrid (<i>F. japonica</i> x <i>F. balschuanica</i>)
<i>sachalinensis</i> ex. UK	Giant knotweed
<i>sachalinensis</i> ex. NA	
<i>convolvulus</i> ex. UK	Black bindweed
<i>convolvulus</i> ex. NA	
<i>cinodis</i>	Fringe Black bindweed
<i>baldschuanica</i>	Russian vine
<i>scandens</i>	Climbing bindweed
<i>dumetorum</i>	Copse bindweed
<i>Polygonum</i>	

<i>douglasii</i>	Douglas' knotweed
<i>aviculare</i>	Knotgrass
<i>anchoreum</i>	(none available)
<i>ramossisimum</i>	Bushy knotweed
<i>paronychia</i>	Beach knotweed
<i>arenastrum</i>	Equal leaved knotgrass
<i>shastense</i>	Shasta knotweed
<i>boreale</i>	Northern knotgrass
<i>maritimum</i>	Sea knotgrass
<i>oxyspermum</i>	Ray's knotgrass
<i>rurivagum</i>	Cornfield knotgrass
<i>pensylvanicum</i>	Pennsylvania smartweed
<i>phytolaccaefolium</i>	Poke knotweed

Polygonella

<i>robusta</i>	Largeflower jointweed
<i>articulata</i>	Coastal jointweed

Persicaria

<i>affinis</i>	Himalayan fleece flower
<i>amplexicaulis</i>	Red bistort
<i>hydropiperoides</i>	False water-pepper smartweed
<i>lapathifolia</i>	Pale persicaria
<i>microcephala</i>	Red dragon knotweed
<i>orientalis</i>	Oriental/Prince's knotweed
<i>sagittata</i>	Arrowleaf tearthumb
<i>virginiana</i>	Virginia knotweed
<i>amphibia</i>	Amphibious bistort
<i>bistorta</i>	Common bistort
<i>campanulata</i>	Lesser knotweed

<i>capitata</i>		Himalayan persicaria
<i>hydropiper</i>	ex. NA	Water pepper
<i>hydropiper</i>	ex. UK	
<i>maculosa</i>		Redshank
<i>minor</i>		Small Waterpepper
<i>mitis</i>		Tasteless Waterpepper
<i>mollis</i>		Soft knotweed
<i>polystachum</i>		Himalayan knotweed
<i>tinctoria</i>		Dyer's knotgrass
<i>vivipara</i>		Alpine bistort

Fagopyrum

<i>esculentum</i>		Buckwheat
<i>dibotrys</i>		Tall buckwheat
<i>tataricum</i>		Tartary buckwheat

Muehlenbeckia

<i>complexa</i> (syn. <i>axillaris</i>)		Wire plant
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Rheum

<i>palmatum</i>		Rhubarb
<i>hybridum</i>		Rhubarb
<i>rhabarbarum</i>		Rhubarb

Rumex

<i>acetosa</i>		Common sorrel
<i>acetosella</i>		Sheep's sorrel
<i>arcticus</i>		Arctic dock
<i>britannica</i>		British dock
<i>fueginus</i>		= syn. <i>R. maritimus</i>

<i>oxidentalis</i>	Dorn western dock
<i>orthoneurus</i>	Blumer's dock
<i>alpinus</i>	Alpine dock
<i>aquaticus</i>	Scottish dock
<i>conglomeratus</i>	Clustered dock
<i>crispus</i>	Curled dock
<i>longifolius</i>	Northern dock
<i>maritimus</i>	Golden dock
<i>obtusifolius</i>	Broad-leaved dock
<i>orbiculatus</i>	Greater water dock
<i>palustris</i>	Marsh dock
<i>pulcher</i>	Fiddle dock
<i>rupestris</i>	Shore dock
<i>sanguineus</i>	Wood dock
<i>scutatus</i>	French sorrel
<i>hydrolapathum</i>	Water dock
<i>triangulivalvis</i>	Mexican dock
Oxyria	
<i>digyna</i>	Mountain sorrel
Antigonon	
<i>letopus</i>	Mexican creeper
Brunnichia	
<i>ovata</i>	American buckwheat vine
Coccoloba	
<i>uvifera</i>	Sea Grape

Eriogonum

umbellatum

Sulphur buckwheat

parishii

Mountainmist

cernuum

Nodding buckwheat

elatum

Tall wooly buckwheat

nudum

Naked buckwheat

pyrolifolium

Shasta buckwheat

Oxytheca

dendroidea

Narrowleaf oxytheca

Chorizanthe

membranaceae

Pink spineflower

Other representative species within Caryophyllales

Family Chenopodiaceae

Beta

vulgaris

Beetroot

Chenopodium

album

Fat hen

Family Caryophyllaceae

Cerastium

glomeratum

Sticky mouse-ear

fontanum

Common mouse-ear

Dianthus

gratianopolitanus

Cheddar pink

Family Nyctaginaceae

Bougainvillea
spp.

Bougainvillea

Family Aizoaceae

Delosperma

cooperi

Trailing Ice plant

Family Plumbaginaceae

Armeria

maritima

Sea thrift

Limonium

bellidifolium

Matted sea lavender

binervosum

Rock sea lavender

carolinianum

Carolina sea lavender

Family Frankeniaceae

Frankenia

laevis

Sea-heath

Family Portulacaceae

Lewisia

columbiana

Columbian lewisia

Family Phytolaccaceae

Phytolacca

americana

American Pokeweed

Family Cactaceae

Notocactus

magnificus

Green pumpkin cactus

Echinocereus

subinermus

Carpobrotus

edulis

Kaffir Fig

Family Amaranthaceae

Celosia

argentea

Plumed cockscomb

Family Tamaricaceae

Tamarix

gallica

French Tamarisk

Morphologically Similar

Calystegia

sepium

Hedge bindweed

Houttuynia

cordata

Chameleon plant

Biochemically Similar

Vitis

vignifera

Grape

Rubus

fruticosus

Blackberry

Agent Specific Species

Cytisus

scoparius

Scotch broom

Malus

domestica

Crab apple

Economically Important

Hordeum

vulgaris

Barley

Lycopersicon

esculentum

Tomato

Phaseolus

vulgaris

French bean

Vicia

fab

Broad bean

Solanum

tuberosum

Potato

melongena

Aubergine/Eggplant

Triticum

aestivum

Wheat

Zea

mays

Corn

Rosa

spp.

Rose

Brassica

napus

Rapeseed

oleraceae

Wild mustard

Vaccinium

macrocarpon

Large cranberry

Pseudotsuga

mensiezii

Douglas fir

Later Additions

Lemna

minor Duckweed

Larix

decidua European Larch

Populus

tremula Aspen

Table 2 – The Netherlands Test Plant List for *A. itadori* (Kyushu biotype) (N.B. All plants sourced from The Netherlands, tested in the UK)

Species

Common name

Order Caryophyllales

Family Polygonaceae

Fallopia

japonica Japanese knotweed

sachalinensis Giant knotweed

x bohemica Hybrid (*F. japonica* x *F. sachalinensis*)

Rumex

thyrsoiflorus Compact dock/Thyrse sorrel

Other representative species within Caryophyllales

Family Chenopodiaceae

Beta

vulgaris subsp. *maritima* Sea beet

Chenopodium

bonus-henricus Good King Henry

quinoa Quinoa

Family Plumbaginaceae

Limonium

vulgare Common sea lavender

Economically Important

Capsicum

annuum Pepper

Cucurbita

maxima Squash

Fragaria

sp. Strawberry

Rubus

idaeus Raspberry

Triticum*spelta*

Spelt

Table 3 – Swiss Test Plant List for *A. itadori* (Kyushu biotype)**Species****Common name****Order Caryophyllales****Family Polygonaceae*****Fallopia****japonica*

Japanese knotweed

convolvulus

Black bindweed

dumetorum

Copse bindweed

Polygonum*alpinum*

Alpine knotweed

lapathifolium

Pale smartweed

mitis

Tasteless water pepper

Fagopyrum*esculentum*

Buckwheat

Rheum*rhabarbarum*

Rhubarb

Rumex

alpestris (syn. *arifolius*)

Maiden sorrel

patientia

Patience dock

thyrsoiflorus

Compact dock/Thyrse sorrel

nivalis

Snow dock

acetosella subsp. *Pyrenaicus*

Round-seeded Sheep's sorrel

Justification for the inclusion of plant species

***Fallopia baldschuanica* (Russian vine)**

Fallopia baldschuanica (non-native horticultural plant but invasive) included in the test plant list due to its close relatedness to the target species. *Fallopia baldschuanica* (also known as Russian vine) is found growing wild in a number of EU countries and is established in The Netherlands (Duistermaat *et al.*, 2012). It is naturalised in Austria, Belgium, Britain, Czech Republic, Romania and Spain. Russian vine is a non-native species and often considered invasive in Europe just as it is in the USA where it is sold under its synonym *Polygonum aubertii* or Japanese fleecflower. Still available in garden centres and grown as a garden covering vine, Russian vine has the potential to grow in close proximity to *Fallopia japonica* especially in urban areas and on brown field sites.

***Fallopia convolvulus* (Black bindweed)**

Fallopia convolvulus (native, garden weed) is a very common annual native species found throughout western Europe, certainly introduced in the extreme north and perhaps elsewhere. Regarded as an archaeophyte, this species was formerly regarded as a weed of cultivated sites. Included in the test plant list due to its close relatedness to the target. As a weed of waste ground and roadsides *Fallopia convolvulus* is often found in close proximity to the target species.

***Fallopia dumetorum* (Copse bindweed)**

Fallopia dumetorum (native, restricted) is the only native *Fallopia* in Europe and was previously listed as a Red Data book species (classification: vulnerable), although it is absent from the 2014 list suggesting the population is stable or increasing. This species occurs over much of Europe northwards to northern Sweden, it is widely distributed across N.W. Europe, including The Netherlands and areas of Austria, Belgium, Denmark, France and Germany (GBIF), although sometimes has local distributions, and is absent from the Mediterranean and Iberian Peninsula. Preston *et al.* (2002) regards the plant as having an erratic appearance in its preferred habitat of hedgerows and woodland margins. Included in the test plant list due to its close relatedness to the target.

***Fallopia sachalinensis* (Giant knotweed)**

Fallopia sachalinensis was introduced into St Petersburg in 1864 and was reported as growing in the city's botanical gardens in the same year. It has subsequently spread throughout many European countries when it became commercially available to gardeners and recommended as a forage plant for cattle. This non-native species is found on waste

ground, roadsides and river banks, mainly in lowland areas where it forms dense monocultures, sometimes mixed in with the target. It can also be a pollen partner producing the highly invasive *F. x bohémica*. The species has no environmental benefits. Included in the test plant list due to its relatedness to the target.

***Fallopia japonica* var. *compacta* (Dwarf variety)**

Fallopia japonica var. *compacta* records do not indicate it to be present in the wild in European countries. It is however sold in the horticultural trade. Unlike *F. japonica*, which spreads clonally throughout Europe, compact knotweed has both functional female and male plants (Duistermaat *et al.*, 2012). It is also found in Belgium and Germany.

***Fallopia x bohémica* (hybrid)**

This hybrid of *Fallopia japonica* x *F. sachalinensis* is regarded as a rampant non-native weed and is distributed throughout Europe. Found mainly on waste ground and along roadsides and river banks in lowland areas, *F. x bohémica* was not collected from the wild until 1954. This hybrid is competitive and spreading at a fast rate in Central Europe. Again, included in the test plant list due to it being closely related to the target species.

***Fallopia x conollyana* (hybrid)**

A non-native hybrid of *Fallopia japonica* x *Fallopia baldschuanica*, known at only one location in Europe (in Great Britain, Bailey, 2001) and there are no records of its presence in wider European countries.

Polygonum

The genus *Polygonum* contains a group of species which are the most closely related to *Fallopia* and this is represented by both *Fallopia* and *Polygonum* being classified together under the tribe Polygoneae.

***Polygonum alpinum* (Alpine knotweed)**

Hardy perennial found along roadsides, streambeds, and disturbed ground in high altitude areas such as the Alps. Due to the high altitude distribution of this species, it is unlikely *Polygonum alpinum* will grow in or near stands of Japanese knotweed. Red Data book status = Vulnerable.

***Polygonum arenastrum* (Equal leaved knotgrass)**

A native archaeophyte which is found throughout most of Europe, often found on waste ground, trampled ground and growing out of gaps in concrete. *Polygonum arenastrum* is a low-growing mat-forming annual species. It is feasible that the plant will grow in similar habitats to that of the target.

***Polygonum aviculare* (Knotgrass)**

Native to Europe, an annual species commonly found in gardens, arable fields, pavements, tracks and waste ground. This plant has a more sprawling and erect form to that of *Polygonum arenastrum*. It is feasible that the plant will grow in similar habitats to that of the target.

***Polygonum maritimum* (Sea knotgrass)**

A native perennial herb of sand and shingle along the shores of the Atlantic, Mediterranean and Black Sea, northwards to S.E. Ireland. Japanese knotweed does not favour this habitat so is unlikely to grow in any of the locations where *Polygonum maritimum* occurs. In the UK it is listed under the JNCC Red Data list as a vulnerable species and protected under schedule 8 of the Wildlife and Countryside Act 1981.

***Polygonum oxyspermum* (Ray's knotgrass)**

Found predominately along the coasts of Europe, but rare in the south-west, this native annual herb has fluctuating populations from one year to the next. Although *Fallopia japonica* does not prefer sandy habitats near the coast it is occasionally found in such habitats.

***Polygonum rurivagum* (Cornfield knotgrass)**

Polygonum rurivagum is classed by Preston *et al.* (2002) as a native archaeophyte found mainly in S., W. and W.C. Europe, extending northwards to Sweden. Found mainly on arable land and chalk soils this species is unlikely to grow in similar habitats to that of the target.

***Polygonum boreale* (Northern knotgrass)**

A rare UK native species with a very localised distribution in Scotland and the surrounding islands of Orkney and Shetland. It is found in N.W. Europe, from N. Scotland onwards. *Polygonum boreale* is similar in form and structure to *Polygonum aviculare*, and therefore it is difficult to tell the two species apart.

Persicaria

The genus *Persicaria* is a large group with a widespread cosmopolitan distribution. Due to their economic value 14 species (8 European natives and 6 introduced /ornamental species) represent this group in the test plant list.

***Persicaria amphibia* (Amphibious bistort)**

Persicaria amphibia is a widespread European native. It has two distinct forms - an aquatic free floating form found on slow moving water bodies and a terrestrial erect form common in grassland and damp areas.

***Persicaria bistorta* (Common bistort)**

A European native perennial herb commonly found though absent from the higher altitude areas. Common along road sides and river banks. It is therefore feasible that *Polygonum bistorta* will grow near stands of Japanese knotweed.

***Persicaria campanulata* (Lesser knotweed)**

A tall attractive non-native species often grown as a garden ornamental. Found scattered throughout Europe in localised patches along roadsides hedge banks and streams, it is feasible this species can grow close to the target species.

***Persicaria hydropiper* (Water pepper)**

An attractive European native commonly found, except in the extreme north. Growing on damp mud this species is known to grow in and near Japanese knotweed patches along river banks and roadsides.

***Persicaria lapathifolia* (Pale persicaria)**

Very similar to the above species, *Persicaria lapathifolia* is a European native absent only from high altitude areas. Found along streams, ditches and cultivated land this species has the potential to grow around stands of Japanese knotweed.

***Persicaria capitata* (Himalayan persicaria)**

This mat forming non-native annual species can be found along roadsides, paths and in urban areas. As an introduced species and garden escapee this plant is not renowned for its environmental value to Europe.

***Persicaria mollis* (Soft knotweed)**

An introduced-naturalised garden escapee, growing in rough ground and where it does grow it has the potential to form dense clumps. As an introduced species this plant is not renowned for its environmental value to Europe.

***Persicaria affinis* (Himalayan fleece flower)**

A mat-forming perennial ornamental species, not known to occur naturally in European countryside. However, as a species grown in urban areas, *Persicaria affinis* has the potential to grow near stands of Japanese knotweed.

***Persicaria maculosa* (Redshank)**

A European native *P. maculosa* is a widespread species similar to *Persicaria hydropiper*. Found along river banks, roadsides and waste ground this species is likely to grow in and near stands of Japanese knotweed.

***Persicaria vivipara* (Alpine bistort)**

A short tufted European native perennial herb growing on wet rocks and grassland, often abundant in montane grassland and common in the Arctic. Due to the high altitude distribution of this species, it is unlikely *Persicaria vivipara* will grow in or near stands of Japanese knotweed.

***Persicaria tinctoria* (Dyer's knotgrass)**

An eastern European native present in limited populations, *Persicaria tinctoria* is grown for the natural blue dye the plant's leaves produce when crushed.

***Persicaria polystachum* (Himalayan knotweed)**

Persicaria polystachum (non-native, invasive). A tall, rhizomatous perennial herb found along streams and hedge banks in Europe. A garden escapee naturalised on roadsides this non-native, considered invasive in some areas, *Persicaria polystachum*, also known as *Persicaria wallichii*, has been observed growing in close proximity to Japanese knotweed.

***Persicaria mitis* (Tasteless Waterpepper)**

A rare annual European native species of wet soils. *P. mitis* (*Persicaria laxiflora*) is widespread in Europe between 40°N and 55°N, but is rare in the North and Mediterranean region. Found alongside ponds, lakes and rivers and in damp meadow and cattle trampled pastures. Unlikely to be found growing near the target species. Red Data book status = Vulnerable.

***Persicaria minor* (Small Waterpepper)**

A rare annual European native species of wet marshy soils. *P. minor* is widespread between 45°N and 65°N, but absent from most of the Mediterranean and arctic. Found alongside ponds, lakes and rivers and in damp meadow and cattle trampled pastures. Unlikely to be found growing near the target species. Red Data book status = Vulnerable.

Fagopyrum

The genus *Fagopyrum* is represented on the test plant list by two non-native species *Fagopyrum esculentum* and *F. dibotrys*.

***Fagopyrum dibotrys* (Tall buckwheat)**

Fagopyrum dibotrys (non-native) cultivated for ornament, a non-native buckwheat species that may be becoming naturalised at a few localities on the coasts of W. Europe. This species has long bamboo-like stems and unlike its close relative, *Fagopyrum esculentum*, it is a rhizomous species with vigorous growth and spread. Unlikely to be found growing near the target species.

***Fagopyrum esculentum* (Buckwheat)**

Fagopyrum esculentum (non-native, occasional crop) is an economically important crop species in many countries throughout the world. It was formerly extensively cultivated in most of Europe. The seeds can be crushed to produce flour which is free of gluten making it an alternative to wheat flour. The plant is naturalised in disturbed land through much of Europe.

Rumex* and *Rheum

Rumex and *Rheum* genera belong to the tribe Rumiceae. *Rumex*, also known as docks and sorrels are represented in this list by 19 European species, many of which are widespread across Europe and others with more local distributions.

***Rumex acetosa* (Common sorrel)**

A widespread dock species found throughout most of Europe, but rarer in much of the south. Found in a multitude of habitats including, riparian, coastal and urban. This species will grow in and near stands of Japanese knotweed.

***Rumex acetosella* (Sheep's sorrel)**

A widespread dock species present throughout the wider PRA area. Found in a multitude of habitats including, riparian, coastal and urban. This species will grow in and near stands of Japanese knotweed.

***Rumex acetosella* subsp. *pyrenaicus* (Round-seeded Sheep's sorrel)**

A common subspecies of *R. acetosella* native to Europe. Currently undergoing testing with *A. itadori* in Switzerland (CABI).

***Rumex alpestris* [syn. *arifolius*] (Maiden sorrel)**

Native to southern Europe, but also found in mountain meadows and pine forests of North and Central Europe, with populations located at elevations of 200-700 metres. Currently undergoing testing with *A. itadori* in Switzerland (CABI).

***Rumex aquaticus* (Scottish Dock)**

Found in very isolated patches in 3 sites in Scotland. This UK native species of damp marshy land can hybridise with the close relative *Rumex obtusifolius*. There are no known records of Japanese knotweed growing in close proximity to this species. IUCN Red Data book status = Vulnerable.

***Rumex conglomeratus* (Clustered Dock)**

A widespread and short-lived species of wet meadows and streams. Present across the PRA area. This species potentially can grow alongside Japanese knotweed.

***Rumex crispus* (Curled Dock)**

A widespread ruderal dock species found in a multitude of habitats throughout Europe including, waste ground, roadside, arable, coastal and urban. This species will grow in and near stands of Japanese knotweed.

***Rumex hydrolapathum* (Water Dock)**

Rumex hydrolapathum (native, environmental value). A tall, tufted perennial species found along slow moving rivers and lakes. *Rumex hydrolapathum* does not survive well in closed vegetation. Present in most of Europe and all of the focus PRA countries, from 62°N. in Finland to 40°N, in Italy, but very local in the south. This species can grow alongside stands of Japanese knotweed.

***Rumex longifolius* (Northern dock)**

A ruderal species native to Europe, and found on open ground, roadsides, riverbanks and lake shores. This species can potentially grow alongside Japanese knotweed.

***Rumex maritimus* (Golden dock)**

An annual species growing near lakes, rivers and ditches throughout central England. It is feasible this species can grow near stands of Japanese knotweed- though records are not available.

***Rumex nivalis* (Snow dock)**

A dock species endemic to Europe, found in the stony terrain of mountains above 1500m. Distribution restricted to the Alps (including Germany, Austria and Switzerland). Currently undergoing testing with *A. itadori* in Switzerland (CABI).

***Rumex obtusifolius* (Broad-leaved dock)**

A ruderal dock species found throughout Europe except in the extreme north and south. Found in a multitude of habitats including, riparian, coastal and urban. This species will grow in and near stands of Japanese knotweed.

***Rumex palustris* (Marsh Dock)**

Found throughout southern and central Europe, with a distribution extending to Denmark. Growing mainly on marsh land and river banks. This species can grow alongside stands of Japanese knotweed.

***Rumex patientia* (Patience Dock)**

A dock species, native to eastern Europe but naturalised and ruderal in North and West Europe. Currently undergoing testing with *A. itadori* in Switzerland (CABI).

***Rumex pulcher* (Fiddle Dock)**

Found commonly in South and West Europe, this species is a biennial or short-lived perennial herb of dry coastal pastures and disturbed grassland. This species can potentially grow alongside of Japanese knotweed.

***Rumex rupestris* (Shore dock)**

A perennial herb of sand and shingle beaches. This species' distribution is restricted to the coasts of SW Britain, NW France and NW Spain. Unlikely to grow near stands of Japanese knotweed. Protected under Schedule 8 of the Wildlife and Countryside Act, 1981. IUCN Red Data book status = Endangered.

***Rumex sanguineus* (Wood dock)**

A common and widespread European species found in wooded margins, hedgerows, roadsides and waste ground. It is feasible that Wood dock will grow around stands of Japanese knotweed.

***Rumex scutatus* (French sorrel)**

A species native to Central and Southern Europe, this dock is cultivated elsewhere and is locally naturalised.

***Rumex thyrsiflorus* (Compact dock/Thyrses sorrel)**

A species found in meadows and wastelands of Eastern and Central Europe. This species is an addition to the original UK test plant list, and was selected by The Netherlands and Switzerland due to its relatedness to the target and its occurrence in the wider PRA area.

The economic importance of *Rheum* (Rhubarb) warrants the inclusion of both an ornamental species *Rheum palmatum* and a food plant *Rheum x hybridum*.

***Rheum x hybridum* (Rhubarb) (“Glaskins”)**

A rhizomatous non-native perennial herb found in gardens, urban areas, railway banks, rivers and stream and on waste ground. Hybrid derived from *R. rhabarbarum*. Wild populations are regarded as a relic of cultivation, as it is grown widely in parts of NW Europe for its edible petioles. Several species cultivated and may be naturalized locally. Can potentially be found growing near stands of Japanese knotweed.

***Rheum palmatum* (Rhubarb) and *Rheum x hybridum* “Glaskins” (Rhubarb)**

Originating from West China, and found in scattered isolated populations in Britain, Sweden, Germany and The Netherlands. An introduced species occurring in the countryside as a result of cultivation.

***Rheum rhabarbarum* (Rhubarb)**

Native to Mongolia and surrounding areas, and cultivated in parts of Europe (see *R. x hybridum*). This species is an addition to the original UK test plant list, and was selected by The Netherlands and Switzerland due to its economic importance within the PRA area.

***Muehlenbeckia complexa* (Wire plant)**

Muehlenbeckia complexa (non-native ornamental). There are no native species of *Muehlenbeckia* in Europe, but *M. complexa* has naturalised on the coasts of western Europe (Azores, Britain, France, Portugal). *Muehlenbeckia complexa* has been included in the proposed test plant list due to its close relatedness to *Fallopia*.

***Oxyria digyna* (Mountain sorrel)**

Oxyria digyna (native, alpine species). The genus *Oxyria* is represented in the test plant list by *Oxyria digyna*, which is the only species native to Europe in this group of two species. Present in Arctic Europe and in mountainous regions southwards (to Corse and C. Greece), on ungrazed alpine meadows and by streams. Unlikely to grow near stands of Japanese knotweed due to its high altitudinal distribution.

***Eriogonum umbellatum* (Sulphur buckwheat)**

A non-native species grown as a garden ornamental.

N.B. The following 19 Polygonaceae species have also been tested using *A. itadori* (Kyushu biotype) in North America. These species were selected for their relatedness to the target and their relevance to the native or economically important flora of North America.

***Rheum rhabarbarum* (Rhubarb)**

***Rumex arcticus* (Arctic dock)**

***Rumex britannica* (British dock)**

***Rumex fueginus* [syn. *R. maritimus*]**

***Rumex occidentalis* (Dorn western dock)**

***Rumex orthoneurus* (Blumer's dock)**

***Rumex alpinus* (Alpine dock)**

***Rumex orbiculatus* (Greater water dock)**

***Rumex triangulivalvis* (Mexican dock)**

***Antigonon leptopus* (Mexican creeper)**

***Coccoloba uvifera* (Sea grape)**

***Brunnichia ovata* (American buckwheat vine)**

***Eriogonum parishii* (Mountainmist)**

***Eriogonum cernuum* (Nodding buckwheat)**

***Eriogonum elatum* (Tall woolly buckwheat)**

***Eriogonum nudum* (Naked buckwheat)**

***Eriogonum pyrolifolium* (Shasta buckwheat)**

***Oxytheca dendroidea* (Narrowleaf oxytheca)**

***Chorizanthe membranacea* (Pink spineflower)**

Other representative species within Caryophyllales

***Limonium bellidifolium* (Matted sea lavender)**

A member of the Plumbaginaceae, *Limonium bellidifolium* has a restricted distribution in Europe, occurring primarily in the upper salt marshes of the UK, France, Spain and Greece. Unlikely to grow near stands of Japanese knotweed due to its high coastal distribution.

***Limonium binervosum* (Rock sea lavender)**

A group of apomictic perennial herbs comprising of nine species (sub-species). Endemic to Europe and present in some coastal areas of the British Isles, France, Spain and Portugal. Unlikely to grow near stands of Japanese knotweed due to its high coastal distribution.

***Limonium vulgare* (Common sea lavender, marsh rosemary)**

Predominantly found in the maritime salt marshes of South and West Europe, with a range extending northeastwards to SW Sweden. This species is an addition to the original UK test plant list, and was selected by The Netherlands due to the frequency of its occurrence on the Dutch coastline.

***Cerastium glomeratum* (Sticky mouse-ear)**

A species found in disturbed areas, often in places where there is nutrient-enriched soil. Found throughout Europe. Likely to grow in close proximity to Japanese knotweed stands.

***Cerastium fontanum* (Common mouse-ear)**

A mat-forming perennial species of grasslands. Found throughout almost all of Europe. *Cerastium fontanum* could potentially be found growing near stands of Japanese knotweed.

***Carpobrotus edulis* (Kaffir Fig)**

A non-native ornamental species with the tendency to become invasive along coastal areas and cliffs. Highly invasive in the coastal Mediterranean and unlikely to grow near stands of Japanese knotweed.

***Chenopodium album* (Fat hen)**

Widespread across Europe, this annual species grows on disturbed nutrient rich soils, cultivated field and urban areas. *Chenopodium album* can potentially be found growing near stands of Japanese knotweed.

***Chenopodium bonus-henris* (Good King Henry)**

Native to central and southern Europe, but rare in the east and doubtfully native in the north; mainly on mountains in the south and naturalised elsewhere. Once cultivated but now considered a weed. This species is an addition to the original UK test plant list, and was selected by The Netherlands for its relevance to the native flora.

***Chenopodium quinoa* (Quinoa)**

Originating from the Andes, quinoa is a pseudocereal grown for its edible seeds, which have become popular in European countries over recent years. This species is an addition to the original UK test plant list, and was selected by The Netherlands for its potential economic importance.

***Beta vulgaris* (Beetroot)**

A commonly grown garden species. As beetroot can be found in urban areas it has the potential to grow near stands of Japanese knotweed.

***Beta vulgaris* subsp. *maritima* (Sea beet)**

Present primarily on the coasts of South and West Europe . This species is an addition to the original UK test plant list, and was selected by The Netherlands for its relevance to the native flora.

***Frankenia laevis* (Sea-heath)**

A native to Western Europe, this coastal, low-growing mat-forming species occurs in salt marshes and sand dunes.

***Tamarix gallica* (French Tamarisk)**

A tree species found in coastal areas of Europe, particularly common in Mediterranean regions.

N.B. The following 10 species (Order: Caryophyllales) have also been tested using *A. itadori* (Kyushu biotype) in North America. These species were selected for their relevance to the horticultural trade or native flora of North America.

***Armeria maritima* (Sea thrift)**

***Bougainvillea* sp. (Bougainvillea)**

***Celosia argentea* (Plumed cockscomb)**

***Delosperma cooperi* (Trailing Ice plant)**

***Dianthus gratianopolitanus* (Cheddar pink)**

***Echinocereus subinermis* (Cactus - no common name available)**

***Lewisia columbiana* (Columbian lewisia)**

***Limonium carolinianum* (Carolina sea lavender)**

***Notocactus magnificus* (Green pumpkin cactus)**

***Phytolacca americana* (American pokeweed)**

Morphologically similar species

The following two species were included in the test plant list due to their morphological similarities to Japanese knotweed.

***Calystegia sepium* (Hedge bindweed)**

A bindweed species. Common in hedgerows and in urban areas across Europe. Recorded as growing within stands of Japanese knotweed. Leaf morphology is similar to that of Japanese knotweed.

***Houttuynia cordata* (Chameleon plant)**

It is often hard to tell the leaves of this species apart from Japanese knotweed. Non-native species grown as a garden plant.

Biochemically similar species

The following two species share a common chemical with the target species, namely resveratrol.

***Vitis vignifera* (Grape)**

Widely cultivated in much of Europe, as well as being a garden and greenhouse plant, this species was included in the test plant list due to the high concentrations of resveratrol found in the plants fruits.

***Rubus fruticosus* (Blackberry)**

A species very common in north-west Europe. Included in the test plant list due to the high concentrations of resveratrol found in the plants fruits. The species grows within and near stands of Japanese knotweed.

Agent specific species

***Cytisus scoparius* (Scotch broom)**

Native and common to western and central Europe. This species was included because it is known to be a host of another psyllid species, *Arytainilla spartiophila*.

***Malus* spp. (Crab apple)**

A commonly-grown garden tree found throughout the UK. *Malus* has the potential to be grown near the target species. This was included because at the time a sawfly *Amatastegia polygoni* was under consideration and another sawfly *Ametastegia glabrata* which specialises on dock can also feed on apples.

Economically important species

***Rosa* sp. (Rose)**

A commonly-grown garden species. Rose species have the potential to grow near the target species.

***Phaseolus vulgaris* (French bean)**

A commonly grown garden and allotment species. French bean has the potential to grow near the target species.

***Vicia faba* (Broad bean)**

A commonly-grown garden and allotment species. Broad bean has the potential to grow near the target species.

***Lycopersicon esculentum* (Tomato)**

A commonly-grown glasshouse, garden and allotment species. Tomato has the potential to grow near the target species.

***Solanum tuberosum* (Potato)**

A commonly-grown garden and allotment species. Potato has the potential to grow near the target species.

***Triticum aestivum* (Wheat)**

A commonly-grown arable species. Likely to be close to roadside knotweed.

***Zea mays* (Corn)**

A commonly-grown garden, arable and allotment species. Corn has the potential to grow near the target species.

***Hordeum vulgare* (Barley)**

A commonly-grown arable species.

***Brassica napus* (Rape seed)**

A commonly-grown arable species of cultivated land. Found along roadsides and waste ground. Rape has the potential to grow near the target species.

The following 5 species have also been tested using *A. itadori* (Kyushu biotype) on behalf of The Netherlands. These species were selected for their importance to Dutch agricultural and horticultural trade.

***Capsicum annuum* (Pepper) (cultivated, not wild) Sweet pepper, Cayenne pepper, Chili pepper, Christmas pepper, Red pepper, Ornamental chili pepper**

***Cucurbita maxima* (Squash)**

***Fragaria* sp. (Strawberry)**

***Rubus idaeus* (Raspberry)**

***Triticum spelta* (Spelt)**

The following 4 species have also been tested using *A. itadori* (Kyushu biotype) in North America. These species were selected for their relevance to the agricultural and timber trade of the region.

***Brassica oleraceae* (Wild mustard)**

***Pseudotsuga mensiezii* (Douglas fir)**

***Solanum melongena* (Aubergine/Eggplant)**

***Vaccinium macrocarpon* (American cranberry)**

Other additions

As a result of interactions with regulators focussing on plant pathogens, the following three plant species were added to the test plant list as a result of them being host to other *Mycosphaerella* spp.

***Lemna minor* (Duckweed)**

An aquatic mat-forming native species present across most of Europe.

***Larix decidua* (European Larch)**

Native to the mountains of central Europe, a popular fast growing and widely planted tree species.

***Populus tremula* (Aspen)**

A small tree species found throughout most of Europe.

Source: Flora Europaea Volume 1: Psilotaceae to Platanaceae, 2nd edition

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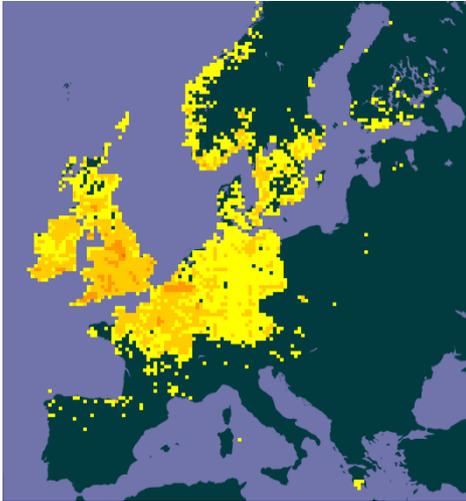
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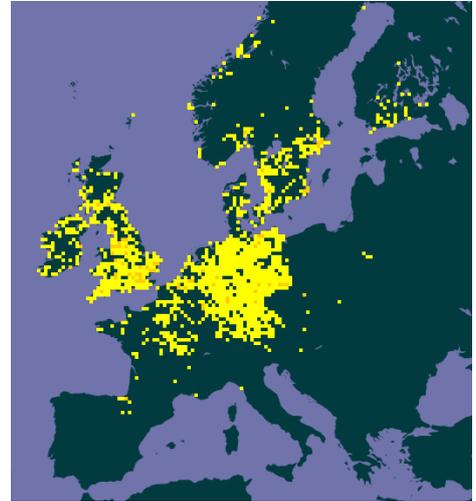
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APPENDIX 2

European species distribution maps for those plants which received eggs during oviposition studies Data from GBIF Portal



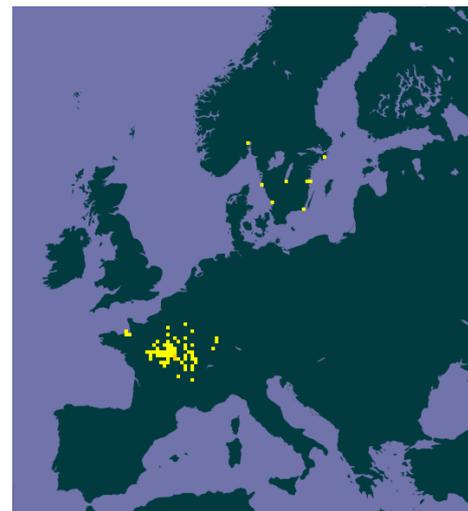
Fallopia japonica



Fallopia sachalinensis



Fallopia x bohemica
to be more



syn. = *Reynoutria x bohemica* (known

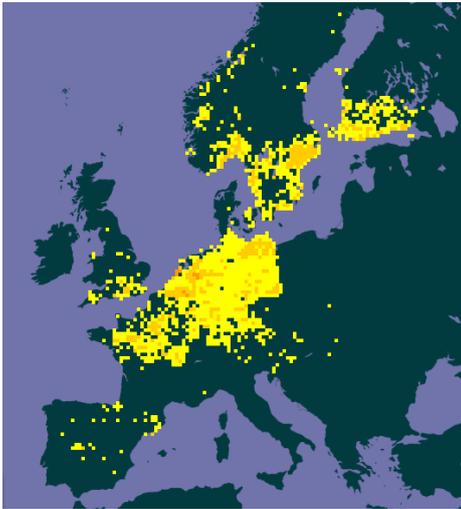
widely distributed but difficult to distinguish from *F. japonica*)

Maps unavailable for:

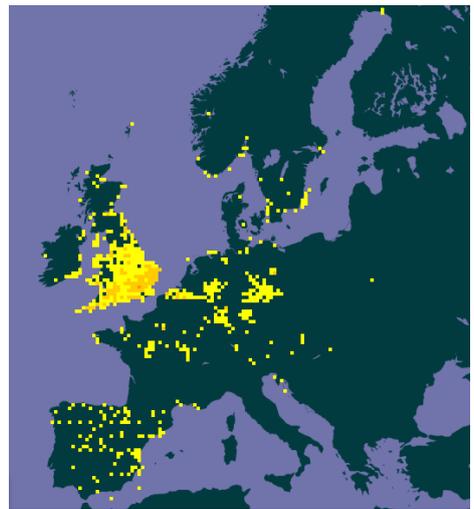
Fallopia conollyana – a non-native hybrid

Fallopia var. compacta - a cultivated ornamental.

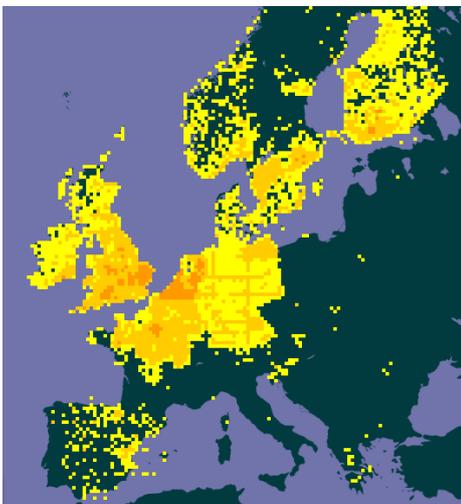
N.B the following non-target species will not support development and therefore a population



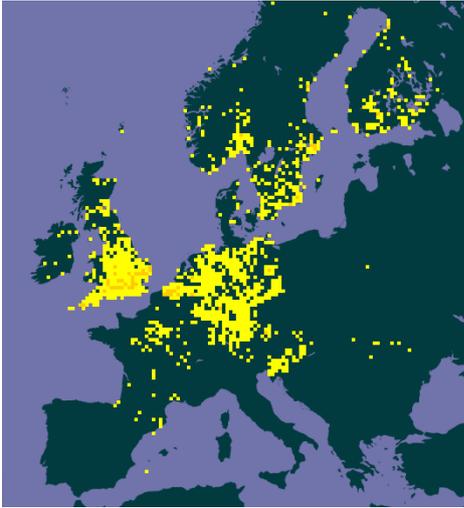
***Fallopia dumetorum*
*baldschuanica***



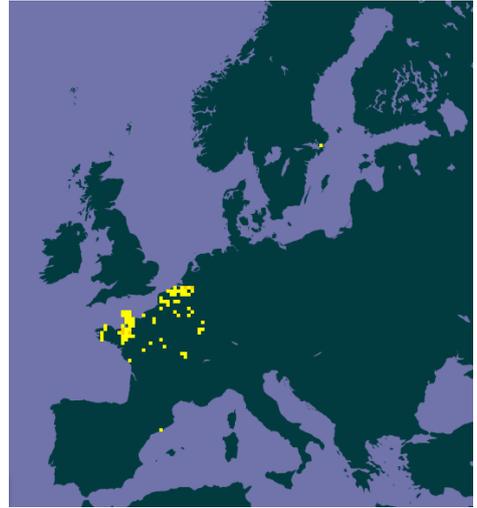
Fallopia



Fallopia convolvulus



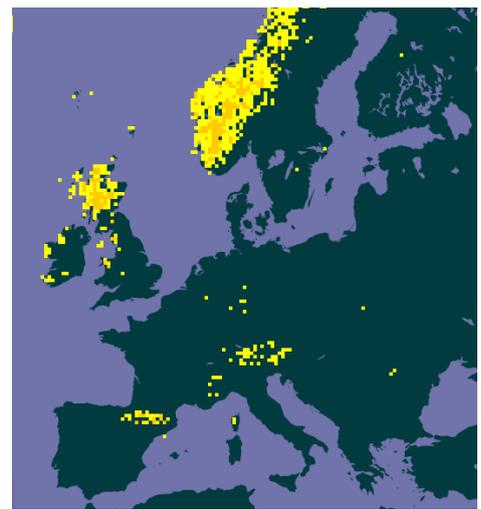
Fagopyrum esculentum



Polygonum (Persicaria) polystachyum



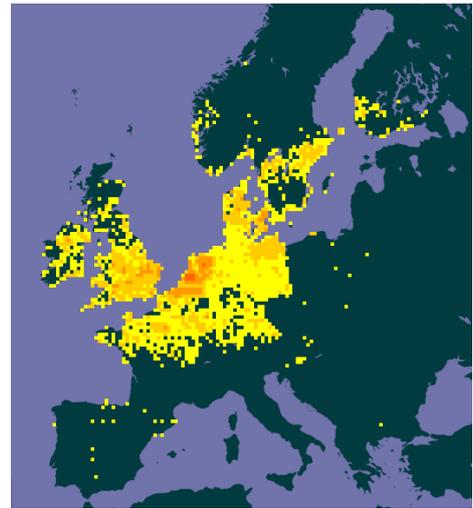
Muehlenbeckia complexa



Oxyria digyna



Rheum palmatum



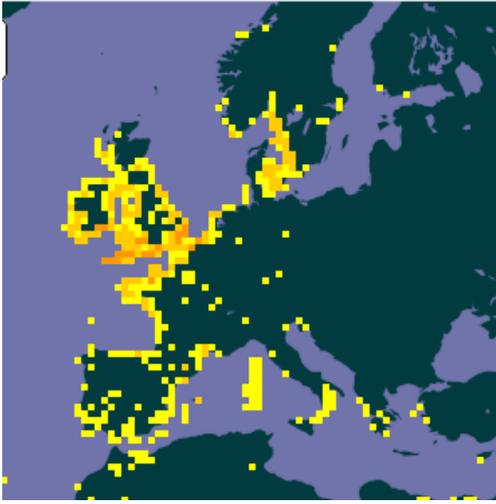
Rumex hydrolapathum



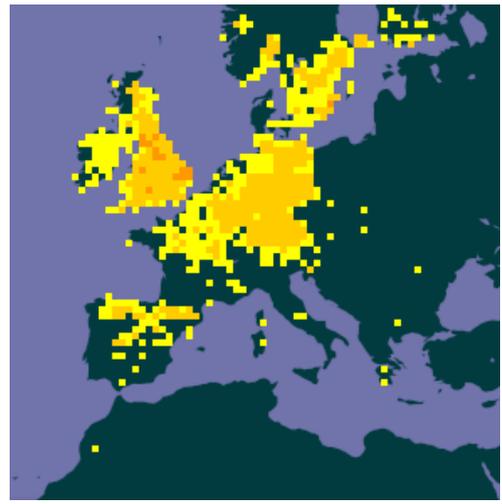
Rheum x hybridum



Fagopyrum dibotrys



Beta vulgaris subsp. *maritima*



Chenopodium bonus-henricus

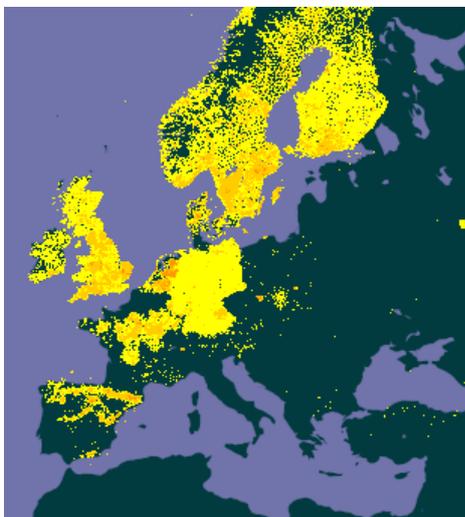
Species distribution maps for four of the possible *A. itadori* overwintering shelter plants



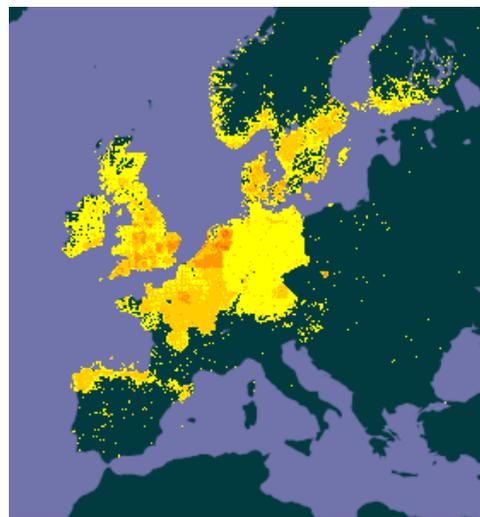
Cryptomeria japonica



Pinus densiflora



Pinus sylvestris



Quercus robur

APPENDIX 3

Climatic comparisons

Any classical biological control program needs to compare the climate of the country from where the biological control agent is collected to the country or region of the proposed introduction. Matching areas of similar climate and then focusing collections in such areas should increase the likelihood of a successful establishment, and the fitness of the introduced organism due to climatic pre-adaptation.

Indeed temperature is one of the key factors in determining if a biological control agent will establish in an area outside of its native range (Boivin *et. al.*, 2006). If, in the area of introduction the temperature extremes are significantly higher or lower than the area of origin, the agent may have difficulty establishing and have a higher risk of death.

The climate of Japan is strongly influenced by the Asian continent, the Pacific Ocean and the associated air masses. It is the collision of the Siberian air mass into the moist Pacific air mass that causes high snow fall in the west of the country. Three main ocean currents influence the climate, the Kuro-shio current, a warm current flowing north washing the southern and eastern side of Japan, the Tsushima current, another warm current flowing west of Kyushu and the Oya-shio current, a cold water current flowing south past the east side of Hokkaido. Overall Japan and the EU have temperate climates with four clearly defined seasons. There is considerable variation in temperature within each country with Japan showing the greater extremes.

Japan extends over 25° of latitude, 3,200km from northeast to south west. The southernmost part of Japan, including the islands of Yaku-shima and Tokara-retto, have a sub-tropical climate, where the average winter temperature rarely drops below 13°C. The yearly average temperature range is 7°C to 17°C and average precipitation per year ranges from 1000-2500mm. Figure 1 shows the average monthly temperature of 5 regions in Japan at an altitude of 200 metres above sea level.

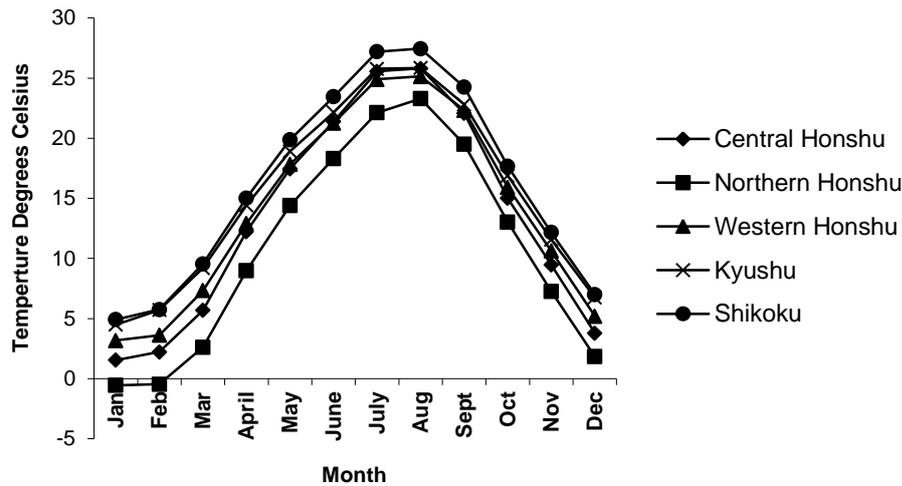


Figure 1: The average monthly temperature from 5 regions of Japan

The EU has less extreme variation in climate compared to that of Japan. The climate is strongly influenced by the Gulf Stream which keeps mild air over the high latitude northwestern region over the winter months. Western Europe has a more Oceanic climate whilst Eastern Europe a drier, more continental climate. The mean annual temperature range across the selected European countries ranges from 7.7°C to 12.4°C with a range of average annual precipitation from 470 to 1098mm per year.

Figure 2 shows the mean temperatures of ten EU member States: Austria (Vienna), Belgium (Brussels), Czech Republic (Prague), Denmark (Arhus), France (Paris), Germany (Berlin), the Netherlands (Amsterdam), Poland (Warsaw), Switzerland (Kusnacht) and the UK (London), alongside the average monthly temperature in Kumamoto, Japan.

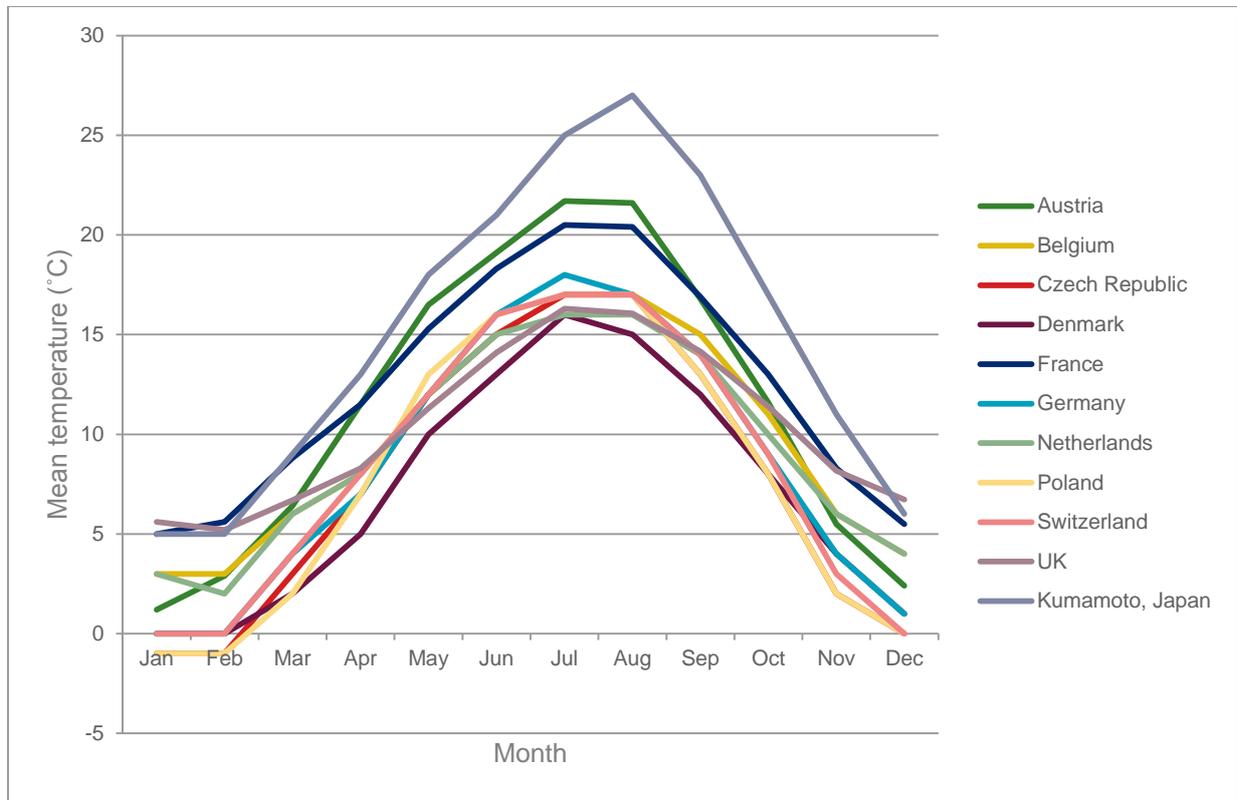


Figure 2: Mean monthly temperatures in ten European countries and Kumamoto, Japan (origin of Kyushu psyllid biotype).

The Netherlands has a less extreme variation in temperature compared to Japan. It has a temperate maritime climate influenced by the North Sea and Atlantic Ocean, with cool summers and moderate winters. Daytime temperatures vary from 2-6°C in the winter and 17-20°C in the summer. Since the country is small there is little variation from region to region, although the marine influences are less inland. Rainfall is distributed throughout the year with a dryer period from April to September. In winter strong Atlantic low-pressure systems can bring gales. Sometimes easterly winds can cause a more continental type of weather, warm and dry in the summer, but cold and clear in the winter with temperatures sometimes far below zero. The Netherlands is a flat country and has often breezy conditions, although more in the winter than in the summer, and more among the coastal areas than inland. The Koeppen-Geiger classification classifies the climate of the Netherlands as a warm temperate humid climate with the warmest month lower than

22°C over average and four or more months above 10°C over average. (<http://www.weatheronline.co.uk/reports/climate/The-Netherlands.htm>).

Figure 2 shows the average temperature of four regions in the Netherlands and the average Netherlands temperature overall. Comparing figure 1 and 2 it is evident the Netherlands is less prone to climatic extremes than Japan.

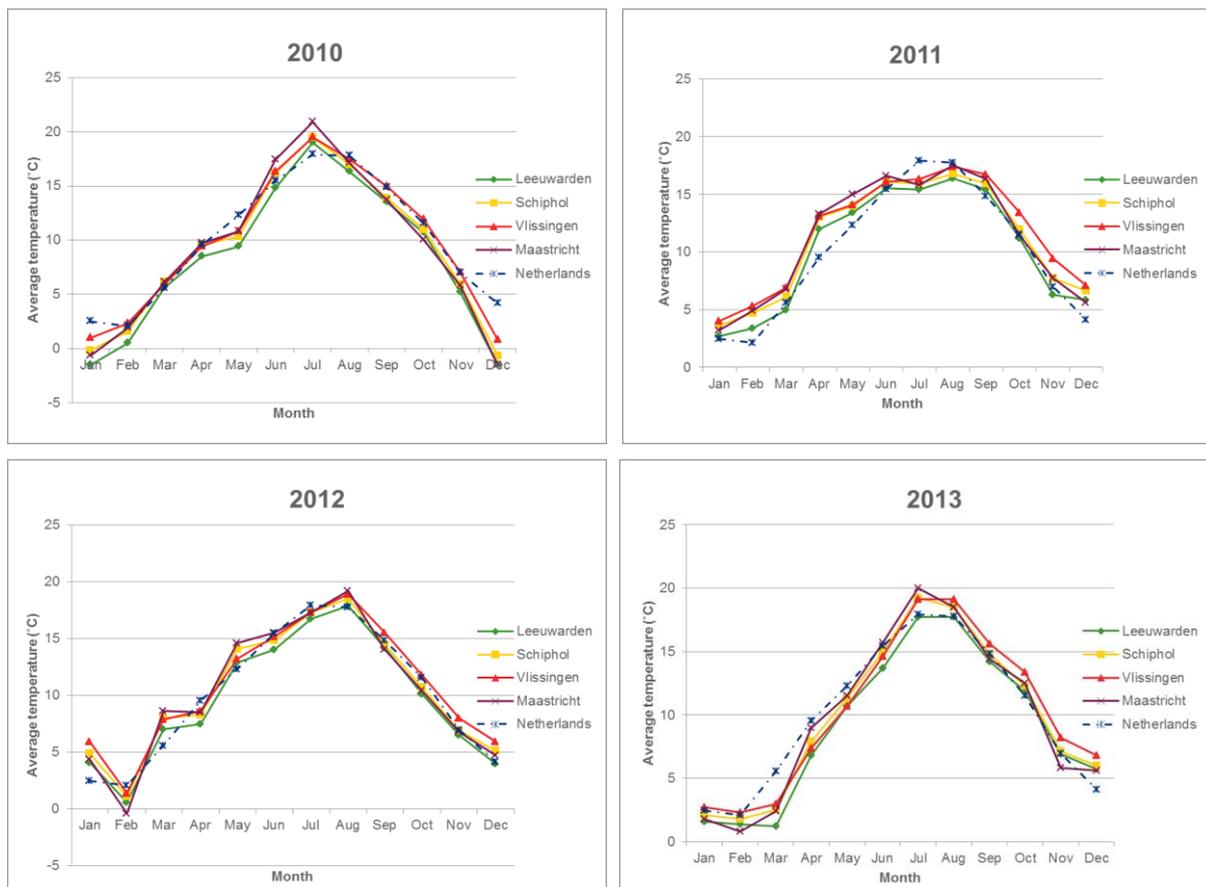


Figure 2. The average monthly temperature for four regions of the Netherlands, and the Netherlands overall temperature in 2010, 2011, 2012 and 2013.

Japan's topography is also very different to that of the PRA area countries almost 75% of Japan is mountainous. The steep undulating landscape adds to climatic variation throughout Japan. Temperature decreases with altitude or increases with descent, at a rate of 0.0065°C per metre (0.65 °C /100m - the Standard Atmosphere Temperature Gradient SATG). Figure 3 shows the mean annual temperatures for the Netherlands and Kyushu (Mt. Aso).

The Netherlands and Mt Aso

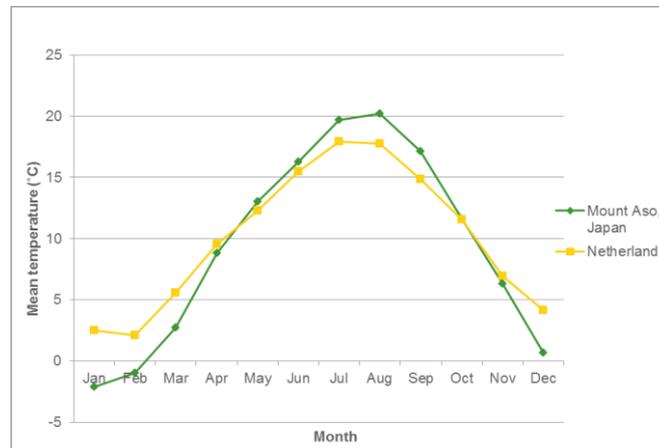


Figure 3: Mean monthly temperatures for the Netherlands (average of 2010-2013) and Mt. Aso, Kyushu, Japan (1961-1990).

Temperature controls the development of an organism throughout its life cycle- effecting the time of development, fecundity, ovioposition and mortality. The upper and lower development threshold of an organism determines when the development of an organism stops. Following the method of Olsen *et al.*, (2003), we determined the lower development threshold of *A. itadori* under controlled temperature quarantine conditions. We recorded the development times of individual psyllids under 7 constant temperatures (10°C, 12 °C, 17 °C, 21 °C, 23 °C and 28 °C). Figure 4 shows the relationship between the development rates (1/day) at the 7 temperatures. Extrapolation of the line indicates the lower development threshold is 8°C. However there was no development at 10°C and very limited development at 12°C. The fitted line includes the zero observations for 10°C to reflect the absolute lack of development at this temperature, and identify a slope for the linear model. Given the poor survivorship at 12°C , the insect's response is likely non linear at low temperatures, however additional experimentation is required to fit this more explicit model.

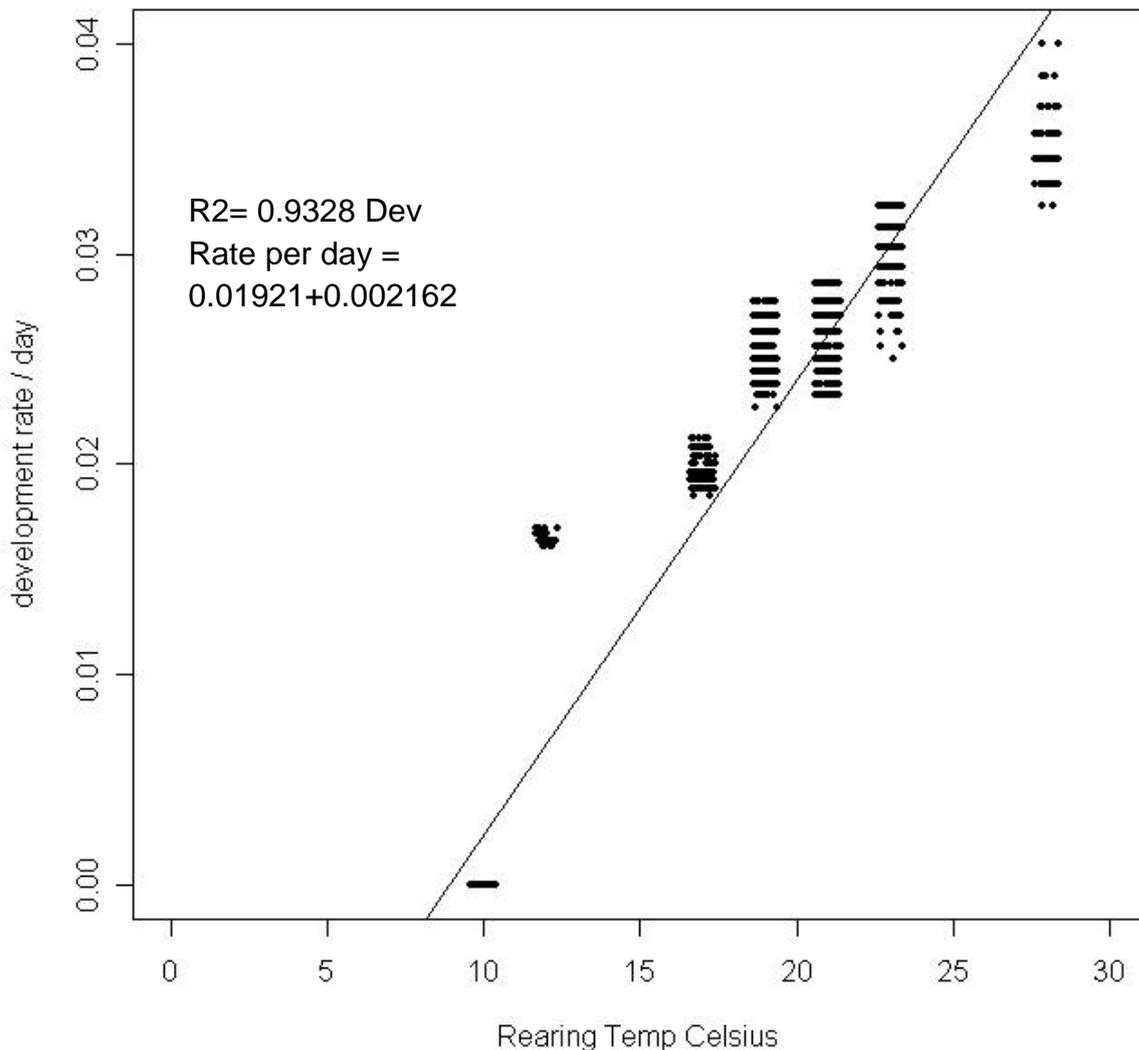


Figure 4. The relationship between development rates at seven constant temperatures

Using the graph and the equation line in figure 4 we were able to calculate the day-degrees requirement for the psyllid by calculating the reciprocal of the slope of the regression line (1/y). This gave us a day-degrees requirement of 462.5 from egg to adult. To define, day-degrees are the total amount of heat required, above the development threshold, for an organism to develop from one stage to another stage of its life cycle.



Figure 5. A map indicating likely psyllid generations per year based on accumulated day-degrees in the Netherlands (2008-2014). The markers indicate number of generations expected per year (green = 1-2 generations, white = 2 generations).

Using the calculated day-degree figure of 462.5 and referring to the map of the Netherlands (Figure 5), we can envisage the possible distribution of the psyllid throughout the Netherlands, based on temperature. A range of Netherlands locations were selected using a date range of 15th March to 1st October over a 7-year average (2008-2014), corresponding with when knotweed tends to start growing until senescence. It can be seen that across most of the Netherlands the psyllid could establish one-possibly two generations, and in the east, south-east and south-west two generations could be possible per year. The UK lies between latitudes 49°N to 61°N and the Netherlands between latitudes 50°N and 54°N, therefore the Netherlands corresponds to similar latitudinal coordinates as southern, central and northern England without the higher altitude areas where the psyllid may struggle to establish. This suggests the psyllid could establish in areas across the Netherlands where Japanese knotweed is present (Figure 6).

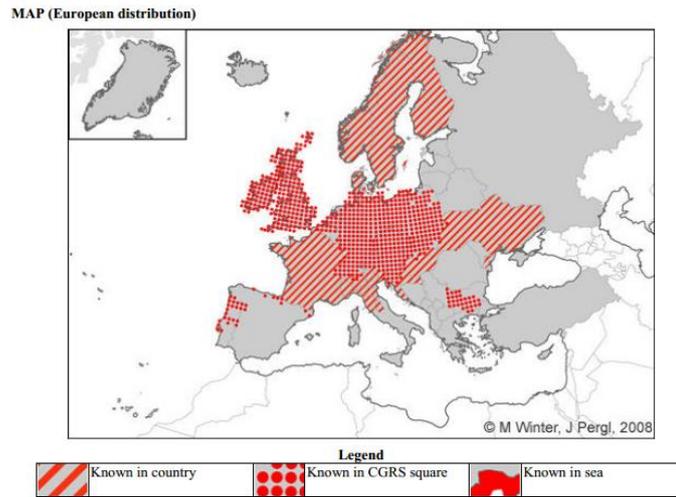


Figure 6 The area covered by *Fallopia japonica* in Europe (http://www.europe-alien.org/pdf/Fallopia_japonica.pdf)

We would like to acknowledge and thank Dr Rob Bouchier from the Agriculture and Agri-food Canada-Lethbridge Research Centre for the work he conducted on the climatic matching and statistical analysis.

APPENDIX 4

The life history and host range of the Japanese knotweed psyllid, *Aphalara itadori* Shinji: Potentially the first classical biological weed control agent for Europe

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Reference:

Shaw, R.H., Bryner, S. and Tanner, R. (2009) The life history and host range of the Japanese knotweed psyllid, *Aphalara itadori* Shinji: Potentially the first classical biological weed control agent for the European Union. *Biological Control* 49: 105-113.

Figures only:

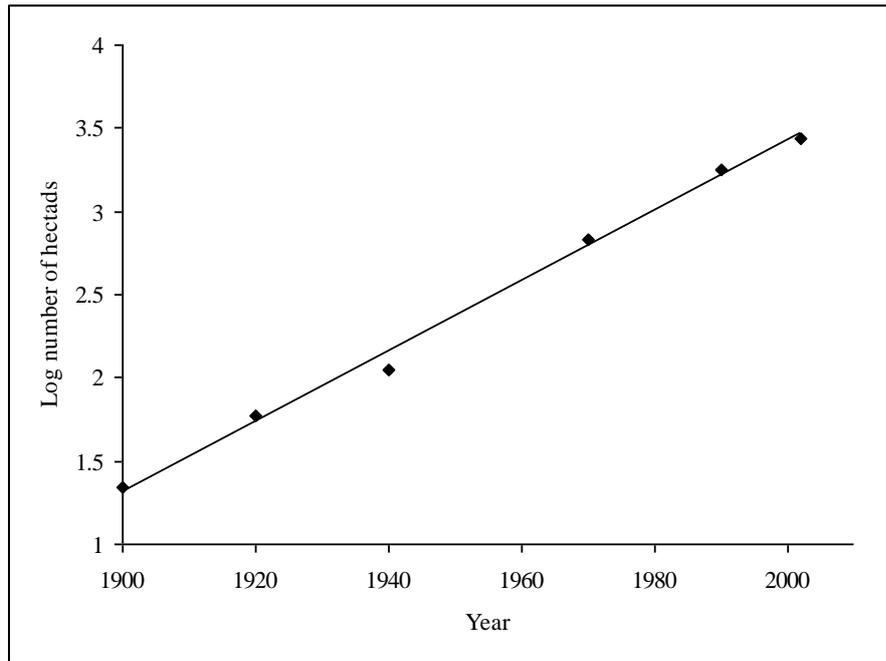


Figure 1: Plot of log number of UK hectads containing *F. japonica* over time (Data from BSBI, 2000)

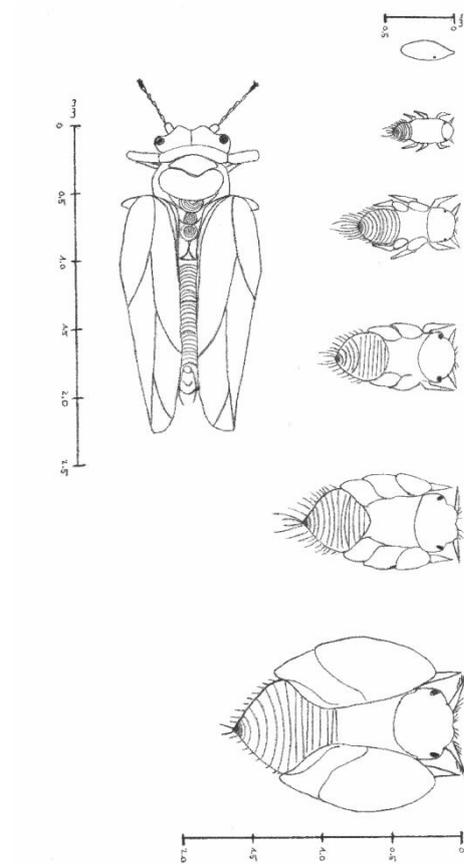


Figure 2: The individual stages of the life cycle of *A. itadori* (clockwise from top right – egg, N1, N2, N3, N4, N5 and adult)

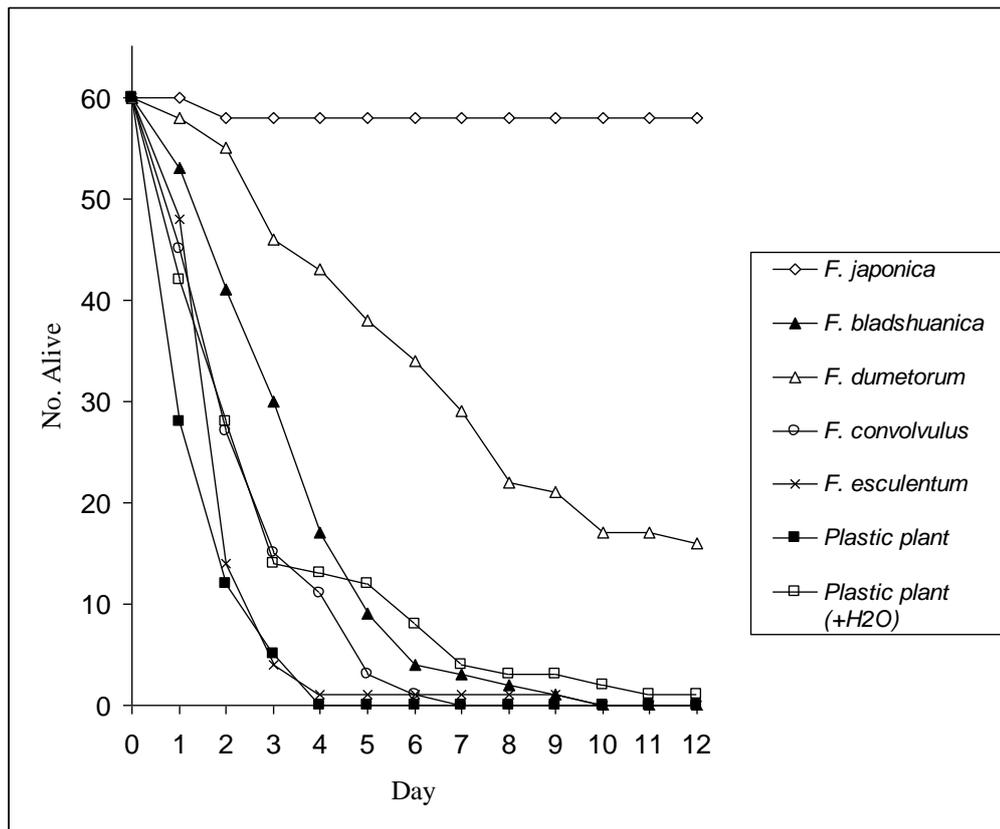


Figure 3: Survivorship curves showing the number of adult *A. itadori* alive over time on different hosts (see legend)

Table 1 - Mean developmental periods for all stages of *A. itadori* in days ($\pm 1SE$, n = 21)

	Egg	1st instar	2nd instar	3rd instar	4th instar	5th instar	Comple t e life cycle
Mean \pm 1S	9.2 \pm 0.1	4.8 \pm 0.2	3.3 \pm 0.2	3.9 \pm 0.3	4.5 \pm 0.1	7.1 \pm 0.3	32.9 \pm 0.8
Range	9 - 10	4 - 6	2 - 5	3 - 8	4 - 6	5 - 11	28 - 42

Table 2 - Summarised oviposition and subsequent development data generated during multiple-choice oviposition studies carried out on *A. itadori* under quarantine conditions at constant temperature (sorted above the line by number of eggs laid/rep and then alphabetically).

Test plant	No replicates	Eggs laid	Eggs laid/rep	Development
<i>Fallopia japonica</i>	324	140,517	433.7	Good
<i>Fallopia Japonica USA (Ithaca)</i>	3	1,523	507.7	Good
<i>Fallopia x bohémica</i>	15	2,033	135.5	Good
<i>Fallopia conolliana</i>	15	866	57.7	Poor
<i>Fallopia japonica var compacta</i>	12	441	36.8	Poor
<i>Fallopia sachalinensis</i>	18	547	30.4	Moderate
<i>Muehlenbeckia complexa</i>	7	132	18.9	-
<i>Rheum palmatum</i>	12	208	17.3	-
<i>Fagopyrum esculentum</i>	14	237	16.9	-
<i>Fallopia dumetorum</i>	12	93	7.8	-
<i>Fallopia convolvulus ex. USA</i>	11	78	7.1	-
<i>Fallopia baldshuanica</i>	15	100	6.7	-
<i>Oxyria digyna</i>	12	43	3.6	-
<i>Persicaria polystachyum</i>	6	15	2.5	-
<i>Rheum Glaskins</i>	9	8	0.9	-
<i>Fallopia convolvulus</i>	14	11	0.8	-
<i>Rumex hydrolaphum</i>	6	2	0.3	-
<i>Fagopyrum dibotrys</i>	9	1	0.1	-
<i>Beta vulgaris</i>	6	0	0.0	-
<i>Bougainvillea sp.</i>	9	0	0.0	-

<i>Brassica napus</i>	6	0	0.0	-
<i>Calystegia sepium</i>	9	0	0.0	-
<i>Carpobrotus edulis</i>	6	0	0.0	-
<i>Celosia argentea</i>	6	0	0.0	-
<i>Cerastium fontanum</i>	6	0	0.0	-
<i>Cerastium glomeratum</i>	15	0	0.0	-
<i>Chenopodium album</i>	6	0	0.0	-
<i>Cytisus scoparius</i>	3	0	0.0	-
<i>Delosperma cooperi</i>	6	0	0.0	-
<i>Echinocereus subinermis</i>	6	0	0.0	-
<i>Erigeron umbellatus</i>	6	0	0.0	-
<i>Frankenia laevis</i>	6	0	0.0	-
<i>Hordeum vulgare</i>	6	0	0.0	-
<i>Houttuynia cordata</i>	7	0	0.0	-
<i>Lewisia columbiana</i>	9	0	0.0	-
<i>Limonium bellidifolium</i>	5	0	0.0	-
<i>Limonium binervosum</i>	6	0	0.0	-
<i>Lycopersicon esculentum</i>	6	0	0.0	-
<i>Malus domestica.</i>	9	0	0.0	-
<i>Nepenthes fusca</i>	6	0	0.0	-
<i>Notocactus magnificus</i>	3	0	0.0	-
<i>Persicaria affinis</i>	6	0	0.0	-
<i>Persicaria amphibia</i>	6	0	0.0	-
<i>Persicaria bistorta</i>	9	0	0.0	-
<i>Persicaria campanulata</i>	12	0	0.0	-
<i>Persicaria capitata</i>	9	0	0.0	-
<i>Persicaria hydropiper</i>	6	0	0.0	-

<i>Persicaria hydropiper</i>	6	0	0.0	-
<i>Persicaria hydropiperoides</i>	10	0	0.0	-
<i>Persicaria lapathifolia</i>	6	0	0.0	-
<i>Persicaria maculosa</i>	6	0	0.0	-
<i>Persicaria mollis</i>	6	0	0.0	-
<i>Persicaria tinctoria</i>	9	0	0.0	-
<i>Persicaria vivipara</i>	6	0	0.0	-
<i>Phaseolus vulgaris</i>	9	0	0.0	-
<i>Phytolacca americana</i>	6	0	0.0	-
<i>Polygonatum multiflora</i>	6	0	0.0	-
<i>Polygonatum verticilliatum</i>	3	0	0.0	-
<i>Polygonum arenastrum</i>	6	0	0.0	-
<i>Polygonum aviculare</i>	6	0	0.0	-
<i>Polygonum maritimum</i>	9	0	0.0	-
<i>Polygonum oxyspermum</i>	6	0	0.0	-
<i>Polygonum pensylvanicum</i>	6	0	0.0	-
<i>Polygonum persicaria</i>	8	0	0.0	-
<i>Polygonum rurivagum</i>	5	0	0.0	-
<i>Polygonum sagitatus</i>	9	0	0.0	-
<i>Polygonum virginianum</i>	3	0	0.0	-
<i>Rosa spp.</i>	6	0	0.0	-
<i>Rubus fruticosus</i>	6	0	0.0	-
<i>Rumex acetosa</i>	6	0	0.0	-
<i>Rumex acetosella</i>	6	0	0.0	-
<i>Rumex alpinus</i>	6	0	0.0	-
<i>Rumex aquaticus</i>	6	0	0.0	-
<i>Rumex conglomeratus</i>	12	0	0.0	-

<i>Rumex crispus</i>	6	0	0.0	-
<i>Rumex longifolius</i>	6	0	0.0	-
<i>Rumex maritimus</i>	6	0	0.0	-
<i>Rumex obtusifolius</i>	9	0	0.0	-
<i>Rumex orbiculatus</i>	15	0	0.0	-
<i>Rumex palustris</i>	6	0	0.0	-
<i>Rumex pulcher</i>	6	0	0.0	-
<i>Rumex rupestris</i>	6	0	0.0	-
<i>Rumex sanguineus</i>	6	0	0.0	-
<i>Rumex scutatus</i>	6	0	0.0	-
<i>Solanum melongena</i>	6	0	0.0	-
<i>Solanum tuberosum</i>	6	0	0.0	-
<i>Tamarix gallica</i> (cut branchlet)	11	0	0.0	-
<i>Triticum aestivum</i>	6	0	0.0	-
<i>Vicia faba</i>	9	0	0.0	-
<i>Vitis vignifera</i>	6	0	0.0	-
<i>Zea mays</i>	6	0	0.0	-

Table 3 - The mean number of eggs received per test plant in the presence and absence of the knotweed host in caged multiple choice experiments (± 1 SE) and the significance of any difference as determined by a generalised linear model using Poisson error structure. p values corrected for overdispersion. (dispersion parameters for *F. dumetorum*, *F. convolvulus* and *F. baldshuanica* were 12.609, 14.956 and 7.55 respectively).

Test Plant	JK present	JK absent	Chi-square value	p value
<i>F. dumetorum</i>	7.75 \pm 2.895 (n=12)	5.167 \pm 3.188 (n=6)	0.323	0.578
<i>F. convolvulus</i>	3.65 \pm 1.775 (n=20)	6.833 \pm 2.664 (n=6)	0.643	0.431
<i>F. baldshuanica</i>	7.75 \pm 2.336 (n=12)	3.833 \pm 1.887 (n=6)	1.379	0.257

Table 4

Percentage survival over time and development of nymphs of *A. itadori* hand transferred to various host plants. (*) indicates values that have been estimated based on counts on previous and following days).

Test plant	Day 3	Day 7	Day 14	Day 28	Ultimate stage
<i>Fallopia japonica</i>	73.1	70.7	68.6	66.2	Adult (>66%)
<i>Fallopia conolliana</i>	61.7	61.7	53.3	48.3	Adult (48.3%)
<i>Meuhlenbeckia complexa</i>	40.0	26.7	11.7	8.3	Adult (6.7%)
<i>Fallopia dumetorum</i>	33.3	28.3	13.3	3.3	N5 (10%)
<i>Rumex hydrolapathum</i>	38.9*	25.0	13.3	3.3	N3 (8.3%)
<i>Rheum palmatum</i>	27.5*	10.0	1.7	0	N3 (1.7%)
<i>Polygonum arenastrum</i>	11.7*	3.3	1.7	0	N3 (1.7%)
<i>Fallopia baldschuanica</i>	51.7	22.5*	0.8*	0	N4 (1.7%)
<i>Oxyria digyna</i>	16.7	6.7	0	0	N3 (1.7%)
<i>Fallopia convolvulus</i>	40.0	2.2*	0	0	N1 (41.7%)
<i>Fagopyrum esculentum</i>	8.3	1.7	0	0	N3 (15%)
<i>Persicaria polystachya</i>	5*	0.4*	0	0	N2 (8.3%)
<i>Rheum Glaskin's</i>	23.3	0	0	0	N2 (8.3%)
<i>Fagopyrum dibotrys</i>	5.6*	0	0	0	N2 (5%)

Appendix 5

Japanese knotweed biological control testing for The Netherlands

January-December 2013

Final Report (Project code TR10091)

(Note: Photos and *Mycosphaerella* leaf-spot fungus sections removed as not relevant to this PRA)

Summary

In the first quarter of 2013, CABI were contracted by the Bureau for Risk Assessment and Research Programming (BuRO) of the Netherlands Food and Consumer Product Safety Authority to extend testing of the psyllid *Aphalara itadori*, (and to a lesser extent the *Mycosphaerella* leaf-spot fungus – removed from this document), for the Netherlands. Rhizome samples of 3 Dutch knotweeds (*Fallopia japonica*, *F. sachalinensis*, *F. x bohemica*) from a wide geographical area were sent to CABI in March along with ten non-target plant species, which were selected by the sponsor for host-range testing.

The susceptibility to the psyllid of the three knotweeds from 11 sites was tested under no-choice conditions, where individually sleeved plants were exposed to 10 psyllids for 7 days in the laboratory for egg laying. All knotweed species supported development from egg to adult, with high egg counts (mean no. eggs/plant) of 131 on *F. sachalinensis* (site 2), 128 on *F. japonica*, and up to 348 on *F. x bohemica* (site 7). Adult emergence was variable between sites and species but reached 100% in some replicates of *F. x bohemica*.

No-choice tests were also carried out for the individually sleeved non-target test plants. Only 3 eggs in total were laid on any of the 60 test plants, none of which hatched. This

compares with >4,000 eggs counted on 18 *F. japonica* plants. In more realistic multiple-choice tests, one *F. japonica* was exposed to 30 psyllids in a laboratory cage set-up along with 5 test plants for one week. Again, egg numbers were minimal on non-target species (3 eggs on 60 test plants vs. >3,500 on 12 *F. japonica* plants), and the 3 eggs did not hatch.

Two multiple choice tests in large field cages were used to provide an even more natural situation. The first was a multiple choice test between the three knotweed species from 8 different sites with 100 psyllids released per replicate. The results confirmed all species to be susceptible to psyllid oviposition with some variation between sites. Egg counts (mean no. eggs/plant) ranged from 39 on *F. sachalinensis* (site 2) to 122 on *F. japonica* and 204 on *F. x bohemica* (site 2). The second field cage multiple choice trial exposed the three knotweed species alongside three selected non-target species. Oviposition occurred on all knotweed species with a total of 3,516 eggs laid, however no eggs were laid on any of the non-target plants.

The results so far confirm the high host specificity of the psyllid for invasive knotweeds in the Netherlands, revealing an apparent preference for the hybrid *F. x bohemica* and confirming that *F. sachalinensis* is a less suitable host.

Introduction

Japanese knotweed (*Fallopia japonica*) is an invasive alien species that causes increasing problems in Europe, including The Netherlands. One of the main difficulties is the lack of cost-effective management options to control this invasive weed.

Japanese knotweed is one of the top 100 invasive species in the world according to the IUCN listing, and has spread primarily through redistribution of fragments of its extensive rhizome system throughout Europe. Interestingly its first appearance outside Japan was in Leiden in the Netherlands in Philipp von Siebold's garden of acclimatisation, from where it was sold around the world. The plant is able to displace native species and reduce biodiversity with negative impacts on the riverbanks it favours for spread. When it dies down in the winter the dead canes can fall into the water body and accumulate to form significant blockages which can cause flooding events. It is most famous as a problem in the built environment where it has a reputation for disrupting drainage, foundations and pushing through asphalt, so much so that in England some mortgage providers refuse to lend money for the purchase of infested properties.

Knotweed is extremely difficult to control due to its extensive rhizome system spreading many metres from the parent plant. Both mechanical and chemical controls are difficult to achieve without repeated applications or the use of persistent non-selective herbicides. Biological control has the potential to be a very (cost) effective solution to fight Japanese knotweed and related knotweed species. In 2012, CABI presented a collection of potential biocontrol projects to the Netherlands, one of which was prioritised. In 2013, this project investigated the potential for biological control of Japanese knotweed and other invasive knotweed species in the Netherlands, using primarily the natural enemy *Aphalara itadori*, but to also the *Mycosphaerella* leaf-spot fungus which CABI holds. It builds upon the large knotweed programme in the UK, funded by UK sponsors since 2003.

The project encompasses all invasive knotweed species in the Netherlands:

1. Japanese knotweed (*Fallopia japonica* var. *japonica*)
2. Sakhalin knotweed (*Fallopia sachalinensis*)
3. Bohemian knotweed (*Fallopia x bohemica*)

These species are collectively called “knotweeds” in the remainder of this report.

Methods

Plant sourcing

Rhizome

Rhizome, measuring up to 1cm in diameter with nodes, from 16 sites was requested by CABI. In March and April 2013, shipments of clean knotweed rhizome were sent to CABI's UK labs by the Netherlands Food and Consumer Product Safety Authority (NFCPSA). The rhizome pieces were individually wrapped in damp tissue and placed together in labelled plastic bags within a padded box for shipment (Figure 1 (A)). The rhizome pieces were unpacked upon arrival and placed into boxes or trays where they were kept moist to allow the first shoots to sprout (Figure 1 (B)). When the rhizome began to sprout they were potted in multi-purpose compost (Figure 1(C)) and kept in a heated glasshouse facility until reaching a suitable size for testing (Figure 1(D)). Some rhizome pieces were retained in a cooler environment to delay sprouting, in order to stagger planting and ensure a continual supply throughout the sequence of trials.

The collection consisted of material from two *F. japonica* sites; two *F. sachalinensis* sites; and twelve *F. x bohemica* sites. There were more *F. x bohemica* sites due to the expected increased variability of the hybrid. Subsequently five shipments arrived, in total containing approximately 225 pieces of *F. japonica* rhizome, 103 pieces of rhizome from the *F. sachalinensis* sites and 300 pieces collectively from the twelve *F. x bohemica* sites.

Unfortunately, only one of the *F. japonica* sites could be used as the other source material appeared to have been chemically treated, judging by its abnormal growth form. Since *F. japonica* is thought to be largely clonal, this was not considered to be a problem. A selection process also took place for the *F. x bohemica* sites, where eight sites were selected according to the healthiness and abundance of the rhizome material and distribution of sites as plotted on a map. This resulted in a total of eleven sites being used in the tests, the locations of which are mapped in Appendix 1.

Test plants

CABI provided the NFCPSA with the list of non-target plant species against which the psyllid has already been tested for the UK, USA and Canada. On this basis, the NFCPSA

selected a total of ten species which included both native species and others of horticultural importance: *Beta vulgaris* subsp. *maritima*, *Capsicum annuum*, *Chenopodium bonus-henricus*, *Chenopodium quinoa*, *Cucurbita maxima*, *Fragaria* sp., *Limonium vulgare*, *Rubus idaeus*, *Rumex thyrsiflorus* and *Triticum spelta*.

Shipments of seeds or plant cuttings (whichever was most suitable) of the above plant species were sent by the NFCPSA and propagated in a heated glasshouse facility until they reached a suitable size for testing (Figure 2).

Psyllid mass rearing and release

CABI's pre-existing culture of the Kyushu biotype of *Aphalara itadori* was augmented to provide the 3,000 or so psyllids required for all of the tests. Mass-rearing was carried out in Perspex cages in a controlled environment, set at artificial summer temperatures and light regime (23°C, 13/11). In each cage 60 adult psyllids were exposed to 4 UK *Fallopia japonica* plants, with the eggs developing into adults from 28 days (Figure 3). Setup of these cages was staggered to allow a continual supply of psyllids that could be removed for testing as and when required. Sex ratios were assumed to be 50:50.

Activity 1: Susceptibility and host range testing with the psyllid, *Aphalara itadori*

Activity 1A: Susceptibility testing with *Aphalara itadori*

The aim of this activity was to test the relative vulnerability of the Netherlands knotweed species, *Fallopia japonica*, *F. sachalinensis* and *F. x bohemica* to the psyllid *A. itadori*.

No-choice oviposition/development studies

This set of no-choice oviposition tests involved the exposure of knotweed plants from each of the 11 sites plus UK *F. japonica* control (x6 replicates) to mated adult psyllids. UK *F. japonica* plants were used to provide a control and allow comparability with previous UK data. Each plant was placed in a single mesh sleeve with 10 psyllids for 7 days in a temperature controlled chamber (average temperature 23/24°C). At the end of the 7 day exposure period, the psyllids were collected and preserved in 70% alcohol, with the

number of living or dead psyllids noted. The eggs on each plant were then counted using a hand lens and recorded according to position on the plant (leaf upper surface, lower surface, node, and stem) and leaf measurements taken to enable the calculation of eggs per leaf area (Figure 4(A)).

In order to show the different knotweeds' suitability as hosts for development, all the plants described above were re-sleeved and maintained in a temperature controlled chamber to monitor development from eggs to adults. Adult emergence counts took place 7 weeks (51 days) post set-up, to ensure optimal adult numbers were recorded with collected adults preserved in 70% alcohol (Figure 4 (B)).

Multiple-choice oviposition studies

The aim of this study was to determine the relative preference of the adult psyllids in their selection of host plants for oviposition. Based on the results generated above, plants from 5 *F. x bohémica* sites were selected for testing in a multiple-choice experiment, along with the two *F. sachalinensis* sites and *F. japonica* site 1. Rather than carrying out this study in Perspex cages as previously planned, the unexpectedly good UK summer weather allowed us to perform these tests inside large mesh field cages (approx. 2 m³), to give a more realistic set up (Figure 5). One plant from each of the 8 sites was placed randomly within a grow bag inside a large mesh field cage on the grounds of CABI, with 6 replicate field cages set up in total (for site selection and plant location within in each cage see Appendix 2, A). In each cage 100 psyllids were released from a central point, and after 7 days the psyllids were removed, eggs were counted and leaf measurements were taken as for the no-choice tests.

Activity 1B: Host-range testing with *Aphalara itadori*

The aim of the host-range testing was to demonstrate the safety of the agent with regards to 10 non-target plant species of importance to the Netherlands.

No-choice oviposition/development studies

These tests were set up in the same way as the Activity 1A no-choice tests, but this time using the 10 non-target plant species (listed in Test Plant section). Six replicates of each species were placed each in a single sleeve and exposed to 10 psyllids for 7 days alongside *F. japonica* controls (Figure 6). At the end of the 7 day exposure period, psyllids were collected and preserved, and the plants were studied carefully for eggs. Under an extreme no-choice scenario, it is possible that these tests could generate false positives, with eggs being laid on non-targets. In this case, any plants with eggs were maintained in a Perspex cage in a controlled environment, and any development from egg to nymph to adult was monitored closely.

Multiple-choice oviposition/development studies

This more realistic trial allows the psyllids the opportunity to choose between their normal knotweed host (*Fallopia japonica* ex. NL) and non-target plants in a multiple-choice arena. The tests were carried out in Perspex cages in a controlled environment, to provide data that can be combined with previous research carried out for the UK and North America.

The capacity of the Perspex cages meant that the tests had to be carried out in two rounds. Five of the 10 test plant species were selected and one plant of each was placed in a cage (x6 replicates), plus the *F. japonica* control plant (Figure 7). The plants were exposed to 30 psyllids for 7 days, after which the psyllids were removed and preserved. The number of eggs laid on all plants was recorded after thorough inspection with a hand lens, and leaf measurements taken as in Activity 1A. If any eggs were laid on non-target species, their fate was followed closely. This was then repeated with the remaining 5 non-target species.

Activity 1C: Field cage testing with *Aphalara itadori*

This approach to safety trials involved the use of large field cages in closer-to-natural conditions, to better reflect the situation that would exist post-release. It is however precautionary, in that it ensures a high population of psyllids choosing between the normal host and non-target plants that may never occur in the vicinity of knotweed in the real world.

The three most important and/or closely related non-target species were selected by NFCPSA for this study: *Fragaria* sp., *Rumex thyrsiflorus*, and *Capsicum annuum*. These, along with one plant of each knotweed species (*F. japonica* [site 1], *F. sachalinensis* [site 1] and *F. x bohemica* [site 6]) were placed randomly in each field cage (x8 replicates) and exposed to 100 psyllid adults, released from a central point (Figure 8). See Appendix 2(B) for a diagram of the layout of this experiment. After 7 days, the location of any resting adult psyllids on the plants was noted before removing them. Eggs were counted and leaf measurements taken, and any plant that had received eggs was sleeved and monitored for development.

Results

Activity 1A: Susceptibility testing with *Aphalara itadori*

No-choice oviposition/development studies

All plants were susceptible to *A. itadori* oviposition with a total of 16,202 eggs laid over the course of the test. Figure 11 below presents the findings.

F. x bohemica appeared particularly susceptible with six out of the eight sites receiving more eggs than the UK *F. japonica* control. *F. x bohemica* (site 2) received the highest number of eggs with a mean of 347.7 ($\pm 79.8 = 1SE$) eggs/plant, followed by *F. x bohemica* (site 7) with a mean of 320.3 (± 77.9) eggs/plant. The remaining *F. x bohemica* sites received the following mean number of eggs (in descending order): (site 4) 246.2 (± 41.9), (site 5) 240.5 (± 33.6), (site 3) 205.0 (± 50.7), (site 6) 203.0 (± 33.2), (site 1) 147.2 (± 67.6) and (site 8) 133.2 (± 31.6).

F. sachalinensis (site 1) received a mean of 131.0 (± 31.6) eggs/plant, with (site 2) receiving the fewest eggs with a mean of 111.5 (± 26.8) eggs/plant. The Netherlands *F. japonica* received a mean of 128.2 (± 45.6) eggs/plant and the UK *F. japonica* control received a mean of 162.2 (± 22.3) eggs/plant.

In terms of mean eggs per cm² *F. x bohemica* received the highest with site 5 receiving 0.62 (± 0.13) eggs/cm², followed by site 7 (0.56 \pm 0.14), site 6 (0.52 \pm 0.10), site 2 (0.36 \pm 0.10), site 1 (0.34 \pm 0.16), site 4 (0.31 \pm 0.07), site 3 (0.28 \pm 0.08) and site 8 (0.20 \pm 0.05) eggs/cm². *F. japonica* (site 1) received 0.20 (± 0.07) eggs/cm² compared to *F. japonica* UK (0.28 \pm 0.04) eggs/cm². *F. sachalinensis* site 1 received 0.19 (± 0.02) eggs/cm² and *F. sachalinensis* site 2 the least with 0.13 (± 0.04) eggs/cm². See Figure 12 below and Table 1 in Appendix 3 for all figures.

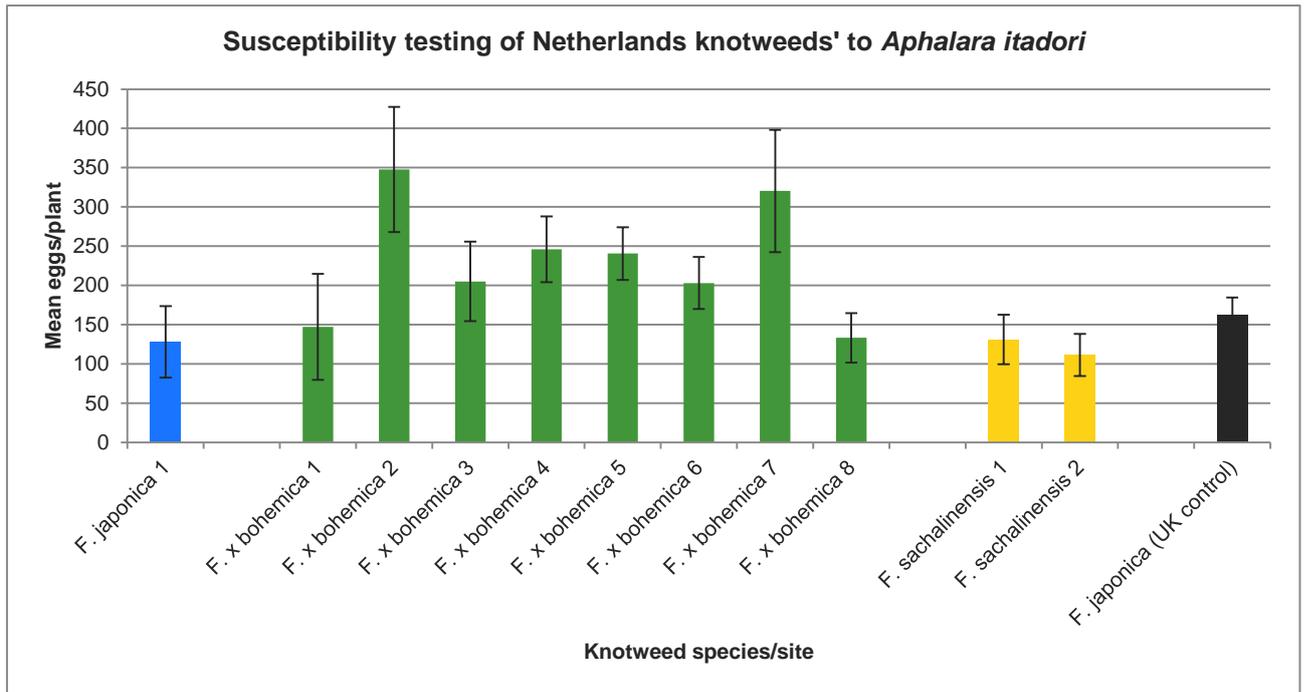


Figure 1: Susceptibility of Netherlands knotweeds' to *A. itadori* including *F. japonica* UK control: mean eggs/plant

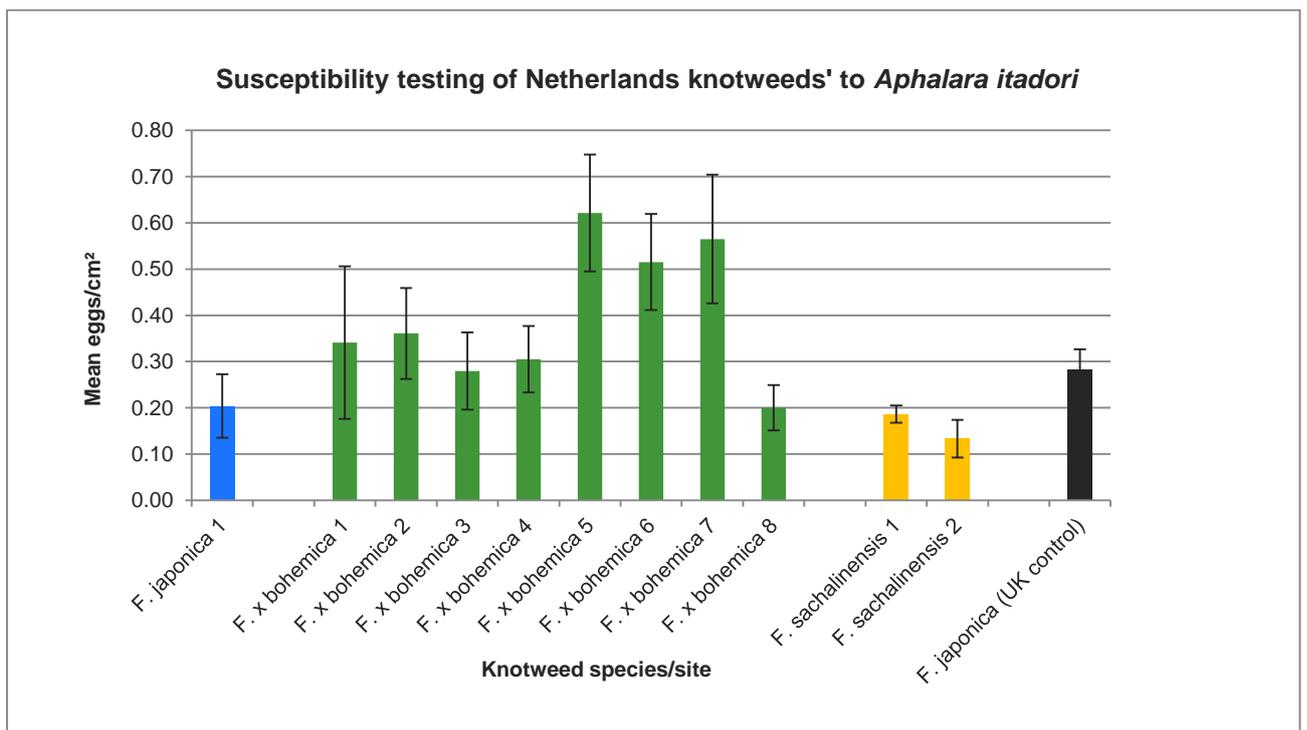


Figure 2: Susceptibility of Netherlands knotweeds' to *A. itadori* including *F. japonica* UK control: mean eggs/cm²

It is important to note that due to the different times of growth of the various knotweeds from the Netherlands it was not possible to run a single experiment incorporating all

material at the same time. Therefore three batches of experiments were conducted as and when material was available. Combining the data across the three batches was not possible to due to the variation between these three e.g. there were significant differences between the mean number of eggs laid per cm² of leaf between batches 1 and batches 2 and 3. Therefore all three batches were analysed separately (always including a UK *F. japonica* control). There was no significant difference within the three batches of experiments. All species received eggs and demonstrate suitability as hosts for *A. itadori*. For the purpose of the statistical analyses the data were log transformed due to non-normal distribution and results are presented in Appendix 4.

All the plants were kept to monitor development of eggs to adults with adult emergence counts taking place 7 weeks (51 days) post set-up. The highest mean number of adults to emerge was from *F. x bohemica* 281.2 (± 48.0) (site 5); 268.33 (± 42.6) (site 7); and 207.67 (± 51.5) (site 6). The lowest mean adult emergence was found for *F. sachalinensis* (site 2), 33.50 (± 11.7) and the Netherlands *F. japonica* (site 1) 37.83 (± 10.4). The mean adult emergence for the *F. japonica* control was 83.00 (± 17.2).

In three instances on *F. x bohemica* (site 1, 5 and 6) the number of emerged adults appears to exceed the number of eggs laid (>100%) this is because some eggs were missed during the non-destructive counting as those concealed in the sheaths around nodes would be damaged otherwise. In these instances the percentage figures were rounded to 100% emergence. See Figure 13 and Table 2 in Appendix 3.

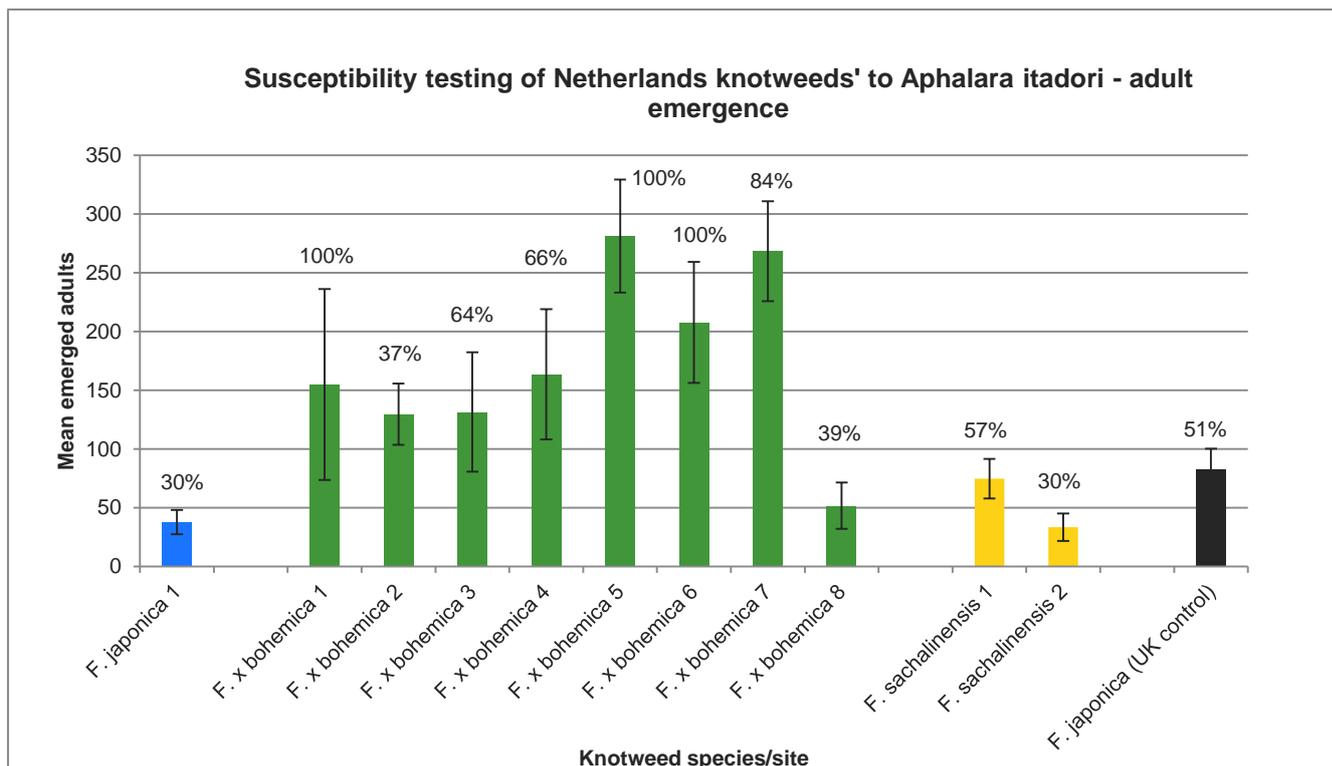


Figure 3: Susceptibility of Netherlands knotweeds' to *A. itadori* including *F. japonica* control: mean emerged adults per plant and mean percentage emergence figures above bars

Multiple-choice oviposition studies

All knotweed plants received eggs with a total of 3,987 eggs laid throughout this test. The highest mean number of eggs per plant was found on *F. x bohemica*: 148.33 (± 31.5); 142.83 (± 28.7); 105 (± 34) (sites 8, 2 and 5 respectively). *F. japonica* (site 1) received a mean number of eggs per plant of 92.17 (± 32.4), and *F. sachalinensis* (site 2) received the least mean eggs per plant of 24.83 (± 12.5). See Figure 14 and Table 3, Appendix 3.

Variation in leaf area available for egg laying was recorded and used to calculate eggs per cm^2 . Figure 15 and Table 4, Appendix 3.

The statistical tests applied to the logged data showed egg laying on *F. japonica* was not significantly different from that on *F. x bohemica* or *F. sachalinensis*. However there was a significant difference between *F. x bohemica* and *F. sachalinensis* ($0.020 < 0.05$) See Appendix 4 for statistical analysis.

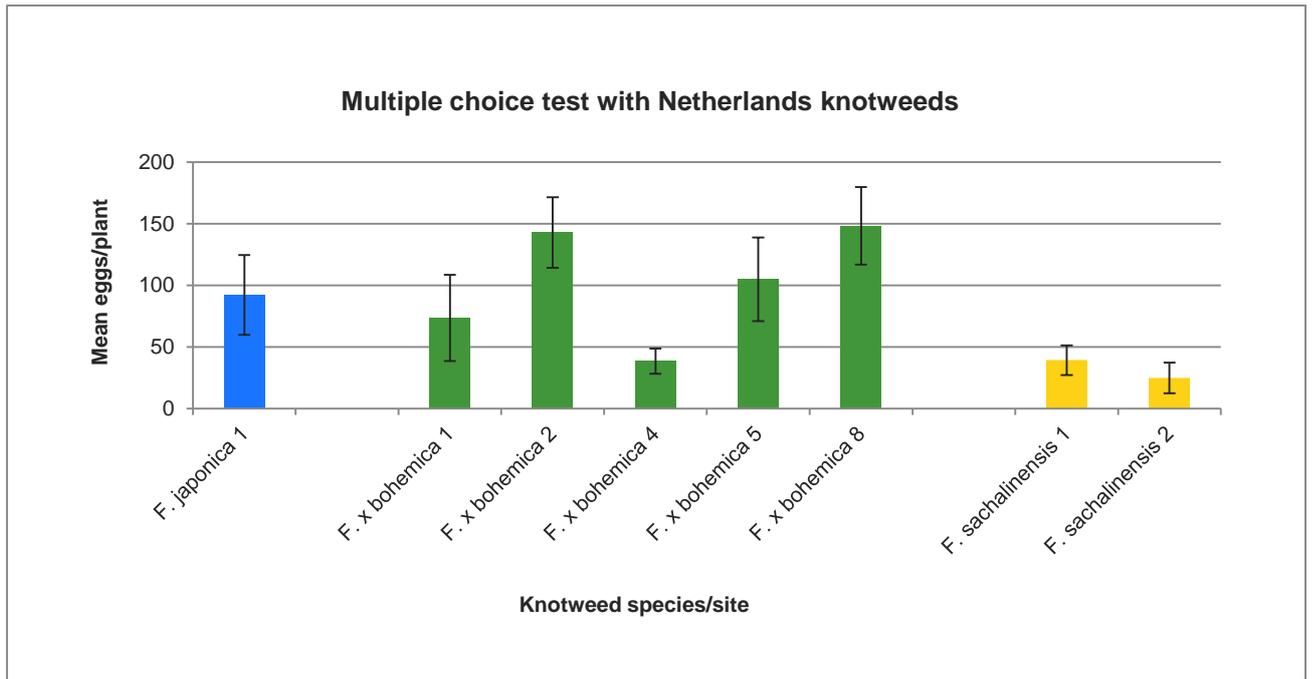


Figure 4: Multiple choice test between selected Netherlands knotweeds' to *A. itadori*: mean eggs/plant

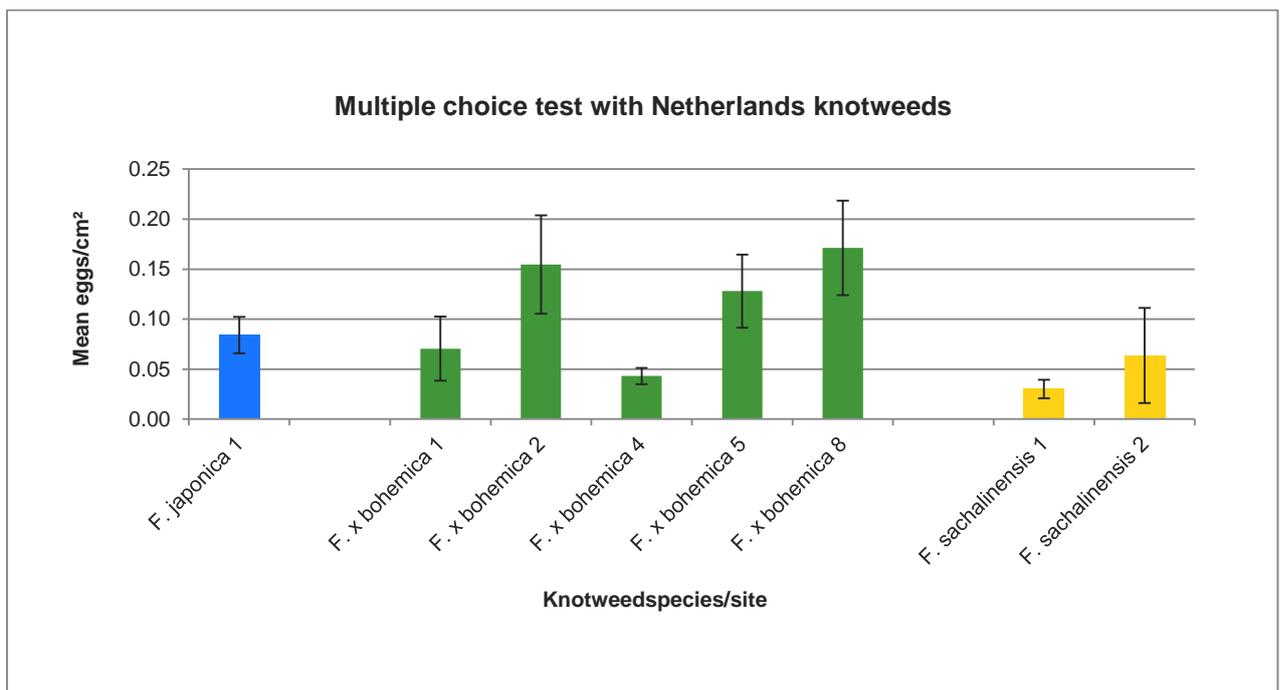


Figure 5: Multiple choice test between selected Netherlands knotweeds' to *A. itadori*: mean eggs/cm²

Activity 1B: Host-range testing with *Aphalara itadori*

No-choice oviposition/development studies

Only three of the 60 non-target plants received a single egg: *Limonium vulgare*, *Beta vulgaris* ssp. *maritima* and *Capsicum annuum*. None of these eggs developed into nymphs or subsequent adults. The *F. japonica* (UK) controls (total 18 reps) received 4,035 eggs (mean eggs per plant 224 ± 36). See Table 5, Appendix 3.

Multiple-choice oviposition/development studies

Two of the ten non-target species received eggs in this multiple-choice situation: *Chenopodium bonus-henricus* received a total of two eggs on one plant (mean eggs/plant 0.33 ± 0.33) and *Beta vulgaris* ssp. *maritima* received a single egg (mean eggs/plant 0.17 ± 0.17). None of these eggs developed into nymphs or subsequent adults. In total the twelve replicates of *F. japonica* controls (ex. NL site 1) received 3,529 eggs (mean eggs per plant 294 ± 48). See Table 6, Appendix 3.

Activity 1C: Field cage Testing with *Aphalara itadori*

All of the three knotweed species received eggs. *F. japonica* received the highest number with a mean of 184.4 (\pm 70.9) eggs per plant. *F. x bohemica* received a mean number of eggs per plant of 157 (\pm 47.4) and *F. sachalinensis* 98.1 (\pm 18.2). None of the three non-target species received eggs. Adult development was also recorded with the following mean number of adults emerging on the knotweed species: *F. japonica* 128.4 (\pm 43.9), *F. x bohemica* 92.4 (\pm 30.6) and *F. sachalinensis* 34.8 (\pm 12.5). See figure 16 below and Table 7, Appendix 3. Table 8, Appendix 3 provides the resting adult psyllid location at the end of the oviposition period.

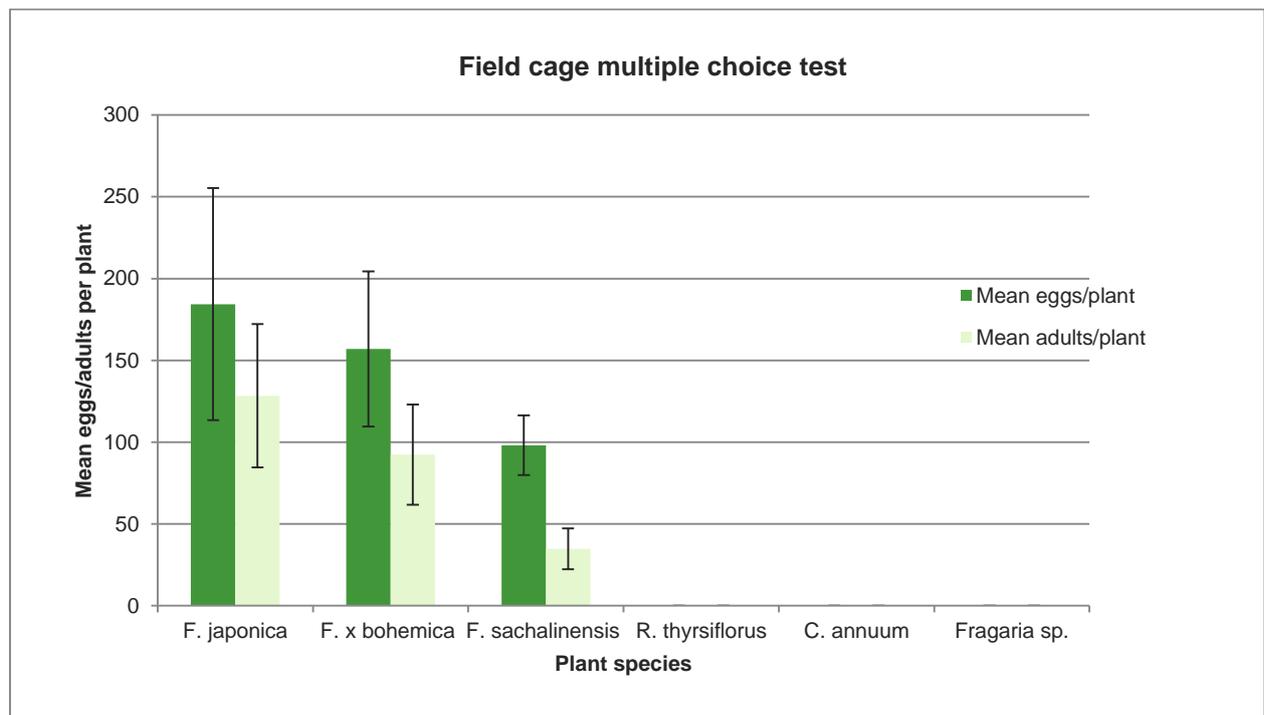


Figure 6: Field cage multiple choice oviposition and development of *A. itadori* on three knotweed species and three non-target test plants

Discussion

The results from Activity 1A, knotweeds' susceptibility testing, demonstrate that all 3 Dutch knotweed species *F. japonica*, *F. x bohemica* and *F. sachalinensis* support oviposition and adult development of *Aphalara itadori*, based on observations of 16,202 eggs across the tests. *F. x bohemica* appeared particularly susceptible with six of the eight sites receiving the greatest number of eggs. *F. sachalinensis* (site 2) received the fewest eggs suggesting it is a less preferable host, although *F. sachalinensis* (site 1) received eggs close to the number received by the UK *F. japonica* control. An analysis of variance showed no significant differences in oviposition between species.

All plants were kept in a controlled environment to monitor development, with adult emergence counts taking place 51 days post set-up (this was determined to be the time when most first generation adult psyllids had emerged but no second generation adults could have emerged). All knotweed species supported adult development with mean emergence ranging from 34 to 281 adults per plant (32% to >100%). The highest adult emergence was found for *F. x bohemica*, with seven of eight sites having higher mean emergence per plant than either *F. japonica* (Dutch and UK control) or *F. sachalinensis*. In three instances over 100% emergence was recorded, which is to be expected when conducting non-destructive egg counts since a high number of eggs are often laid around the plant nodes and are hidden without removing the node or leaf from the plant. The lowest mean adult emergence was found for *F. sachalinensis* (site 2). It is interesting to note the similarity between the relative number of eggs laid and the relative percentage emergence of each species tested, suggesting that the ultimate ability of the plants to maintain development of the insect to adult is influencing the egg laying "preference" even in no-choice situations.

The multiple choice studies between knotweed species were conducted in large field cages to replicate more natural field conditions. The plants included in the study were *F. japonica* (ex. Netherlands, site 1), both the *F. sachalinensis* sites (1 and 2) and five *F. x bohemica* sites. The *F. x bohemica* sites were selected based on the previous susceptibility testing and the health of plants (sites 1, 2, 4, 5 and 8). Overall all the knotweeds received eggs in this setup. The Analysis of Variance indicates a significant

difference in oviposition in terms of eggs/cm² between *F. x bohémica* and *F. sachalinensis*, but none between *F. japonica* and either of the other species. This finding supports the results that *F. x bohémica* is a particularly good host for *A. itadori*.

The mean number of eggs per plant was reduced in the field cage tests compared to the susceptibility tests conducted in a controlled environment, except for *F. x bohémica* (site 8) which received a higher mean number of eggs in addition to the highest number of eggs overall in the multiple choice test (compared to receiving the lowest in susceptibility test). This reduction in oviposition is expected when comparing an extreme no choice test (single sleeved plant) to a large field cage where (1) the psyllids are less concentrated and (2) can choose between plants plus (3) the conditions are more variable.

The leaf area in cm² was calculated for all knotweed species (utilising a pre-determined formula developed for knotweed leaves). There was a difference in area available between knotweed species however this did not appear to affect psyllid oviposition and eggs numbers. There were however morphological differences between the species, *F. sachalinensis* for example had the greatest leaf area available but received fewer eggs and morphologically the leaves were rougher with many trichomes and took a longer time to grow. *F. x bohémica* leaves were softer and faster growing resulting in many leaves that may have been more attractive to the psyllids, whereas it was noted that the *F. japonica* grew slightly thicker, more 'leathery' leaves.

The extreme no-choice host-range testing with non-target species confirms the host specificity of the psyllid. A total of 3 eggs were laid on three non-target plants (out of 60 plants) on *Limonium vulgare*, *Beta vulgaris* ssp. *maritima* and *Capsicum annuum*. However, none of these eggs developed into nymphs or adults and these plants are certainly not to be considered hosts. In comparison, a total of 4,035 eggs were laid on the *F. japonica* controls.

Under multiple-choice conditions (choice test between non-targets with *F. japonica* control) only two of the 10 non-target species received eggs on a single plant (single replicate out of six) these were *Chenopodium bonus-henricus* where 2 eggs were found on one plant and *Beta vulgaris* ssp. *maritima* which received a single egg. Again none of

these eggs developed into nymphs or subsequent adults and compared to the 3,529 eggs laid on the *F. japonica* controls indicates the plants to be unsuitable hosts. In all instances where eggs were found on non-target plants it appears more a case that eggs were 'dumped' on the plant with poor host suitability resulting in no further development to even nymph stage.

The field cage tests allowed for a more natural choice situation between the three knotweed species and three species of non-target plants *R. thyrsiflorus*, *C. annuum* and *Fragaria* sp. It is reassuring that *C. annuum* did not receive a single egg in this choice situation, despite receiving an egg in the susceptibility test and suggests that the egg was accidentally dumped on the plant. *R. thyrsiflorus* was the most closely related non-target test species to *F. japonica* (Polygonaceae family) however it received no eggs. All of the knotweed species received a high number of eggs in this choice scenario with the mean ranging from 98 to 184 eggs per plant. Subsequent mean adult emergence ranged from 35 to 128 adults per plant (30 to 90%). The psyllid resting locations revealed just one psyllid on the upper leaf surface of one *C. annuum* plant, the remaining psyllids were always found to be resting on knotweed plants (n=303). The field temperatures reached up to 31.2°C over the 7 day exposure period with an average temperature of 24.1°C.

Overall the results confirm the psyllid's high specificity to knotweeds, with all knotweed species supporting oviposition and development through to adult. Results further suggest that *F. x bohemica* is the most preferred host next to *F. japonica*, whereas *F. sachalinensis* is a less preferred host with reduced oviposition and subsequent adult development.

None of the ten non-target species supported any development of the psyllid, confirming their poor host potential for the Kyushu biotype of *Aphalara itadori*. The results suggest that in a natural field situation where *A. itadori* achieves a high population in the presence of knotweed the risk to the tested non-target species is extremely low to zero.

In previous host range testing with two biotypes of *A. itadori*, the populations were found to have very narrow fundamental host ranges. In the majority of cases no oviposition or

extremely low oviposition without development to adult stages was recorded on any non-target species (Grevstad et al., 2013).

There is a difference in preference of knotweed by psyllid biotype; the Kyushu psyllid has a preference for *F. japonica* and *F. x bohemica*, whereas the Hokkaido population of *A. itadori* have been reported to prefer *F. sachalinensis*. A combined approach with both biotypes might therefore be preferable for control of the suite of knotweed plants. It is good news that the Kyushu strain (as tested in this reported research) shows good acceptance for *Fallopia x bohemica* as well as *F. japonica* as these are the key targets of concern, above giant knotweed (*F. sachalinensis*) in mainland Europe.

As with any biocontrol program it would take time for the psyllid population to establish and subsequently reach levels capable of reducing the biomass of knotweed plants. However once this is achieved the narrow host range of this specialist insect will contribute to a sustainable and long-term method of controlling knotweeds with the population being in accordance with the availability of its preferred host plant. Indeed, “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” (Grevstad et al., 2013).

It is expected that as populations of the psyllid build up and suck the sap from the host plant, then the plant itself and the plant population as a whole will suffer and be less able to support the high populations of the psyllid. Attacked plants would be smaller and less able to build up resources to put into the rhizome and therefore less able to compete with native plants etc. Since the psyllid is unable to maintain populations on any other species it will be forced to either seek other knotweed plants or succumb to a lack of food. This drives a classic cyclical response driven by the availability of the host plant which eventually settles at an equilibrium.

Recommendations

With regards to *Aphalara itadori*, consideration should be given to the completion of an official Pest Risk Analysis (PRA) using these data combined with data generated on behalf of the UK, USA and Canada, much of which is already in the public domain. See Appendix

6 (Grevstad et al., 2013) for the test plant list for *Fallopia japonica* in the UK, Canada and Switzerland.

There are a number of recommendations for further work in addition to the PRA such as preparing for release in the Netherlands which would involve training on mass rearing techniques, optimised release strategies and monitoring approaches. It is also important to consider lessons learnt from the knotweed biocontrol program in the UK and the need for effective communication in both public and political arenas (Shaw *et al.*, 2011). Subject to approval of a release in the Netherlands, investigations to improve the release strategy would also be beneficial to ensure optimal conditions for psyllid establishment after release. It would also be wise to consider new rearing stock from the same region in Japan as the current culture being used for UK releases has already been in culture under Japanese summer conditions for over 100 generations.

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Species	Mean eggs/plant	S.E.	Mean eggs/leaf	S.E.	Mean eggs/cm²	S.E.
<i>F. japonica</i> 1	128.17	45.55	3.73	0.89	0.20	0.07
<i>F. x bohemica</i> 1	147.17	67.59	6.97	3.60	0.34	0.16
<i>F. x bohemica</i> 2	347.67	79.78	10.61	2.56	0.36	0.10
<i>F. x bohemica</i> 3	205.00	50.67	6.72	2.29	0.28	0.08
<i>F. x bohemica</i> 4	246.17	41.90	7.07	1.00	0.31	0.07
<i>F. x bohemica</i> 5	240.50	33.55	8.71	0.92	0.62	0.13
<i>F. x bohemica</i> 6	203.00	33.20	9.45	1.67	0.52	0.10
<i>F. x bohemica</i> 7	320.33	77.90	10.58	2.78	0.56	0.14
<i>F. x bohemica</i> 8	133.17	31.61	3.94	0.81	0.20	0.05
<i>F. sachalinensis</i> 1	131.00	31.61	5.25	1.66	0.19	0.02
<i>F. sachalinensis</i> 2	111.50	26.83	7.45	2.47	0.13	0.04
<i>F. japonica</i> UK	162.22	22.30	6.45	0.93	0.28	0.04

Table 1: 1A Susceptibility testing of Netherlands knotweeds' to *A. itadori* (mean eggs/plant; mean eggs/leaf; mean eggs/cm², including standard error)

Species	Total no. adults	Mean emerged adults/plant	S.E.	Mean % emergence
<i>F. japonica</i> 1	227.00	37.83	10.36	29.52
<i>F. x bohémica</i> 1	929.00	154.83	81.24	105.21*
<i>F. x bohémica</i> 2	778.00	129.67	26.03	37.30
<i>F. x bohémica</i> 3	789.00	131.50	50.75	64.15
<i>F. x bohémica</i> 4	982.00	163.67	55.40	66.49
<i>F. x bohémica</i> 5	1687.00	281.17	48.02	116.91*
<i>F. x bohémica</i> 6	1246.00	207.67	51.45	102.3*
<i>F. x bohémica</i> 7	1610.00	268.33	42.56	83.77
<i>F. x bohémica</i> 8	310.00	51.67	19.73	38.80
<i>F. sachalinensis</i> 1	449.00	74.83	16.85	57.12
<i>F. sachalinensis</i> 2	201.00	33.50	11.73	30.04
<i>F. japonica</i> UK	1494.00	83.00	17.18	51.16

Table 2: 1A Susceptibility testing adult emergence (mean emerged adults/plant, including standard error and mean % emergence. Note: * = emergence >100% due to non-destructive sampling represented as 100% emergence).

Species	Mean eggs/plant	S.E.	Mean leaves/plant	S.E.	Mean eggs per leaf	S.E.
<i>F. japonica</i> 1	92.17	32.40	43.83	5.47	1.93	0.53
<i>F. x bohémica</i> 1	73.50	34.95	48.33	4.68	1.44	0.62
<i>F. x bohémica</i> 2	142.83	28.66	184.00	3.04	4.89	1.10
<i>F. x bohémica</i> 4	38.50	10.28	26.83	4.71	1.48	0.36
<i>F. x bohémica</i> 5	105.00	33.95	35.50	2.35	2.96	0.85
<i>F. x bohémica</i> 8	148.33	31.54	47.17	5.70	3.04	0.69
<i>F. sachalinensis</i> 1	39.33	12.04	45.33	5.49	1.00	0.30
<i>F. sachalinensis</i> 2	24.83	12.55	22.00	5.25	2.21	1.61

Table 3: 1A Multiple choice oviposition tests between Netherlands knotweeds' (mean eggs/plant; mean leaves/plant and mean eggs/leaf, including standard error)

Species	Mean leaf area (cm ²)	S.E.	Mean eggs per cm ²	S.E.
<i>F. japonica</i> 1	978.72	0.02	0.08	0.02
<i>F. x bohemica</i> 1	1125.61	0.03	0.07	0.03
<i>F. x bohemica</i> 2	1094.00	0.05	0.15	0.05
<i>F. x bohemica</i> 4	924.67	0.01	0.04	0.01
<i>F. x bohemica</i> 5	851.88	0.04	0.13	0.04
<i>F. x bohemica</i> 8	911.92	0.05	0.17	0.05
<i>F. sachalinensis</i> 1	1381.05	0.01	0.03	0.01
<i>F. sachalinensis</i> 2	812.16	0.05	0.06	0.05

Table 4: 1A Multiple choice oviposition tests between Netherlands knotweeds' (mean leaf area and mean eggs per cm², including standard error)

Species	N	Total no. eggs	Mean no. eggs/plant ± S.E.
<i>Chenopodium bonus-henricus</i>	6	0	0
<i>Rumex thyrsiflorus</i>	6	0	0
<i>Limonium vulgare</i>	6	1	0.17 ± 0.17
<i>Beta vulgaris</i> ssp. <i>maritima</i>	6	1	0.17 ± 0.17
<i>Fragaria</i> sp.	6	0	0
<i>Capsicum annuum</i>	6	1	0.17 ± 0.17
<i>Rubus idaeus</i>	6	0	0
<i>Cucurbita maxima</i>	6	0	0
<i>Chenopodium quinoa</i>	6	0	0
<i>Triticum spelta</i>	6	0	0
<i>F. japonica</i> ex. UK (control)	18	4035	224 ± 36

Table 5: 1B Susceptibility (oviposition and development) of Netherlands non-target test plants to *A. itadori* (Total number of eggs, including standard error)

Species	N	Total no. eggs	Mean no. eggs/plant ± S.E.
<i>Chenopodium bonus-henricus</i>	6	2	0.33 ± 0.33
<i>Rumex thyrsiflorus</i>	6	0	0
<i>Limonium vulgare</i>	6	0	0
<i>Beta vulgaris</i> ssp. <i>maritima</i>	6	1	0.17 ± 0.17
<i>Fragaria</i> sp.	6	0	0
<i>Capsicum annuum</i>	6	0	0
<i>Rubus idaeus</i>	6	0	0
<i>Cucurbita maxima</i>	6	0	0
<i>Chenopodium quinoa</i>	6	0	0
<i>Triticum spelta</i>	6	0	0
<i>F. japonica</i> ex. NL Site 1 (control)	12	3529	294 ± 48

Table 6: 1B Multiple choice oviposition and development on Netherlands non-target plants (Total number of eggs, including standard error)

Species	Mean eggs/plant	S.E.	Mean adults/plant	S.E.
<i>F. japonica</i>	184.4	70.9	128.4	43.9
<i>F. x bohemica</i>	157.0	47.4	92.4	30.6
<i>F. sachalinensis</i>	98.1	18.2	34.8	12.5
<i>R. thyrsiflorus</i>	0.0	0.0	0.0	0.0
<i>C. annuum</i>	0.0	0.0	0.0	0.0
<i>Fragaria</i> sp.	0.0	0.0	0.0	0.0

Table 7: 1C Field cage multiple choice oviposition and development (mean eggs/plant and mean adult emergence/plant, including standard error)

Appendix 6

Aphalara itadori (Shinji) (Insecta: Psyllidae)

Taxonomy & Natural Enemies

Andrew Polaszek, Dept of Entomology, the Natural History Museum, London

Taxonomy

A. itadori was described originally in 1938 in the genus *Psylla*. It was transferred to *Aphalara* by Miyatake (1964). The original description, including a single figure, is reproduced below in its entirety, as is the later and more useful redescription by Burckhardt & Lauterer (1997). *A. itadori* is somewhat unusual in the genus, and gives its name to a species-group containing only itself and one other species, *A. taiwanensis*.

Shinji, O. 1938; Five new species of *Psylla* from North-Eastern Japan. p. 149-150.

12. *Psylla itadori* Shinji n. sp. (Itadori-madarakijirami)

Adult: - Body dirty yellow, with usually brownish tint. Frontal tubercles conical, large, slightly infuscated. Head broader than long, divided in middle by a black longitudinal suture, infuscated a little and black at the margins. Eyes large, black, arising from the sides of the head. Ocelli reddish. Proboscis reaching beyond the front leg, apical article black. Prothorax broader than the head but not so broad as the head including the eyes. Meso- and meta-thoracic parts infuscated with 5 grayish longitudinal stripes on the dorsum of the mesothorax. Legs with tibiae and their spurs and claws black, the remaining portion being concolourous with the body. Hind and often the middle tarsi with about 12 large blunt and black spurs and the first hind tarsi with about 4 of them. Wings subhyaline, broad and rounded at the apex. Fore wings with radius almost perpendicular to subcosta and radial sector which is nearly one and half times as long as the radius, almost straight. Medial stalk arising at about the middle part of the radial stalk, basal portion usually obscure, almost as long as radial sector, both M1 and M2 short and subequal. Cubital stalk arising at about $\frac{1}{4}$ part of medial stalk which arises in turn at about the middle part of the cubital stalk, C1 much curved up, about 3 times as long as C2 which is almost straight. The following places are maculated or infuscated: a wide area on both sides of C1; along C1 and continuing to cover the apical half of the cell enclosed by it, a transverse region extending from the infuscated part of the cell and traversing it to the costal margin, and area extending along the radial sector and M1 and M2 as well. Legs short with femora infuscated, the remaining parts being

concolorous with the body, hind tibia with about 10 black and blunt spurs and the first tarsus with about 4. Abdomen yellowish in colour with infuscated transverse band on each of the segments II~VIII. Genital valves with some long hairs, the upper being much longer than the lower.

Measurements in mm:

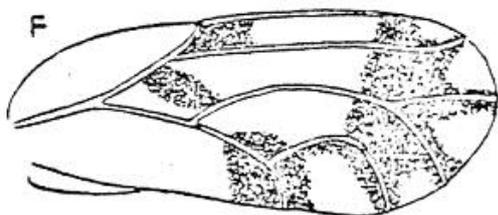
Length of body	1.70		
Length of fore wing	2.00	Width of abdomen	0.90
Length of antenna	0.65	Length of hind tarsus	0.35

Host plant: *Polygonum reynoldi*

Type locality: Morioka, Iwate Prefecture, Japan.

Date of collection: Sept. 20, 1937.

Notes: This species attacks the underside of leaves as well as shoots and flowers. When attacked leaves curl in toward the underside and enclose the insects within. In the case of flowers they become stunted in growth and congregate all in a body. The insects are very common during the months of July (*sic*) to October.



Fore wing of *Aphalara itadori* (from Shinji, 1938)

Redescription of *Aphalara itadori* (Shinji) by Burckhardt & Lauterer, 1997

(*Entomologica Scandinavica* 28: 271-305)

Aphalara itadori (Shinji, 1938)

(Figs 26, 46, 62)

Psylla itadori Shinji, 1938: 149. Syntypes, Japan: Iwate Prefecture, Morioka, 20.ix.1937, *Polygonum reynoldii* {depository?}, not examined.

Aphalara itadori; Miyatake 1964.

Aphalara nebulosa (Zetterstedt); Matsumura 1916 (misidentification).

Aphalara kunashirensis Klimaszewski, 1983: 8. Holotype ♂, Russia: Primorskiy Kray, Amursk region, Sev. Kunashir, okr. volc. Tjatja, 2-4.vii.1976 (V. Zherihin) (ZMUM?), not examined. **Syn. n.**

Description. - Adults. Head. Anteorbital tubercles and macroscopic setae on vertex absent. Anterior tubercles on vertex small. Outer anterior vertex margin straight or concavely rounded. Tubercle between antennal insertion and eye small, flattened. Clypeus short, not or hardly visible from above, conical, apically subacute.

Forewings. Membrane semi transparent to weakly coriaceous; with light or dark brown pattern consisting of well-defined spots and patches. Surface spinules forming regular cellular pattern.

Genitalia. Parameres in profile lamellar, with small antero-basal projection; slender in the middle, flattened apically; anterior subapical process removed from apex, small, claw-like. Apical dilatation of distal segment of aedeagus small relative to shaft; with large dorso-apical membranous sack; apico-ventral process small, pointed, hooklike, strongly sclerotised distally and convex proximally. Female proctiger subacute apically; subgenital plate subacute apically; valvulae dorsalis and ventralis curved.

Measurements and ratios (1♂, 1♀). HW 0.61-0.65; AL 0.61-0.73; ALHW 1-1.12; WL 2.12-2.32; WLHW 3.48-3.57; WLW 1.97-1.98; cell m_1 ratio 1.07-1.23; cell cu_1 ratio 1.86-2.13; TLHW 0.74; MP 0.17; MPHW 0.28; PL 0.22; AE 0.19; FP 0.69; FPHW 1.06; FPCP 3.83; FPFS 1.50.

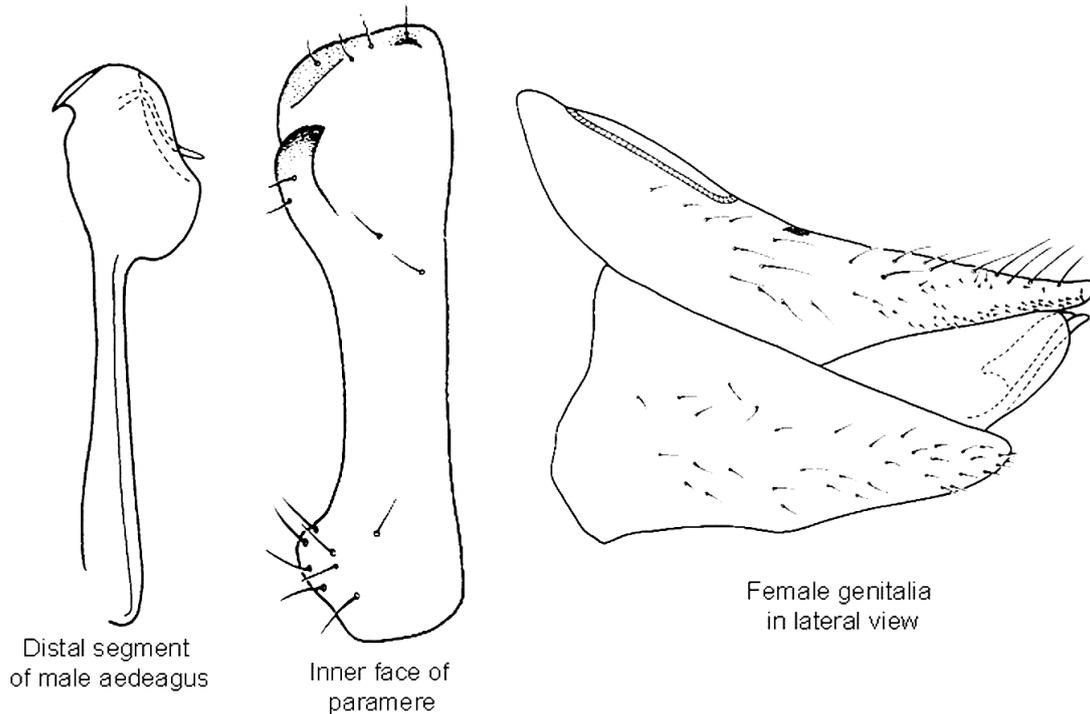
Larvae and eggs unknown.

Host plants. - *Polygonum cuspidatum*, *P. reynoldii*, and *P. sabulosum* (= *P. sachalinense*).

Distribution. - Japan; Korea; Russia: Sakhalin, Kurile Islands (Kwon 1983, Gegechkori & Loginova 1990).

Material. - Japan: Kyushu, Honshu (MHNG). Russia: Kurile Islands (ZISP).

Remarks. - Type material of *Psylla itadori* could not be traced and seems to be lost (Y. Miyatake pers. comm. to DB, 24.viii.1996). Type material of *A. kunashirensis* was unavailable to us; 1♀ labelled as paratype which we could examine (ZMUM) belongs to *Cacopsylla*. No other type I specimen could be traced in the ZMUM (S. Kurbatov pers. comm.) nor in the SIUK, and we think that the type material is lost. A series of *A. itadori* from the Kurile Islands (ZISP) fits the description of *A. kunashirensis* which is therefore synonymised with the former.



(Figures modified from Burckhardt & Lauterer, 1979).

Natural enemies of *Aphalara itadori*

According to R. Shaw (personal communication) a single eulophid parasitoid has been recorded attacking the species in Japan (see photographs below). From the photographs, this appears to be a *Tamarixia* species (considered by some a synonym of *Tetrastichus*), although *Tamarixia* species are usually associated with Triozidae. The six British *Tamarixia* species are all associated with Triozidae, but could conceivably move over to *Aphalara*. *Chrysonotomyia* species (Eulophidae) have been reared from Psyllidae twice, but never from *Aphalara* spp.

Noyes' (2007) "Universal Chalcidoidea Database" ([www. http://internt.nhm.ac.uk/jdsml/research-curation/projects/chalcidoids](http://internt.nhm.ac.uk/jdsml/research-curation/projects/chalcidoids)) lists no known parasitoids of *Aphalara itadori*, and out of a total of 90 records of Psyllidae parasitoids, only two are associated with *Aphalara* species. Both concern North American species (California), and both are *Psyllaephagus* species (Encyrtidae), although one is recorded as "*Encyrtus aphalarae*". At least one of these records is attributable originally to Jensen (1957), who also records a cecidomyiid predator of *Aphalara maculipennis* from Germany. Of the 90 records of chalcidoid parasitoids of Psyllidae in the Noyes database, more than 60 concern *Psyllaephagus* spp. On the face of it, there is therefore some probability that an indigenous British *Psyllaephagus* species, of which there are currently two, could use *Aphalara itadori* as a potential host. However, of these two species, one (*P. lusitanicus* Mercet) is known only from the scale family Asterolecaniidae, and the other (*P. pilosus* Noyes) is known only from *Ctenarytaina eucalypti* – a psyllid living on eucalypts. Furthermore, none of the four indigenous British *Aphalara* species is known to be attacked by any parasitoid species. According to Dr Ian Hodkinson (psyllid specialist) there is very little known about the parasitoids of *Aphalara* species. Having worked extensively on populations of *Craspedolepta* species (the next genus) he has never found high levels of parasitism. It seems, therefore, very unlikely that *Aphalara itadori* will be attacked to any major extent by any known British chalcid parasitoid.



Unparasitised *Aphalara* nymph



Parasitised *Aphalara* nymph



Parasitoid pupa with host remains removed



Adult eulophid parasitoid (?*Tamarixia* sp.)

(photos by Naoki Takahashi)

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Appendix 7

Preliminary studies of generalist predator interactions with the psyllid *Aphalara itadori*

Supplies

Two shipments of *Adalia bipunctata* eggs laid on filter paper and *Orius majusculus* eggs laid in bean pods were received on 16th September and 21st October. Both *Adalia bipunctata* and *Orius majusculus* from the first shipment started to hatch on 17th September. In the second shipment, *Orius majusculus* started to hatch on 24th October and *Adalia bipunctata* on 27th October. Cannibalism was a serious problem, at least in *Adalia* immediately after hatching and *Orius* was revealed to have a major impact on *Adalia* egg survival when they managed to bypass electrical tape barriers on Petri dishes. All eggs were kept in an incubator at 16°C with a day/night cycle of 12 hours to slow development until they hatched and were used in the tests. Unless stated, the tests were set up with freshly hatched larvae in a climatized quarantine chamber at 22°C. Both Thistle aphid and Rosebay willowherb aphids (hereafter called RW aphid) were collected from the field and *Aphis gossypii* from a rearing colony supplied by Syngenta Bionline. The *A. itadori* eggs, nymphs and adults were harvested from the established culture at CABI's quarantine facility in Egham, Surrey.

Methods

***Syrphus ribesii* choice test**

Individual early stage *Syrphus* larvae were placed in each of 10 Petri dishes (diameter 4.5cm) and provided with a choice of 20 mid-stage nymphs each of *A. itadori* and RW aphid. The number of individuals of each species consumed was recorded after 24 hours and the whole experiment repeated. Those cases where the predator died were excluded from a paired T-test (using R version 2.3.2).

***Chrysoperla carnea* choice test**

The same procedure as with *Syrphus ribesii* was followed.

***Adalia bipunctata* no-choice vs. Aphids**

One freshly hatched larva of *Adalia bipunctata* was placed in each of ten a 4.5cm diameter Petri dishes with a moist filter paper and twenty thistle aphids on fresh plant material each. The number of surviving aphids was recorded regularly and the experiment was repeated where possible. The same procedure was followed for *Aphis gossypii* and RW aphids though in the latter case only developmental times were recorded.

***Adalia bipunctata* no-choice vs. Psyllid eggs**

20 replicates were set up as above with leaf sections with a known number of *A. itadori* eggs. The number of remaining eggs was recorded regularly and the number consumed was calculated by deduction. Those cases where the predator larva died were excluded from the analysis.

***Adalia bipunctata* no-choice vs. Psyllid nymphs**

Ten replicates were set up as above, with a known number of psyllid nymphs in excess of the daily possible level of consumption (>25 of mixed stages) and fresh plant material. The number of nymphs consumed was calculated from the number surviving.

***Adalia bipunctata* no-choice vs. Psyllid adults**

One *Adalia bipunctata* larva (stage 3 or 4) was placed in a 12cm diameter Petri dish with a moist filter paper. Eight replicates were set up with plant material and 4 adult psyllids each. The number of psyllids eaten was recorded after 24h and the experiment repeated with new adults.

Adalia bipunctata* single choice vs. psyllid eggs or *A. gossypii

Ten replicates were set up as for no choice psyllid eggs (above) and with a number of aphids similar to the number of eggs. The number of remaining eggs and aphids, as well as the development of *Adalia* was recorded regularly. By the time the tests were recorded, some of the *A. itadori* eggs had hatched but were counted as eggs. New prey were provided after each check and the experiment restarted.

Adalia bipunctata* single choice vs. psyllid nymphs or *A. gossypii

This was carried out under three different conditions, i.e with prey transferred into a relatively bare arena, with prey transferred on their host (excised tissue) in a larger arena and again using living material under a cloche:

Transferred nymphs:

Five replicates were set up as for *Adalia* single choice test with *Aphalara* eggs, but with 15 or 20 psyllid nymphs of 3-4th stage and the same number of aphids transferred onto a piece of leaf with a fine paintbrush. The number of remaining nymphs and aphids were recorded regularly and the Petri dishes were set up again the same way. The development of *Adalia* was monitored until pupation.

Large Petri dishes:

One *Adalia bipunctata* 2nd larval stage was placed in each of ten 12cm diameter Petri dish with moist filter paper. A piece of shoot or leaf with 20 established psyllid nymphs (stage 2-5) and 20 aphids of various stage was placed in the Petri. The number of remaining nymphs and aphids were recorded regularly. The experiment was repeated where possible.

Under Cloches:

One young *Adalia bipunctata* was placed on each of 12 living shoots of Japanese knotweed with a known number of psyllid nymphs (15 to 20 nymphs stage 3-4 transferred) and the same number of transferred *Aphis gossypii*. The bases of the shoots were placed in a vial filled with water and covered with a parafilm lid. The vials were placed into a sealed transparent plastic container. The number of remaining psyllid nymphs and aphids was recorded after 2-3 days.

Orius majusculus

***Orius majusculus* no choice tests vs. Aphids**

One *Orius majusculus* freshly hatched nymph was placed in each of ten 4.5cm diameter Petri dishes with a moist filter paper. Twenty thistle aphids were placed in each dish and the number of surviving aphids was recorded and the experiment restarted. The same procedure was carried out using RW aphids and also *Aphis gossypii*, but an unlimited supply was made available so that development rates could be recorded.

***Orius majusculus* no choice tests vs. Psyllids (eggs and nymphs)**

Ten replicates were set up as for *Adalia* no choice psyllid eggs with a known number of psyllid eggs provided and eggs consumption recorded and compared at regular intervals. In parallel another cohort of *Orius* nymphs were provided with an unlimited supply of psyllid eggs and the rate of development was recorded. The same procedure was repeated using psyllid nymphs.

The few remaining late stage *Orius* nymphs were set up in a 4.5cm diameter Petri dish with a moist filter paper and provided with 4 adult psyllids each in an attempt to see whether adults might be susceptible to *Orius* predation. The number of psyllid adults consumed was recorded.

Orius majusculus* choice test – psyllid egg vs. *Aphis gossypii

A known number of psyllid eggs adhered to a Japanese knotweed leaf were provided to a newly-emerged *Orius* nymph alongside a similar number of various stages of *A. gossypii* in each of fifteen 4.5cm Petri dishes. The number of each prey species consumed in each choice chamber was recorded.

***Orius majusculus* choice test – psyllid nymphs vs. *Aphis gossypii* in Petri dishes**

10 replicates were set up as for the equivalent *Adalia* study using 15 individuals of each prey species

***Orius majusculus* choice test – psyllid nymphs vs. *Aphis gossypii* - live plant**

Since the psyllid nymphs do not behave as they would when “plugged in” to a plant and could be more vulnerable a further choice test was set up between *Aphalara* nymphs and *Aphis gossypii* using live plants under Perspex cloches. 15 replicates were set up with 20 psyllid nymphs stage 3-4 and the same number of *Aphis gossypii*.

Results

Syrphus ribesii

When given a choice between *A. itadori* nymphs and RW aphids the number of psyllids consumed per day 4.53 ± 1.01 ($\pm 1SE$, $n=17$) was significantly lower than the aphid consumption of 10.71 ± 1.98 ($\pm 1SE$, $n=17$) ($p = 1.8 \times 10^{-4}$, $df = 16$)

Chrysoperla carnea

When given a choice between *A. itadori* nymphs and RW aphid the number of psyllids consumed per day, 6.83 ± 0.8 ($\pm 1SE$, $n=18$), was no different to the equivalent aphid consumption of 5.33 ± 0.86 ($\pm 1SE$, $n=18$) ($p = 0.14$, $df = 17$)

***Adalia bipunctata* no-choice vs. Aphids**

When given only nymphs of the thistle aphid of various stages 5.88 nymphs were eaten per day ± 0.31 ($\pm 1SE$, $n=10$). When supplied with *Aphis gossypii* all *Adalia* nymphs inexplicably died within two days. When presented with RW aphid development to pupa was successful in 5 though the initial stage of nymph was not always clear so the mean development time from N1 to adult of 11.2 days ± 0.74 ($\pm 1SE$, $n=5$) cannot be relied upon.

***Adalia bipunctata* vs. Psyllid eggs no-choice**

When *A. itadori* eggs were the sole food source the *Adalia* larvae were capable of consuming 28.58 eggs per day ± 3.54 ($\pm 1SE$, $n=11$)

***Adalia bipunctata* no-choice vs. Psyllid nymphs**

When given *Aphalara* nymphs of various stages only 3.38 nymphs were eaten per day ± 1.01 ($\pm 1SE$, $n=4$) but mortality was unacceptably high in this study with all but 4 replicates dying in less than two days.

***Adalia bipunctata* no-choice vs. Psyllid adults**

Adalia nymphs are quite capable of catching and eating adults and were found to have fed on 2.8 adults per day ± 0.31 ($\pm 1SE$, $n=21$). However on the recording day in some replicates all the prey had been consumed so this estimated feeding rate is likely to be an under-estimate. Also the arena prevented the psyllids from flying away so this may be a false positive.

Adalia bipunctata* single choice vs. psyllid eggs or *A. gossypii

When the number of prey species eaten per day are compared in these paired tests the mean number of psyllid eggs eaten per day 13.05 ± 1.18 ($\pm 1SE$, $n = 47$) was significantly lower than the number of aphids eaten 16.96 ± 0.92 ($\pm 1SE$, $n = 47$) ($p=2.02 \times 10^{-5}$, $df = 46$). When one considers that the eggs are so much smaller than the aphid prey we can conclude that the beetles consumed much more aphid material than psyllid material in this test.

Adalia bipunctata* single choice vs. psyllid nymphs or *A. gossypii - Small Petri dishes transferred:

When the number of prey species eaten per day are compared in these paired tests the mean number of *A. itadori* nymphs eaten per day 10.7 ± 1.14 ($\pm 1SE$, $n=23$) was lower compared with a mean daily consumption of 12.35 *A. gossypii* ± 1.38 ($\pm 1SE$, $n=23$) ($p = 0.035$, $df = 22$).

Adalia bipunctata* single choice vs. psyllid nymphs or *A. gossypii - Large Petri dishes

A.itadori nymphs and *A.gossypii* (large Petri dishes)

When the number of prey species eaten per day are compared in these paired tests the mean number of *A. itadori* nymphs eaten per day 7.41 ± 0.88 ($\pm 1SE$, $n=28$) was significantly lower compared with 12.02 ± 0.91 ($\pm 1SE$, $n=28$) *A. gossypii* ($p = 0.0005$, $df = 27$).

Adalia bipunctata* single choice vs. psyllid nymphs or *A. gossypii - Live plant material

When a choice of prey on live plants was offered the mean number of *A. itadori* nymphs eaten per day 3.42 ± 0.52 ($\pm 1SE$, $n=12$) was significantly lower compared with 5.13 ± 0.45 ($\pm 1SE$, $n=12$) *A. gossypii* ($p = 0.08$, $df = 11$).

Orius majusculus

***Orius majusculus* no-choice feeding and development – various hosts**

Orius can consume a mean of 19.52 *A.itadori* eggs per day ± 3.19 ($\pm 1SE$, $n=9$) when given no choice. The shortest development time from hatch to adult was 13 days (mean $=15.25$ days ± 1.03 ($\pm 1SE$, $n=4$) on this food source though the number of them that

developed through to adult was low. This compares with the earliest emergence of 14 days on both *Aphis gossypii* and RW aphid and 15 days on a thistle aphid.

When fed solely on *Aphalara* nymphs the development time to adult was similar at 13 days (mean =13.33 days \pm 0.33 (\pm 1SE, n=3) and the few that survived in the feeding trial on psyllid nymphs were able to consume a mean of 3.38 nymphs per day \pm 1 (\pm 1SE, n=4) in their early stages.

The few late *Orius* nymphs that were still available at the end of the experiment did show the capability to feed on *A. itadori* adults in a Petri dish.

Orius majusculus* choice test psyllid eggs vs. *Aphis gossypii

When presented with a choice between psyllid eggs and aphids the *Orius* nymphs showed no significant preference with the consumption of 5.69 eggs \pm 0.94(\pm 1SE, n=15) and 4.99 aphids \pm 0.72 (\pm 1SE, n=15) ($p=0.617$, $df =14$).

***Orius majusculus* choice test psyllid nymphs vs. *Aphis gossypii* in Petri dishes**

When presented with a choice between psyllid nymphs and aphids the *Orius* nymphs showed no significant preference with the consumption of 3.1 nymphs \pm 0.47 (\pm 1SE, n=10) and 2.77 \pm 0.51 (\pm 1SE, n=10) ($p=0.096$, $df = 9$).

***Orius majusculus* choice test psyllid nymphs vs. *Aphis gossypii* - live plant**

Using a live plant the results were much the same in a choice between *A. itadori* nymphs and aphids with *Orius* consuming 3.96 nymphs per day \pm 0.47 (\pm 1SE, n=15) and 4.11 aphids per day \pm 0.78 (\pm 1SE, n=15) ($p=0.83$, $df=14$).

Observations/Discussion

It is clear that every generalist natural enemy that were available for study was capable of feeding on the psyllid, at least in all the no choice laboratory tests within quarantine that were possible. This



Fig 1 *Orius* nymph feeding on *Adalia* eggs

perhaps is not surprising since they are generalist predators in genera that are used for biological control in glasshouses against many target species. One interesting factor was that they fed very just as willingly on themselves and other predators (Fig 1). Indeed, keeping the very mobile *Orius* nymphs out of other collections and experiments proved surprisingly difficult.

When it comes to a choice situation some preferences do become apparent. The syrphid *Syrphus ribesii*, collected from eggs laid amongst Rosebay willowherb aphid colonies in the field, showed a clear preference for this “normal” host over *Aphalara* nymphs. However, this does not hold for the lacewing *Chrysoperla carnea* which fed indiscriminately. Our more detailed studies involving various stages of psyllid in choice situations with aphid prey are mixed. With *Adalia bipunctata* a significant preference is shown for *Aphis gossypii* over psyllid eggs and this preference is also significant when one considers tests using *A. itadori* nymphs with the ability to plug in i.e those in large Petri dishes with large sections of cut material and those using live plants. However, in the tests when the psyllids had been transferred with no suitable host material the difference is present but not significant.

Orius can feed on both *A. itadori* eggs and nymphs developing through to adult with the same development time on both food sources (13 days) which was similar to that when fed on *Aphis gossypii* and RW aphid. In choice tests conducted in petri dishes *Orius* showed no preference between *A. itadori* and aphids both in terms of feeding on their eggs and nymphs. The same is true for a more natural environment using live plants for choice tests between the two kind of preys. In general *Orius* seems to be a more indiscriminate feeder with no clear preference in comparison to *Adalia*. The fact that both predators were capable of catching and consuming the more mobile adult *Aphalara* stage should be received with caution since the test arenas were so small as to make it impossible for the adults to jump and fly effectively.



Fig 2 *Adalia* nymph feeding on *Aphalara* eggs

Whilst we tried to use standard organisms, the limited supply due to the study taking place outside the field and horticultural season meant that we were limited in what was available. Thus, the size and stage of the predators used may have influenced the outcome of some studies since larger organisms may have been immune from predation by very small predators and equally, small prey may be of little interest to larger predators. It was also not possible to

examine situations where larger populations of psyllid nymphs existed with their characteristic waxy covering or were concealed under the knotweed nodal sheaths. This was because counting eggs or nymphs present in these refugia can only be done destructively. The protection provided by these preferred refugia may further limit predation by generalists and could have selected for any specialist parasitoids that co-evolved in Japan and have been removed prior to any release.

In summary, none of the experiments revealed any preference in four generalist predators for any stage of the psyllid over aphid prey. This suggests, at least in the short term, that *A. itadori* will not become their preferred food source.

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