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# Japanese knotweed biological control testing for The Netherlands

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Photo: Scanning for *Aphalara itadori* eggs on a Dutch *Fallopia japonica* plant

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## Summary

In the first quarter of 2013, CABI were contracted by the Team Invasive Aliens (TIE) 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, 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.

Testing of *Mycosphaerella polygoni-cuspidati* commenced in July, to assess the susceptibility of the 3 knotweeds collected from the 11 sites in the Netherlands. Results indicate a low level of susceptibility of both *F. japonica* and *F. bohemica* to the leaf-spot pathogen whilst *F. sachalinensis* is immune.

## 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 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. The rhizome was kept in a moist environment to allow the first shoots to sprout, before the plants were potted in multi-purpose compost and kept in a heated glasshouse facility until reaching a suitable size of testing. Some rhizome pieces were retained and prevented from sprouting, in order to stagger planting and ensure a continual supply throughout the sequence of trials.


**Figure 1: (A) Shipment of NL rhizome to CABI UK; (B) Sorted rhizome; (C) Potted rhizome; (D) NL Knotweed plants growing in the greenhouse.**

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: Test plants being propagated**

## 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. Setup of these cages was staggered to allow a continual supply of psyllids that could be removed for testing as and when required.

**Figure 3:** **Life stages of the Japanese knotweed psyllid *Aphalara itadori***

## 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 knotweeds, *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.

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.

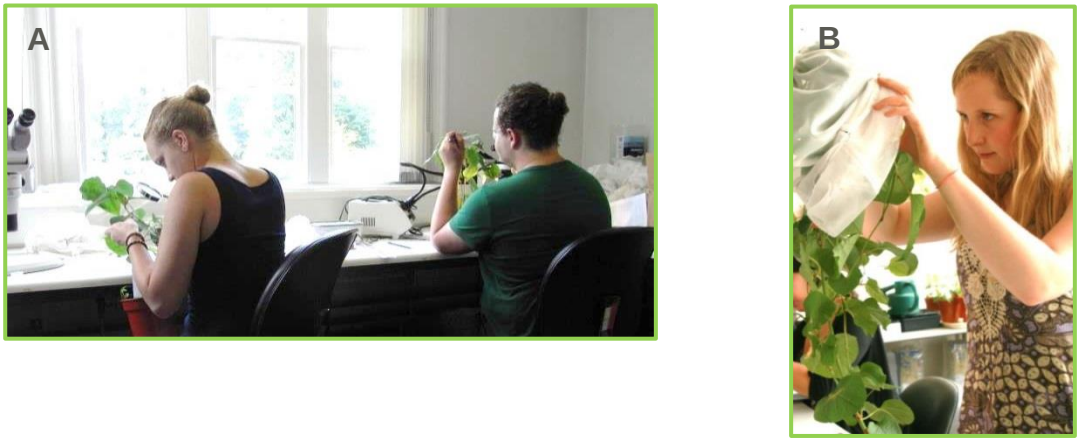


**Figure 4: (A) Egg counting; (B) Emergent adults of *A. itadori* being collected from an *F. x bohemica* plant**

## 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 *bohemica* 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 $m^3$), to give a more realistic set up. 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.

**Figure 5:** **Setting up Activity 1A multiple-choice test plants before releasing the psyllids**

### 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. 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.

**Figure 6: Sleeved knotweed plant for the no-choice studies in Activity 1B**

### 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. 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.

**Figure 7:** **Multiple-choice test after 7 days of exposure to 30 psyllids**

### 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. 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.

**Figure 8:** **Setup of the multiple-choice field cage tests for Activity 1C**

## Activity 2: Susceptibility testing with *Mycosphaerella polygoni-cuspidati*

The leaf-spot pathogen, *Mycosphaerella polygoni-cuspidati* is a highly damaging hemibiotrophic pathogen found ubiquitously associated with Japanese knotweed *(F. japonica*) in Japan. Molecular studies revealed that this pathogen is heterothallic; therefore it requires mating of two complementary mating types to complete its lifecycle. Under evaluation as a potential biological control agent for Japanese knotweed in the UK, the leaf-spot pathogen is currently undergoing full host-range testing under quarantine conditions at CABI E-UK. Like many other *Mycosphaerella* species, *M. polygoni-cuspidati* has a dual infection mechanism inasmuch as both ascospores and mycelial fragments can act as infective propagules. Inoculation studies have shown that comparable symptoms are evoked on both Japanese knotweed and closely related non-target plants by each of these infective propagules. Viable ascospore material of *M. polygoni-cuspidati* is limited by the field season in Japan; however the pathogen can be easily maintained as mycelial cultures and mass produced in liquid medium. Therefore mycelial broth was used for inoculations.

## Methods

### Plant material

A total of 3 knotweed species; *F. japonica* (collected from 1 site), *F.* x *bohemica* (collected from 8 sites) and *F. sachalinensis* (collected from 2 sites) from the Netherlands were propagated as described previously and were assessed for their susceptibility towards the leaf-spot pathogen.

Susceptibility studies of knotweed species from the Netherlands using the *Mycosphaerella* leaf-spot commenced in July 2013 and continued until November 2013. Due to difficulties with plant propagation from rhizome of *F. sachalinensis*, it was only possible to test 5 replicates of *F. sachalinensis* ex site 1 and 4 replicates ex site 2. For this reason, *F. sachalinensis* plants that had been previously used for the psyllid work were pruned and re-used by washing the rhizomes and allowing plants

to reshoot. Assessment of the outstanding replicates from sites 1 and 2 has commenced at the beginning of 2014.

## Broth production

Inoculations were carried out using the *M. polygoni-cuspidati* strain ex Mt Hiko (W2619, recollection of strain IMI 393529, Mt Hiko, Fukuoka, Kyushu island Japan, alt. 488 m.a.s.l. collected 5$^{th}$ July 2005, Kurose *et al.,* 2009). Cultures were established as single ascospore isolates from ascospore drop induced from fresh samples of infected leaf material shipped from Japan. Single spore cultures were maintained on potato carrot agar (PCA) and two different isolates were used separately to set up mycelial broth for the susceptibility studies. To inoculate individual broth media four mycelial plugs, 5 mm in diameter, were taken from the actively growing margin of individual cultures, split into smaller fragments and added to 75 ml of potato dextrose broth (PDB) in a 250 ml conical flask. A flask containing PDB only was used as a control to detect potential contamination. Flasks were sealed with a sterile foam bung and placed on a rotary shaker at 170 rpm at 18 ºC for 8 days. Subsequently the mycelial broth was transferred to a sterilised blender and macerated for 10 seconds in order to produce a more homogenous inoculum before decanting it into sterile plastic Universal tubes which were stored in a freezer at -18 ºC until required.

## Inoculation procedure and susceptibility assessments

Universals were removed from the freezer and allowed to defrost at room temperature for 2-3 hours. Three leaves of different ages (mature, fully expanded and apical) were inoculated on each plant per isolate (6 leaves per plant in total). Plants were lightly misted with sterile distilled water (SDW) before brushing the whole leaf (both leaf surfaces) with the broth mixture using a fine paintbrush. The concentration of the mycelial broth was determined by counting the number of mycelial fragments per ml of broth using a haemocytometer. Concentrations used for inoculations ranged from 5 x10$^4$ to 5 x 10$^5$ fragments/ml. One leaf of each plant was also inoculated with PDA broth only to serve as a control. Plants were re-sprayed with SDW before being placed into a dew chamber running at 19.5/18 ºC for 48 hours. After that time all plants were removed from the dew chamber and transferred to a designated quarantine greenhouse chamber with natural light

conditions and supplement lighting for 15 hours per day and a temperature range of 15 °C night/ 20°C day. The viability of the mycelial broth was confirmed by assessing the growth of mycelial fragments plated on PCA and incubated in the dew chamber with the inoculated plants. Inoculated plants were monitored regularly for the development of macroscopic symptom over a period of 7 weeks (Figure 9). For each knotweed species from the Netherlands, 6 replicate plants were tested on at least 2 separate occasions and a minimum of two inoculated plants of *F. japonica* ex UK were included in each test for reference.


**Figure 9: (A) Knotweed plants inoculated with *M. polygoni-cuspidati* in the quarantine greenhouse, CABI E-UK, Egham; (B) monitoring of leaf-spot symptoms on *Fallopia japonica* site 1 from the Netherlands**

After 7 weeks all test plants underwent a final assessment during which they were rated for their susceptibility to *M. polygoni-cuspidati* on a scale from 0 (no visible macroscopic symptoms) to 5 (whole leaf necrosis) (Figure 10) based upon symptoms observed both with the naked eye, and using a stereomicroscope:

0. no symptoms
1. < 10 necrotic lesions (1- 2 mm in diameter)
2. > 10 necrotic lesions (1- 2 mm in diameter)
3. Lesions beginning to merge ( >2 mm in diameter)
4. Large area of leaf necrosis caused by merging of lesions
5. Whole leaf necrosis

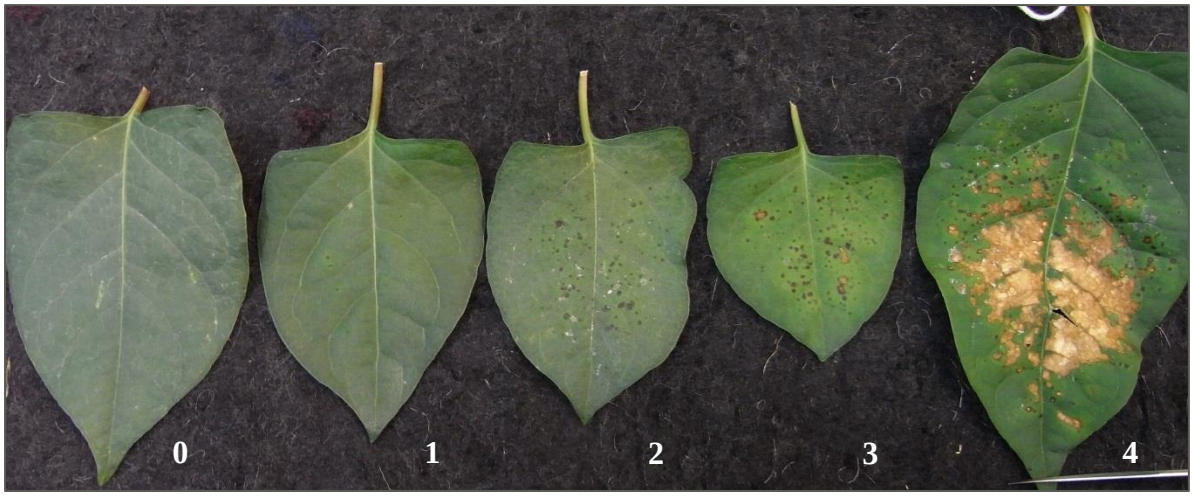


**Figure 10:** **Susceptibility scores recorded for *F. japonica* ex site 1, 7 weeks after inoculation with *Mycosphaerella polygoni-cuspidati.* No image is given for a score of 5 as whole leaf necrosis was not achieved on any of the knotweeds tested. In this case, a susceptibility score of 0 was given to the control leaf**

## 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 (± 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 ± 0.14), site 6 (0.52 ± 0.10), site 2 (0.36 ± 0.10), site 1 (0.34 ± 0.16), site 4 (0.31 ± 0.07), site 3 (0.28 ± 0.08) and site 8 (0.20 ± 0.05) eggs/cm². *F. japonica* (site 1) received 0.20 (± 0.07) eggs/cm² compared to *F. japonica* UK (0.28 ± 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.

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

was significant differences between the mean number of eggs laid per $cm^2$ 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.

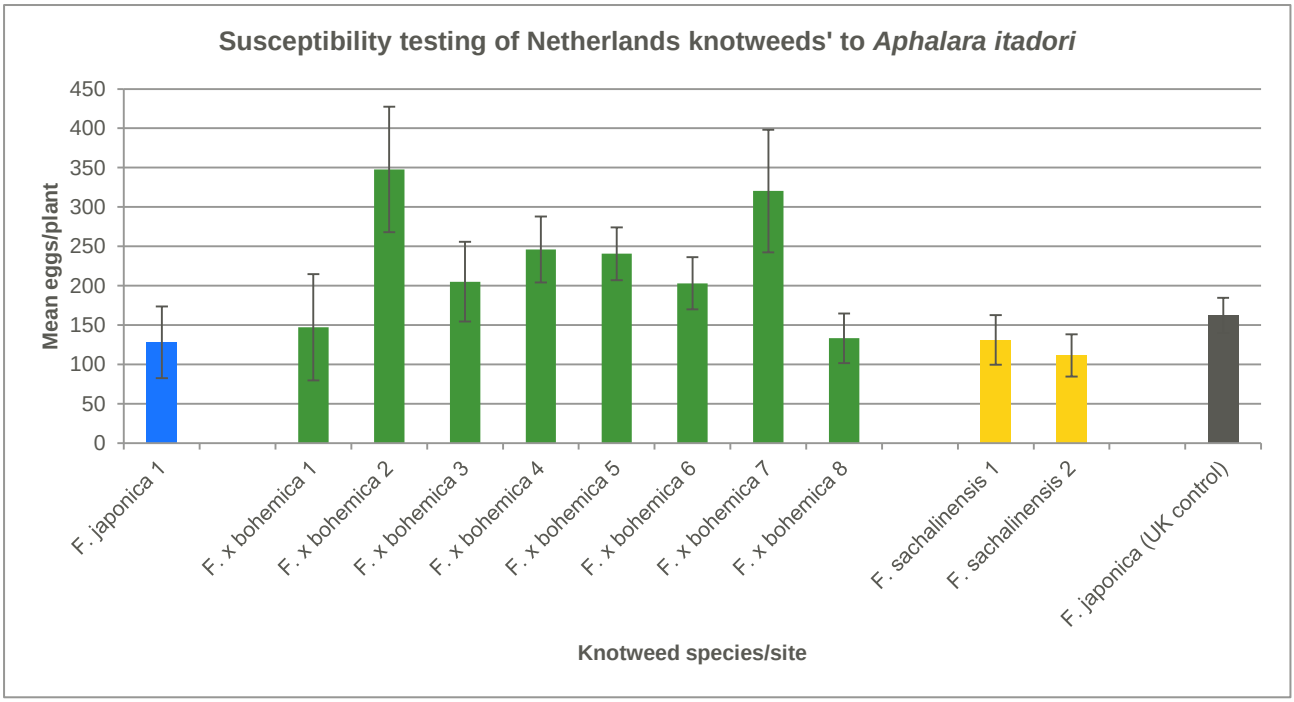


**Figure 11: Susceptibility of Netherlands knotweeds' to *A. itadori* including *F. japonica* UK control: mean eggs/plant**

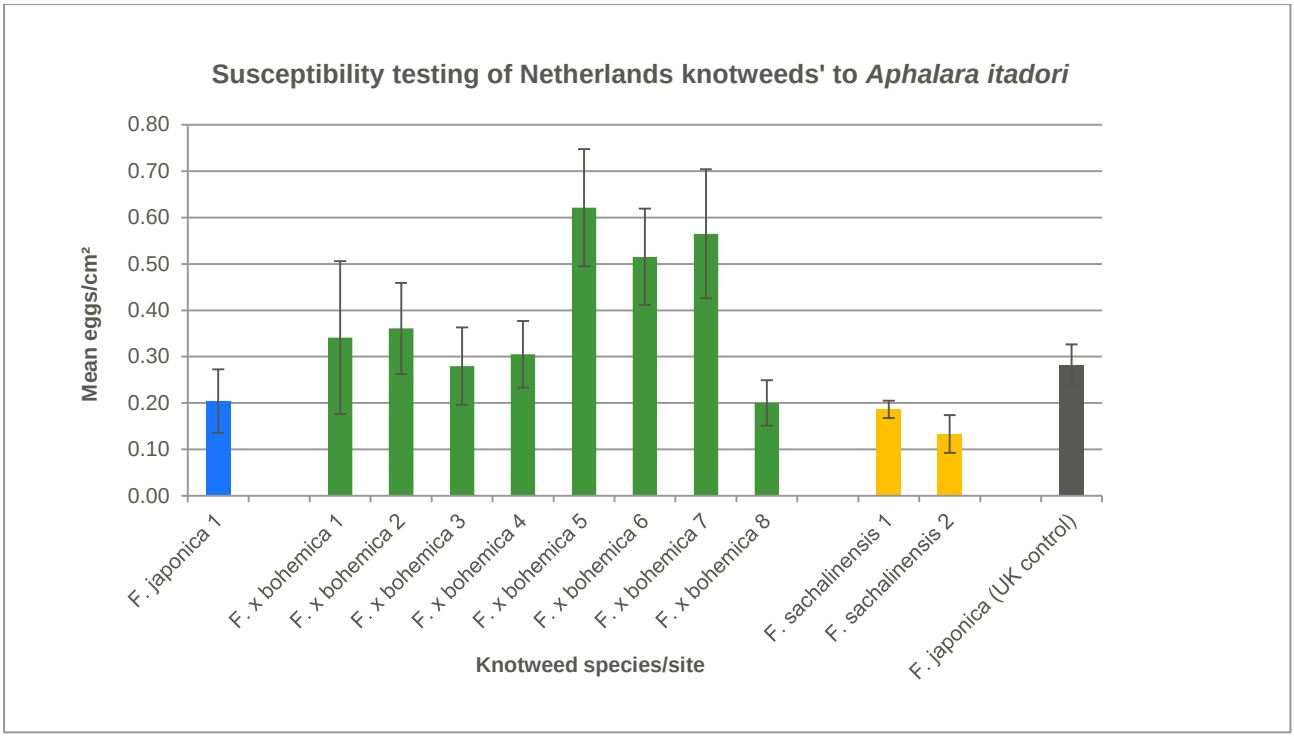


**Figure 12: Susceptibility of Netherlands knotweeds' to *A. itadori* including *F. japonica* UK control: mean eggs/cm²**

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 below and Table 2 in Appendix 3.

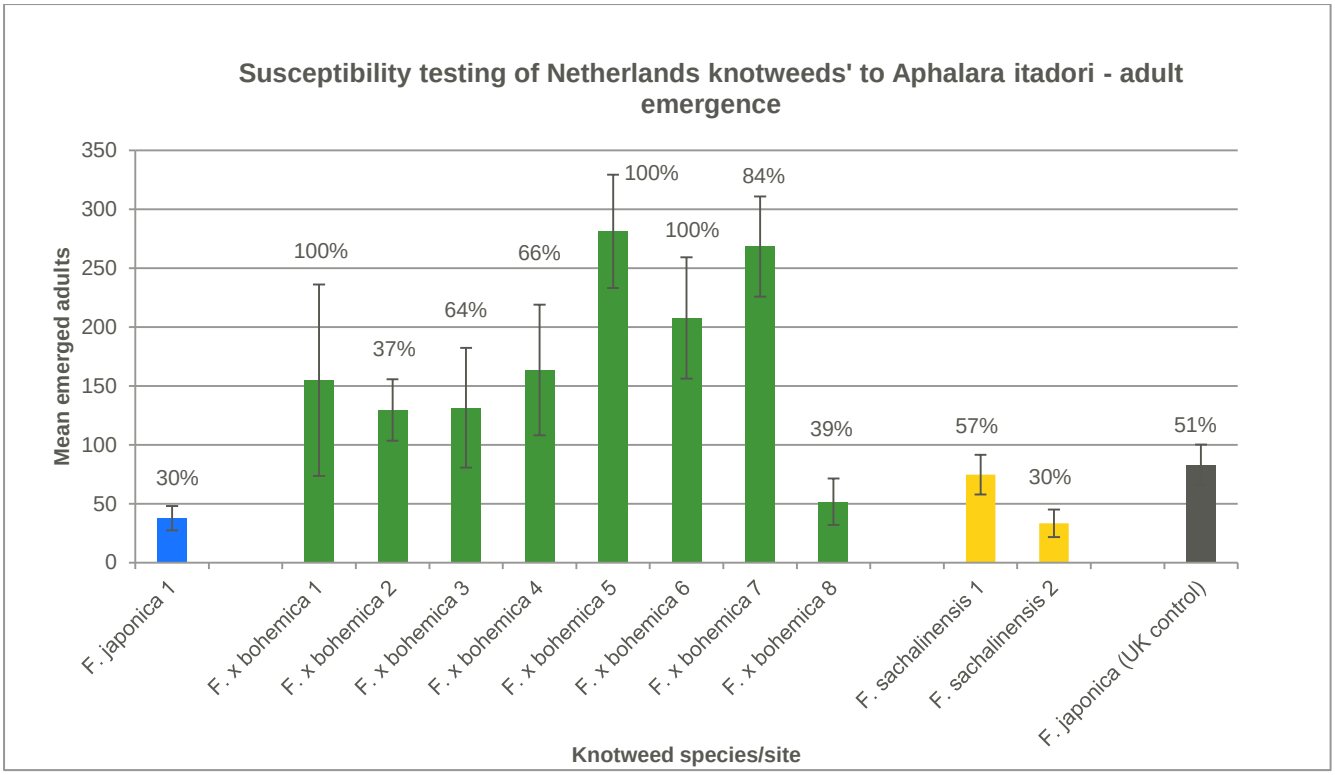


**Figure 13: Susceptibility of Netherlands knotweeds' to *A. itadori* including *F. japonica* control: mean emerged adults per plant and mean percentage emergence figures above bars**

## 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 below 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 below and Table 4, Appendix 3.

*F. japonica* was not significantly different from any *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.

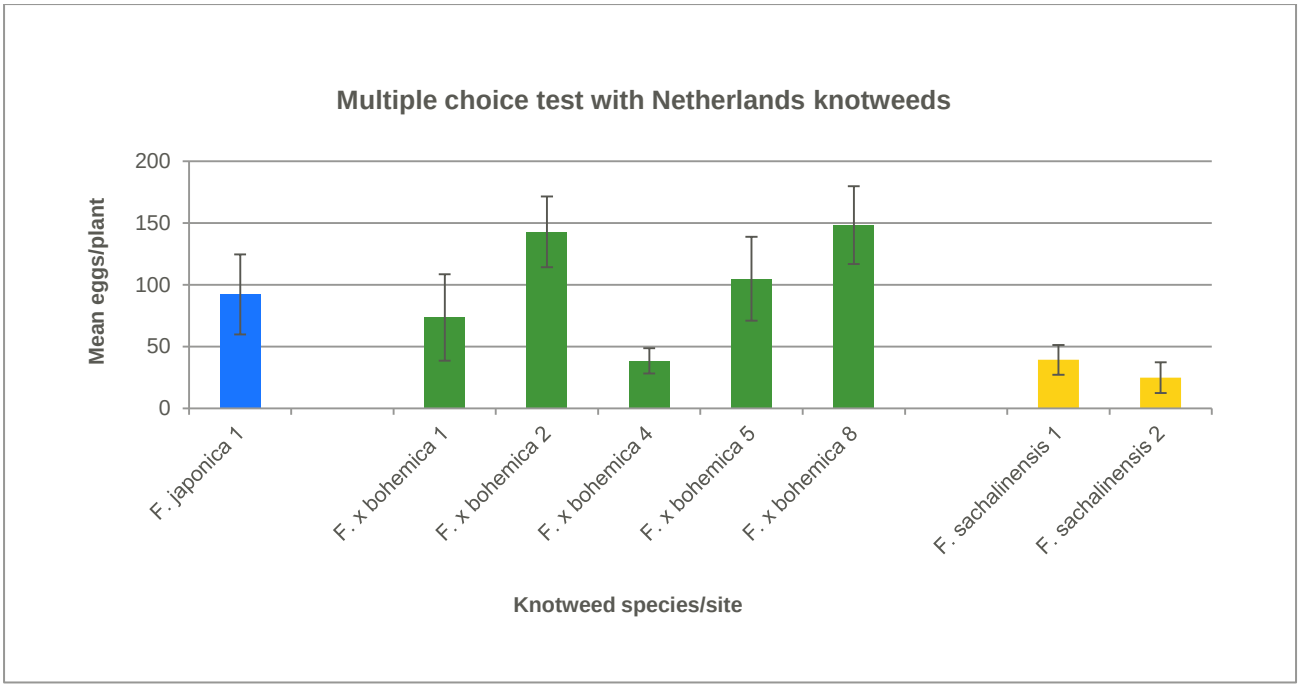


**Figure 14:** **Multiple choice test between selected Netherlands knotweeds' to *A. itadori*: mean eggs/plant**

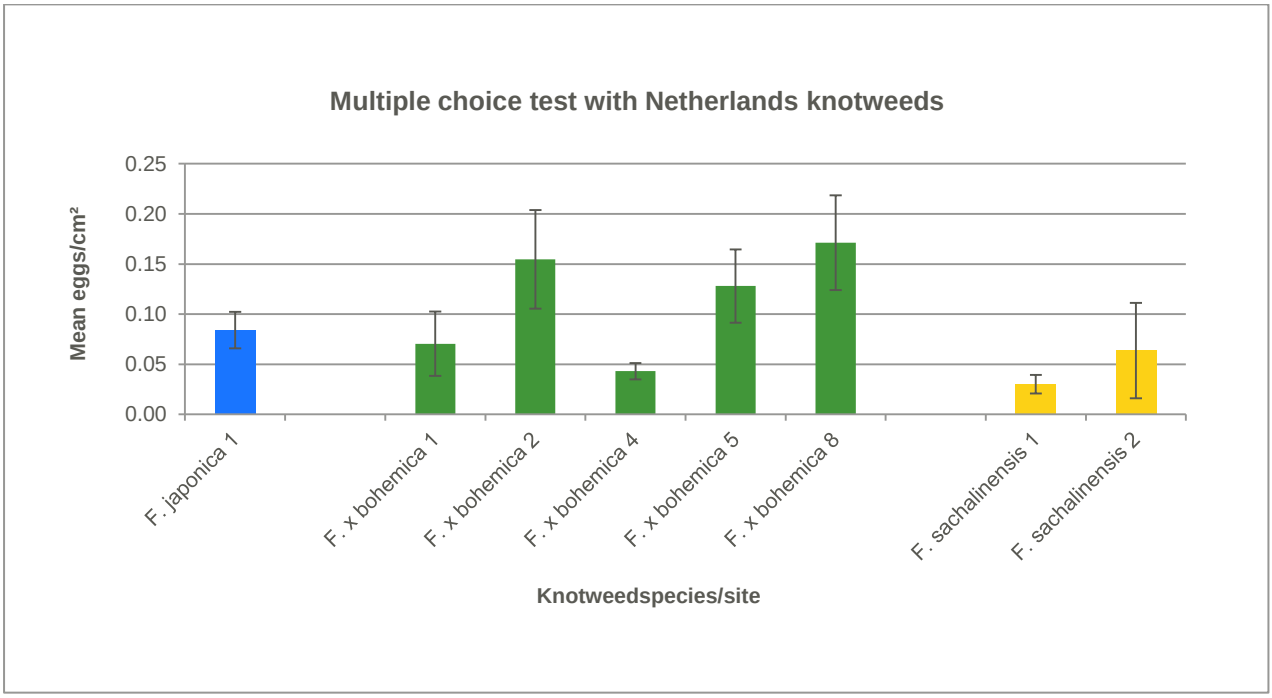


**Figure 15:** **Multiple choice test between selected Netherlands knotweeds' to *A. itadori*: mean eggs/cm²**

### 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 (± 70.9) eggs per plant. *F.* x *bohemica* received a mean number of eggs per plant of 157 (± 47.4) and *F. sachalinensis* 98.1 (± 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 (± 43.9), *F.* x *bohemica* 92.4 (±30.6) and *F. sachalinensis* 34.8 (±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.

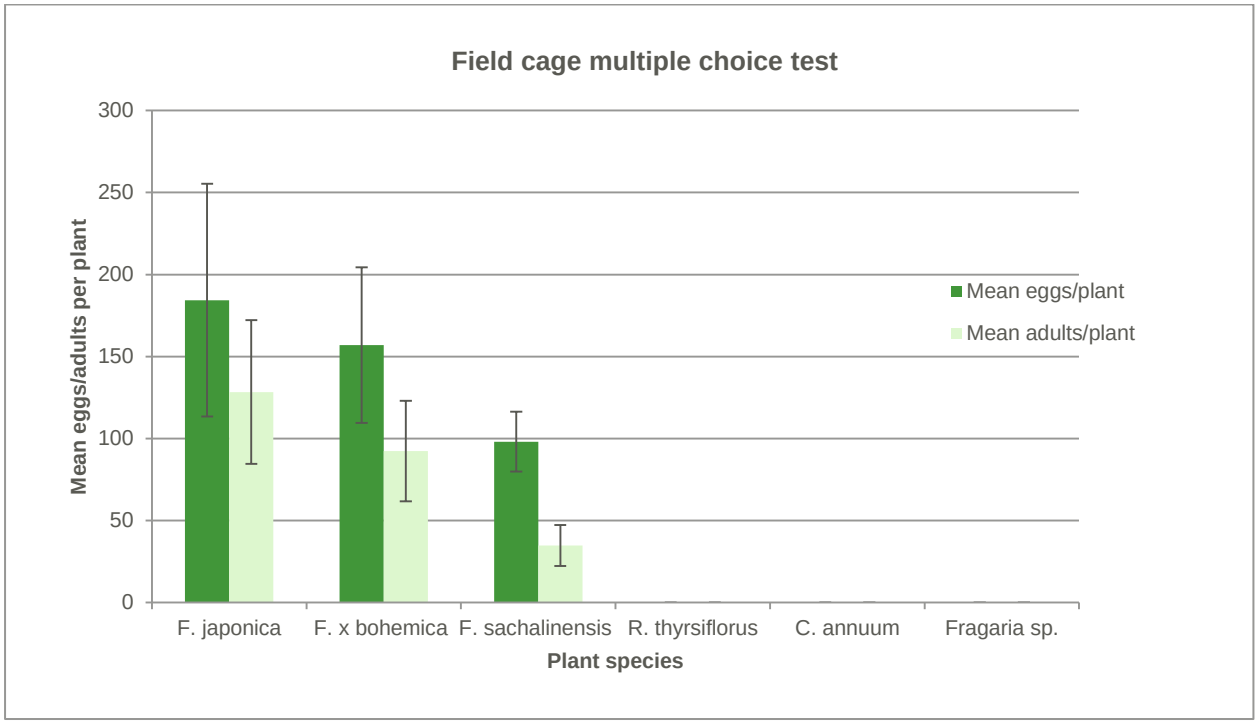


**Figure 16: Field cage multiple choice oviposition and development of *A. itadori* on three knotweed species and three non-target test plants**

**Activity 2: Susceptibility Testing with *Mycosphaerella polygoni-cuspidati***

The results of all inoculation studies undertaken during the months of July to November 2013 are presented in Appendix 5, Table 1. *Fallopia japonica* and *F.* x *bohemica* (excluding site 3) proved to be susceptible towards both isolates of *M. polygoni-cuspidati* ex Mt Hiko, although overall levels of susceptibility were low. Generally, the susceptibility scores for isolate 2 appear to be lower than those for isolate 1 (Figure 17). These differences, however, are less likely to be due to a difference in virulence and more likely to be correlated with a lower number of mycelial fragments in the broth. *Fallopia sachalinensis* and the *F.* x *bohemica* biotype ex site 3 exhibited apparent non-susceptibility or immunity (Figure 17).

Generally a decline in plant susceptibility was recorded during the course of the study. Initial experiments set up during the summer months July and August showed both *F. japonica* ex site 1 and *F.* x *bohemica* ex site 4 to be highly susceptible to the leaf-spot pathogen scoring susceptibility ratings of up to 4 for one isolate. Knotweed plants representing species assessed as susceptible during the first inoculation studies, including *F. japonica* plants ex UK, were given a susceptibility score of 0, and thus rated as non-susceptible during studies established from September onwards. In order to evaluate whether the apparent lack of susceptibility was due to a loss of viability of the stored frozen mycelial broth, fresh broth was prepared from agar cultures of the Mt Hiko pathogen and comparative inoculations were set up in November 2013. Little or no macroscopic symptoms indicating infection with the pathogen were observed on any of the inoculated plants at the time of the final assessment; *F.* x *bohemica* ex site 6 (only 1 of 2 replicate plants) was the only species which exhibited minor necrotic lesions, susceptibility score 1, following inoculation with freshly prepared as well as stored mycelial broth. Overall comparable symptoms were seen on plants inoculated with either type of broth, indicating that the apparent decrease in plant susceptibility cannot be attributed to a decline in viability of the pathogen during storage. Thus, it must be assumed that the susceptibility of individual plants decreased from the summer to the winter months.

Table 1 and Figure 17 show the maximum susceptibility score given to each knotweed species during the experimental period with the majority of knotweeds scoring higher than the mean susceptibility score also shown in Figure 17 by the bar

chart. Although not representative for the whole of the study, this gives an indication of the potential susceptibility levels for the individual knotweeds. However, all replicates of *F. x bohemica* site 3 and both sites of *F. sachalinensis* were tested after September which resulted in susceptibility scores of 0, therefore the potential susceptibility of these knotweeds remains unknown. Full dates of testing can be seen in Appendix 5, Table 2. No clear correlation between leaf age and susceptibility to the leaf-spot pathogen could be seen during this study.

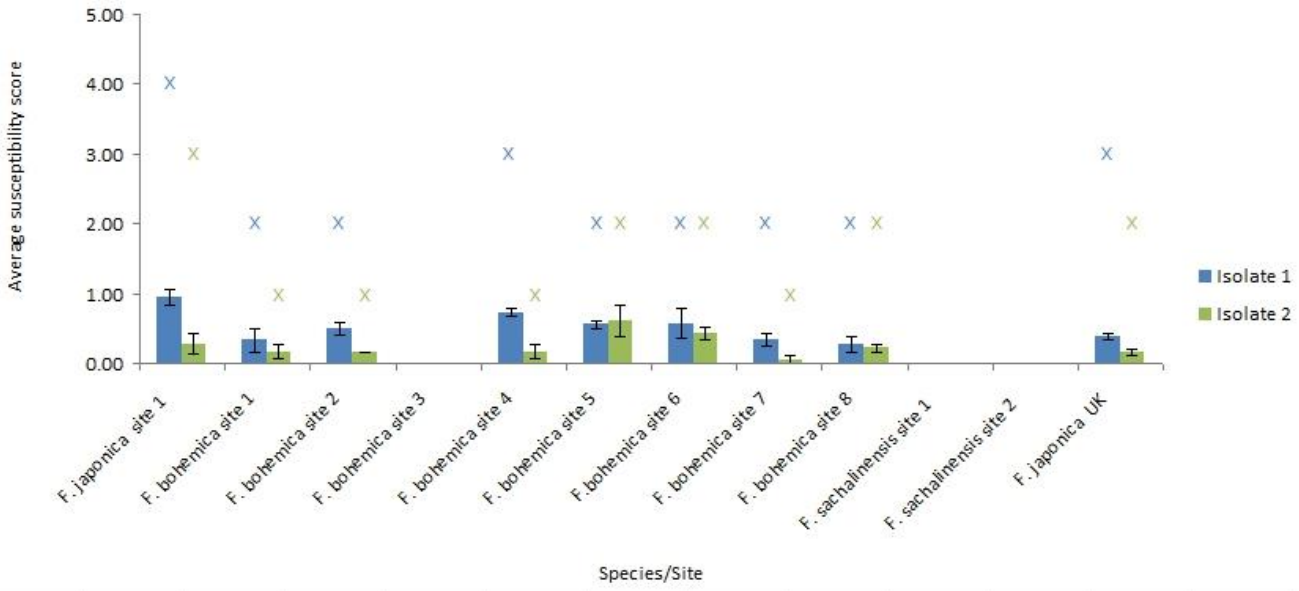


**Figure 17: The susceptibility score of each knotweed species inoculated with each isolate of the leaf-spot pathogen *Mycosphaerella polygoni-cuspidati* isolate ex Mt Hiko. Bars represent the average susceptibility score across all leave ages +/- standard error. Crosses represent the maximum susceptibility score given to each species throughout the experiment for the respective isolate.**

**Table 1: The highest susceptibility score and the number of leaves with that score given for each knotweed species inoculated with both isolates of the leaf-spot pathogen *Mycosphaerella polygoni-cuspidati.* Susceptibility score of 0 = immune to infection by *M. polygoni-cuspidati* whereas 5 = fully susceptible.**

| | Isolate 1 | | | Isolate 2 | | |
|---|---|---|---|---|---|---|
| **Knotweed species** | **Maximum susceptibility score** | **number of leaves with maximum score** | **Leaf age** | **Maximum susceptibility score** | **number of leaves with maximum score** | **Leaf age** |
| *F. japonica site 1* | 4 | 1 | Apical | 3 | 1 | Apical |
| *F. x bohemica* site 1 | 2 | 2 | Mature and fully expanded | 1 | 3 | Mature and fully expanded |
| *F. x bohemica site 2* | 2 | 2 | Fully expanded and apical | 1 | 2 | Mature and apical |
| *F. x bohemica* site 3 | 0 | 0 | / | 0 | 0 | / |
| *F. x bohemica* site 4 | 3 | 2 | Mature and apical | 1 | 3 | Fully expanded and apical |
| *F. x bohemica* site 5 | 2 | 2 | Fully expanded and apical | 2 | 4 | Mature and fully expanded |
| *F. x bohemica* site 6 | 3 | 3 | Mature and fully expanded | 2 | 1 | Mature |
| *F. x bohemica* site 7 | 2 | 1 | Fully expanded | 1 | 1 | Mature |
| *F. x bohemica* site 8 | 2 | 1 | Mature | 2 | 1 | Mature |
| *F. sachalinensis* site 1 | 0 | 0 | / | 0 | 0 | / |
| *F. sachalinensis site 2* | 0 | 0 | / | 0 | 0 | / |
| *F. japonica* ex UK | 3 | 1 | Mature | 2 | 1 | Apical |

## 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 Dutch *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. 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).

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 *bohemica* and *F. sachalinensis*, but none between *F. japonica* and either of the other species. This finding supports the results that *F.* x *bohemica* 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 *bohemica* (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 $cm^2$ 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 *bohemica* 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.

The majority of knotweed species tested in this study were susceptible to both isolates of *M. polygoni-cuspidati* evaluated. However, over the study period a

decrease in susceptibility was noted. Due to work on the psyllid given priority, the majority of the plant material became available only at the beginning of autumn. The apparent lack of susceptibility coincided with seasonal changes from summer to winter months. Over winter, it has been observed that leaves of knotweed become thicker and more "leathery" even in the glasshouse, which may render the plant immune to infection by the leaf-spot pathogen. Similarly, the susceptibility of *F. japonica* ex UK to infection by ascospores (as part of the DEFRA funded project for the UK) also declines during this period. It is believed that the apparent non susceptibility of *F. x bohemica* ex site 3 is likely to be due to these seasonal changes as all *F. x bohemica* tested from the remaining 7 sites were susceptible. The fact that *F. x bohemica* is a hybrid between both *F. japonica* and *F. sachalinensis* would explain why this species is more susceptible to the leaf-spot pathogen than *F. sachalinensis.* However, as testing of all replicates of *F. sachalinensis* was conducted during the winter months it is unknown whether the apparent low susceptibly observed is due to seasonal changes or whether this species is actually immune to *M. polygoni-cuspidati*. Previous testing in the UK gave an indication of lower susceptibility of *F. sachalinensis* to the leaf-spot pathogen compared to both *F. japonica* and *F. x bohemica* (Shaw *et al.,* 2007). During such study *F. sachalinensis* was rated as moderately resistant as microscopic examination revealed only limited colonisation by internal hyphae (Shaw *et al.,* 2007).

## 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.

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. 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.

With regards to the leaf-spot, in order to evaluate the maximum susceptibility of each knotweed it would be recommended to repeat the experiments using fresh rhizome during spring and summer when plants are likely to be most susceptible. Quantitative inoculation studies could be included to compare the virulence of the two isolates of the pathogen and would establish the comparative degree of susceptibility of each knotweed species. In addition to this, distinct strains of *M. polygoni-cuspidati* collected from different locations in Japan could be assessed to determine whether they differ in virulence towards individual knotweed species present in the Netherlands.

## References


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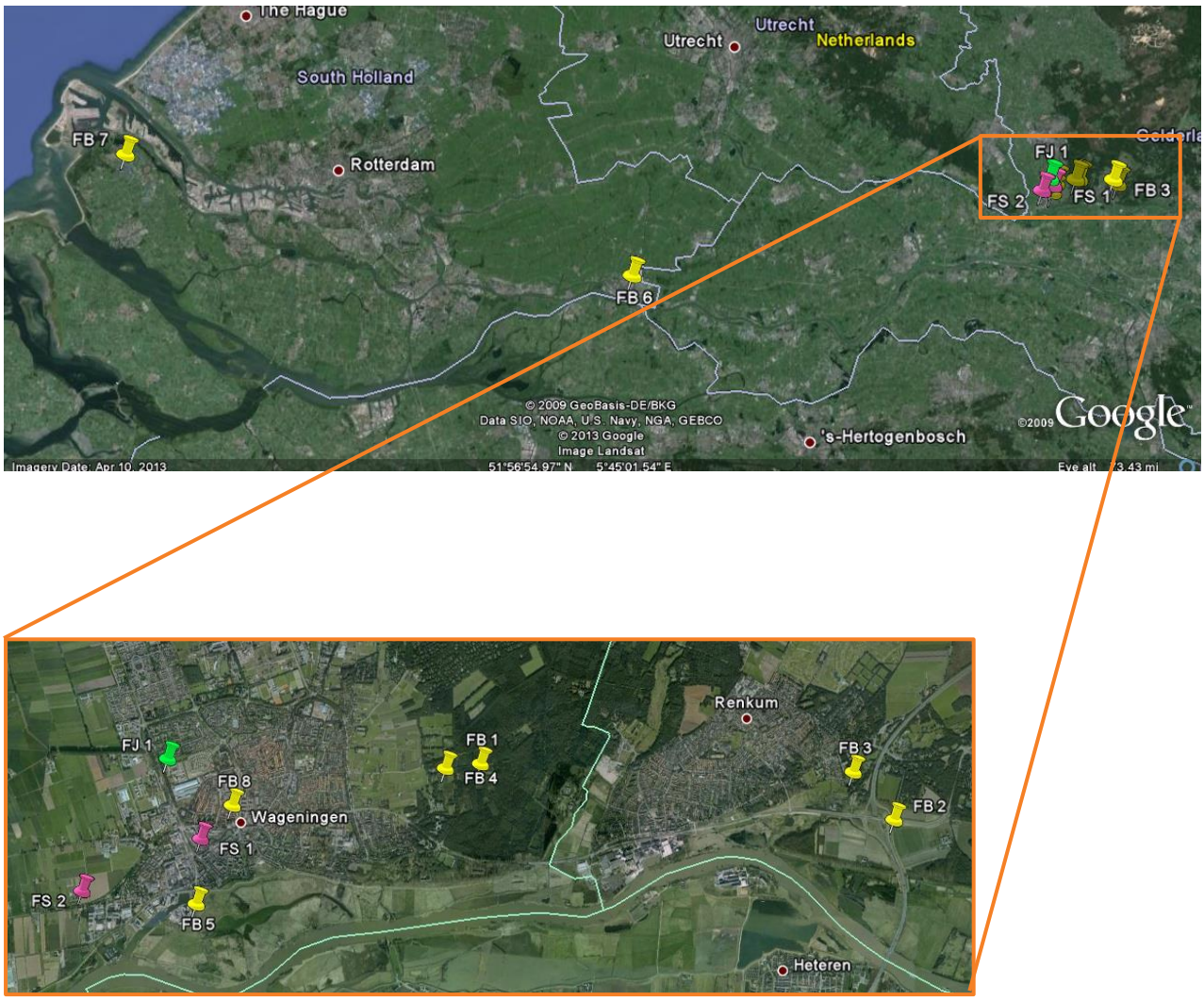
## Appendix 1: Location of Netherlands knotweed rhizome collection sites

A. Locations of the 11 sites from which tested Dutch knotweed rhizome was collected (© 2013 Google). Most collections were concentrated around the City of Wageningen and surroundings, although two sites of *Fallopia* x *bohemica* are located to the south and west of the country. Colours indicate knotweed species (see key).




*Fallopia japonica* *Fallopia sachalinensis* *Fallopia* x *bohemica*

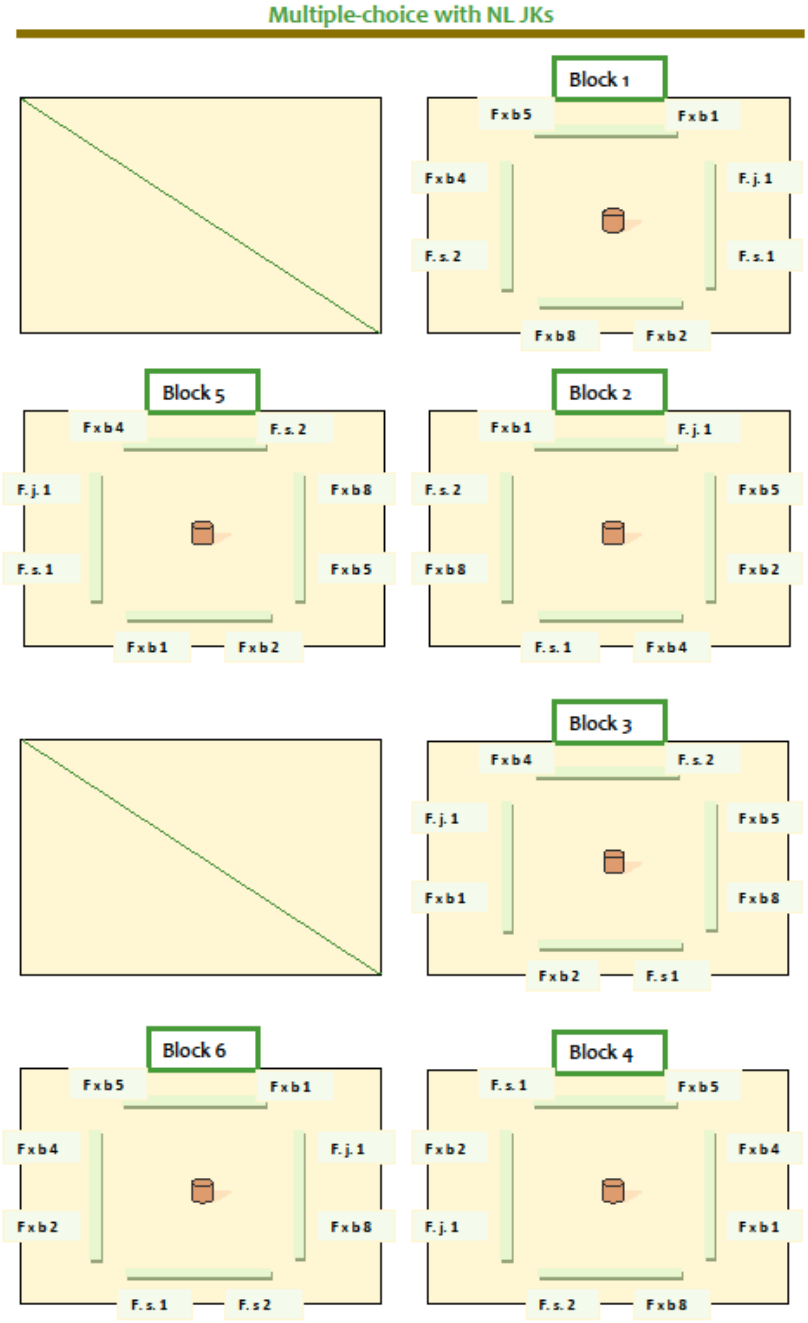
B. Detail of all sites from which rhizome was provided:

| Selected sites: | | |
|---|---|---|
| Species | Site name | Site reference |
| Fallopia japonica | Elstar/Haagsteeg | Site 1 |
| Fallopia sachalinensis | Costerweg/Plantsoen/ Duivendaal | Site 1 |
| Fallopia sachalinensis | Nude/Plant Breeding | Site 2 |
| Fallopia x bohemica | Smalle laan/Forest | Site 1 |
| Fallopia x bohemica | Heelsum/Highway | Site 2 |
| Fallopia x bohemica | Heelsum stream | Site 3 |
| Fallopia x bohemica | Vineyard | Site 4 |
| Fallopia x bohemica | Harbour of Wageningen | Site 5 |
| Fallopia x bohemica | Gorinchem, Spijkse dijk 2 | Site 6 |
| Fallopia x bohemica | Oostvoorne, Ruigendijk 4 | Site 7 |
| Fallopia x bohemica | Lijnbaanstr 1/east side | Site 8 |

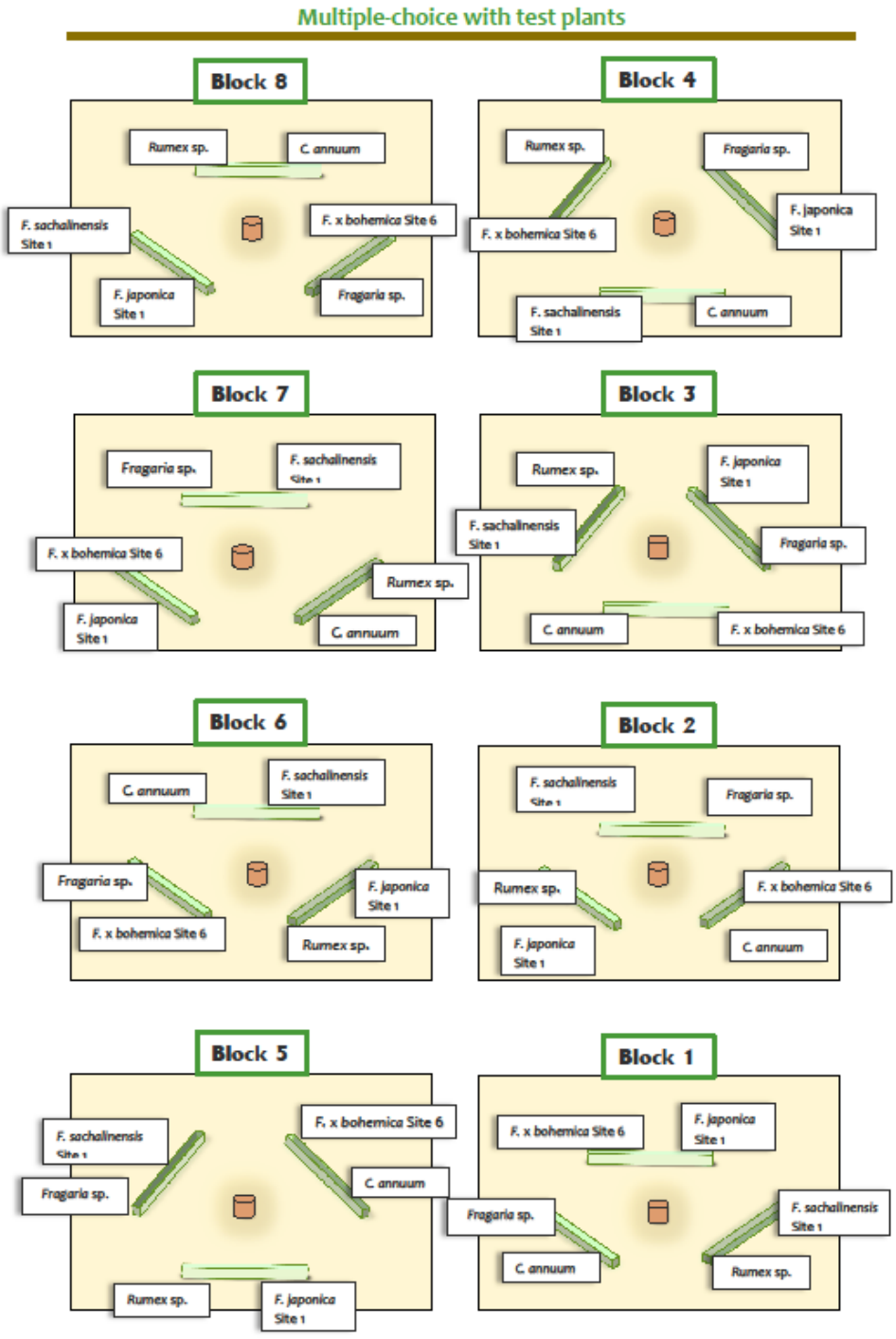
| Rejected sites: | | |
|---|---|---|
| Species | Site name | Reason |
| Fallopia japonica | Geertjesweg/ Scouting/Sport field | Suspected site chemcially sprayed due to plant growth |
| Fallopia x bohemica | Heelsum Keijenbergseweg forest (cycle pad) | Selected sites depending on GPS location and suitability of rhizome |
| Fallopia x bohemica | Diedenweg, Lawickse allee, "de Eng" | Selected sites depending on GPS location and suitability of rhizome |
| Fallopia x bohemica | Berkenrjs oustuoorm | Selected sites depending on GPS location and suitability of rhizome |
| Fallopia x bohemica | Tinte | Selected sites depending on GPS location and suitability of rhizome |
| Fallopia x bohemica | Zaitbommel | Selected sites depending on GPS location and suitability of rhizome |

## Appendix 2: Layout of field cage set up for A) Activity 1A & B) Activity 1C

A. Diagram of the layout of field cages set up for Activity 1A multiple choice. 100 psyllids were released from a central point into each of the 6 replicate field cages containing 8 knotweed test plants (*F. japonica* x 1 site; *F. sachalinensis* x 2 sites; and *F. x bohemica* x 5 sites).



B. Diagram of the layout of field cages set up for Activity 1C. 100 psyllids were released from a central point into each of the 8 replicate field cages containing 3 knotweed and 3 non-target test plants.



## Appendix 3: Results tables for Activity 1 (A-C)

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

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

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

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

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

| Species | Mean leaf area (cm²) | S.E. | Mean eggs per cm² | S.E. |
|---|---|---|---|---|
| *F. japonica* 1 | 978.72 | 0.02 | 0.08 | 0.02 |
| | | | | |
| *F.* x *bohemica* 1 | 1125.61 | 0.03 | 0.07 | 0.03 |
| *F.* x *bohemica* 2 | 1094.00 | 0.05 | 0.15 | 0.05 |
| *F.* x *bohemica* 4 | 924.67 | 0.01 | 0.04 | 0.01 |
| *F.* x *bohemica* 5 | 851.88 | 0.04 | 0.13 | 0.04 |
| *F.* x *bohemica* 8 | 911.92 | 0.05 | 0.17 | 0.05 |
| | | | | |
| *F. sachalinensis* 1 | 1381.05 | 0.01 | 0.03 | 0.01 |
| *F. sachalinensis* 2 | 812.16 | 0.05 | 0.06 | 0.05 |

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. |
|---|---|---|---|
| *Chenopodium bonus-henricus* | 6 | 0 | 0 |
| *Rumex thyrsiflorus* | 6 | 0 | 0 |
| *Limonium vulgare* | 6 | 1 | 0.17 ± 0.17 |
| *Beta vulgaris* ssp. *maritima* | 6 | 1 | 0.17 ± 0.17 |
| *Fragaria* sp. | 6 | 0 | 0 |
| *Capsicum annuum* | 6 | 1 | 0.17 ± 0.17 |
| *Rubus idaeus* | 6 | 0 | 0 |
| *Cucurbita maxima* | 6 | 0 | 0 |
| *Chenopodium quinoa* | 6 | 0 | 0 |
| *Triticum spelta* | 6 | 0 | 0 |
| *F. japonica* ex. UK (control) | 18 | 4035 | 224 ± 36 |

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

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

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

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

Table 8: 1C Field cage multiple choice: Psyllid resting locations at end of oviposition period (14.08.13)

| Block: 8 | | | |
|---|---|---|---|
| NL Test Plants | top | bottom | stem/node |
| *Rumex thyrsiflorus* | 0 | 0 | 0 |
| *Fragaria* | 0 | 0 | 0 |
| *Capsicum annuum* | 0 | 0 | 0 |
| *F. japonica s1* | 1 | 1 | 14 |
| *F. x bohemica s6* | 4 | 2 | 6 |
| *F. sachilensis s1* | 4 | 2 | 6 |

| Block: 4 | | | |
|---|---|---|---|
| NL Test Plants | top | bottom | stem/node |
| *Rumex thyrsiflorus* | 0 | 0 | 0 |
| *Fragaria* | 0 | 0 | 0 |
| *Capsicum annuum* | 0 | 0 | 0 |
| *F. japonica s1* | 2 | 6 | 17 |
| *F. x bohemica s6* | 1 | 0 | 3 |
| *F. sachilensis s1* | 3 | 5 | 6 |

| Block: 7 | | | |
|---|---|---|---|
| NL Test Plants | top | bottom | stem/node |
| *Rumex thyrsiflorus* | 0 | 0 | 0 |
| *Fragaria* | 0 | 0 | 0 |
| *Capsicum annuum* | 0 | 0 | 0 |
| *F. japonica s1* | 3 | 1 | 1 |
| *F. x bohemica s6* | 3 | 0 | 5 |
| *F. sachilensis s1* | 9 | 5 | 16 |

| Block: 3 | | | |
|---|---|---|---|
| NL Test Plants | top | bottom | stem/node |
| *Rumex thyrsiflorus* | 0 | 0 | 0 |
| *Fragaria* | 0 | 0 | 0 |
| *Capsicum annuum* | 0 | 0 | 0 |
| *F. japonica s1* | 3 | 1 | 3 |
| *F. x bohemica s6* | 5 | 5 | 12 |
| *F. sachilensis s1* | 1 | 2 | 6 |

| Block: 6 | | | |
|---|---|---|---|
| NL Test Plants | top | bottom | stem/node |
| *Rumex thyrsiflorus* | 0 | 0 | 0 |
| *Fragaria* | 0 | 0 | 0 |
| *Capsicum annuum* | 1 | 0 | 0 |
| *F. japonica s1* | 4 | 2 | 4 |
| *F. x bohemica s6* | 2 | 2 | 10 |
| *F. sachilensis s1* | 8 | 2 | 2 |

| Block: 2 | | | |
|---|---|---|---|
| NL Test Plants | top | bottom | stem/node |
| *Rumex thyrsiflorus* | 0 | 0 | 0 |
| *Fragaria* | 0 | 0 | 0 |
| *Capsicum annuum* | 0 | 0 | 0 |
| *F. japonica s1* | 2 | 1 | 10 |
| *F. x bohemica s6* | 2 | 1 | 10 |
| *F. sachilensis s1* | 1 | 3 | 0 |

| Block: 5 | | | |
|---|---|---|---|
| NL Test Plants | top | bottom | stem/node |
| *Rumex thyrsiflorus* | 0 | 0 | 0 |
| *Fragaria* | 0 | 0 | 0 |
| *Capsicum annuum* | 0 | 0 | 0 |
| *F. japonica s1* | 5 | 2 | 17 |
| *F. x bohemica s6* | 0 | 1 | 0 |
| *F. sachilensis s1* | 1 | 3 | 1 |

| Block: 1 | | | |
|---|---|---|---|
| NL Test Plants | top | bottom | stem/node |
| *Rumex thyrsiflorus* | 0 | 0 | 0 |
| *Fragaria* | 0 | 0 | 0 |
| *Capsicum annuum* | 0 | 0 | 0 |
| *F. japonica s1* | 2 | 2 | 7 |
| *F. x bohemica s6* | 3 | 1 | 13 |
| *F. sachilensis s1* | 7 | 3 | 5 |

## Appendix 4: Statistical analysis for Activity 1A

Statistical packages: Genstat

Activity 1A Susceptibility Test: Analysis of variance

UK *F. japonica* Batches eggs per $cm^2$

P (0.002) < 0.05

Tests between species in 3 experimental runs:

No significant differences were found between the mean number of eggs per $cm^2$ between species *P* = >0.05 (see below).

Experimental run 1: P(0.079) > 0.05

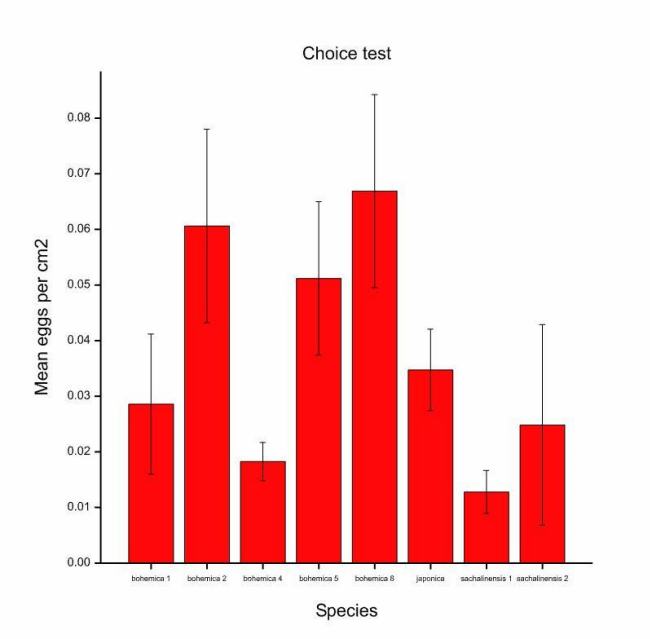
Experimental run 2: P(0.599) > 0.05

Experimental run 3: P(0.058) > 0.05

Activity 1A Multiple choice: Analysis of variance

Significant difference found between *F.* x *bohemica* and *F. sachalinensis* P(0.020) < 0.05

but no significance between *F. japonica* and either *F.* x *bohemica* or *F. sachalinensis*.



## Appendix 5: Susceptibility testing result for Activity 2

**Table. 1**. The average susceptibility score of different knotweed biotypes from the Netherlands towards the leaf-spot pathogen *Mycosphaerella polygoni-cuspidati.* Results are an average of six replicates (apart from *F. sachalinensis* site 1 - 5 replicates and *F. sachalinensis* site 2 – 4 replicates) and a score of 0 = immune and 5 = fully susceptible.

| | | Average susceptibility score | | | | | |
|---|---|---|---|---|---|---|---|
| | | Isolate 1 | | | Isolate 2 | | |
| Fallopia species | Site | O | M | Y | O | M | Y |
| *F. japonica site 1* | 1 | 0.83 | 0.83 | 1.17 | 0.33 | 0.00 | 0.50 |
| *F. x bohemica* site 1 | 1 | 0.50 | 0.50 | 0.00 | 0.33 | 0.14 | 0.00 |
| *F. x bohemica site 2* | 2 | 0.33 | 0.67 | 0.50 | 0.17 | 0.17 | 0.17 |
| *F. x bohemica* site 3 | 3 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *F. x bohemica* site 4 | 4 | 0.67 | 0.67 | 0.83 | 0.00 | 0.33 | 0.17 |
| *F. x bohemica* site 5 | 5 | 0.50 | 0.67 | 0.50 | 0.83 | 0.83 | 0.17 |
| *F. x bohemica* site 6 | 6 | 0.43 | 1.00 | 0.29 | 0.57 | 0.43 | 0.29 |
| *F. x bohemica* site 7 | 7 | 0.33 | 0.50 | 0.17 | 0.17 | 0.00 | 0.00 |
| *F. x bohemica* site 8 | 8 | 0.50 | 0.17 | 0.17 | 0.33 | 0.17 | 0.17 |
| *F. sachalinensis* site 1 | 1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *F. sachalinensis site 2* | 2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *F. japonica UK* | UK | 0.46 | 0.31 | 0.38 | 0.15 | 0.08 | 0.23 |

**Table 2**. The dates and number of replicates (given in brackets) of each individual test for each knotweed variety against the leaf-spot pathogen *Mycosphaerella polygoni-cuspidati.*

| ***Fallopia* species** | **Dates tested and number of replicates ()** |
|---|---|
| *F. japonica site 1* | 24/07/2013 (2), 16/9/13 (1), 30/9/13 (3) |
| *F. x bohemica* site 1 | 12/08/2013 (1), 16/9/13 (2), 30/9/13 (3) |
| *F. x bohemica site 2* | 12/8/13 (3), 23/9/13 (3) |
| *F. x bohemica* site 3 | 23/9/13 (3), 7/10/13 (3) |
| *F. x bohemica* site 4 | 24/07/2013 (2) 23/9/13 (1), 30/9/13 (2), 25/10/13 (1) |
| *F. x bohemica* site 5 | 12/8/13 (3), 30/9/13 (3) |
| *F. x bohemica* site 6 | 16/9/13 (3) 25/11/13 (3) |
| *F. x bohemica* site 7 | 16/9/13 (3), 7/10/13 (3) |
| *F. x bohemica* site 8 | 16/9/13 (2) 23/9/13 (1), 7/10/13 (3) |
| *F. sachalinensis* site 1 | 23/9/13 (2), 25/10/13 (1) 25/11/13 (2) |
| *F. sachalinensis site 2* | 23/9/13 (1), 25/10/13 (2), 25/11/13 (1) |

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