Background document:
2017 protocol for the release of the rust *Puccinia komarovii* var. *glanduliferae* a biological control agent for *Impatiens glandulifera* in the UK

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Key Points

- CABI is to be supplied with Himalayan balsam seeds (~30, mature, black, dried) from each release site, the autumn before the release of the rust, to test for rust susceptibility. Seedlings can be sent in the spring of rust release year if necessary.
- Rust release site selection criteria:
  - sites that are stable i.e. unlikely to be washed away in the winter;
  - have a high natural humidity i.e. near a watercourse;
  - not liable to be submerged by water for extended periods during the winter;
  - have a minimum of ~10m^2 of dense, healthy, actively growing populations of Himalayan balsam
  - open sites or with low shade from trees/shrubs.
- Grid references of sites and permission letter from landowner to release rust.
- Rust spores will be applied on 3 separate occasions during the Himalayan balsam growing season; June, July and August.
- Training will be given by CABI during the first inoculation in June. Equipment, additional rust spores (to be stored in freezer) and information sheet will be supplied.
- Inoculation should be shortly after rain and in the evening (when ambient temperature is dropping).
- Spores are suspended in bottled water + spore dispersant and sprayed from below onto the lower surface of leaves in the top 50cm of plants.
- The inoculated plants should be observed after 4 weeks for the development of brown rust pustules on lower leaf surface.
- Plants surrounding the inoculated area should also be regularly checked for rust spread and approximate distance recorded.
- Continue normal management techniques outside of the 10m^2 experimental zone.
- Check seedlings the following spring for stem infection below seed leaves when first true leaves are starting to develop.
- Feedback results to CABI Himalayan balsam team.

Introduction and background

Himalayan balsam (*Impatiens glandulifera,* Ericales: Balsaminaceae), is an annual weed introduced into the UK, mainland Europe and North America from its native range, the Western Himalayas (India and Pakistan) (Tanner *et al.,* 2014a; Cockel and Tanner 2011). Himalayan balsam was introduced into the UK in 1839 and is now the most commonly occurring non-native plant species on riparian systems, occupying over 13% of rivers in England and Wales (Environment Agency, 2010). This species has an adverse impact on native biodiversity (Tanner *et al.,* 2013; Hulme and Bremner, 2006), ecosystem services (Chittka and Schürkens, 2001), and in Canada, recent research has highlighted the negative impact of the weed on pollination of native plants (Beans and Roach, 2015). A study undertaken in Switzerland also demonstrated that riverbanks, laid bare by the annual weed after it dies back in the winter, renders them more prone to erosion (Greenwood and Kuhn, 2014). The negative environmental impact of this species, together with the high financial costs of control using traditional chemical and mechanical methods (Williamson *et al.,* 2010), resulted in the UK Government asking CABI to investigate the potential of classical biological control (CBC) of Himalayan balsam in the UK (Tanner *et al.,* 2014).

The rust fungus *Puccinia komarovii* Tranz was first identified on *I. glandulifera* in the Indian Himalayas in 2010. *P. komarovii* is known to be a macrocyclic, autoecious (all five spore stages on a single host plant: aeciospores, urediniospores, teliospores, basidiospores and spermatia, see Appendix), host specific, rust fungus, infecting *Impatiens parviflora* DC in both its native (Central Asia) and exotic range (mainland Europe). The presence of *P. komarovii* in the UK on *Impatiens parviflora* was recently confirmed. Cross inoculation studies highlighted species-specific varieties of *P. komarovii*: the rust variety that infects *I. parviflora* (but not *I. glandulifera*) was named *P. komarovii,* and the rust collected on *I. glandulifera* in the Indian Himalayas was subsequently renamed *Puccinia komarovii* var. *glanduliferae* RA Tanner, CA Ellison, HC Evans & L Kiss (Tanner *et al.,* 2014b).

A comprehensive, 4-year research programme was undertaken to assess the safety and suitability of *P. komarovii* var. *glanduliferae* for release in the UK as a CBC agent of *I. glandulifera.* The rust was tested against 74 plant species to ensure its specificity to Himalayan balsam. A Pest Risk Assessment (PRA) which fully detailed the research conducted on the host range, life cycle and ecology of the rust was submitted for official assessment by the Food and Environment Research Agency (FERA) (a Defra Agency) in 2014. The PRA also underwent public consultation, and further evaluation by the European Commission's Standing Committee on Plant Health. Following their feedback, Defra Ministers approved the release of the rust into the wild in England on the 27th July 2014 (Tanner *et al.,* 2015).

On-going work can be found on the following web site, which is regularly updated:
www.himalayanbalsam.cabi.org

A factsheet on the Himalayan balsam rust is provided in the Appendix.

Release strategy employed in 2014 - 2016

After consultation with the Pesticide Safety Directorate, FERA and Natural England, it was agreed that the rust would be released as an infection of its host plant, Himalayan balsam. This required a license from Natural England, to plant a prohibited species in the wild (under Section 14, Schedule 9 of the Wildlife and Countryside Act in England and Wales). In order to obtain this NE license (2015-10527-SPM-NNR-1) the grid reference, map, land use designation and a letter of permission from the land owner of each site was required, and the information collated by CABI. The rust itself, however, was released from the terms of the plant health license (51073/199418/2) that it has been held under in quarantine at CABI, and hence no additional license is required for its release into the wild.

The rust release sites were selected where populations of the weed were not growing too close to river, so unlikely to be washed away in the winter, but with high natural humidity i.e. near a ditch or pond. Large dense, actively growing populations of the weed were sought.

The Himalayan balsam plants were grown outside in 6" diameter, plastic, plant pots, in order to produce field-hardy plants, which were considered more likely to cope with being planted in the field once infected with the rust. Plants were inoculated with the rust at CABI, at the 3-4 whorl stage (10-13 true leaves). Once chlorosis/young pustules were evident, but prior to full eruption of the pustules and release of the spores, plants were taken to the field sites. This is approximately 14-21 days after inoculation. Each release site received 6-10 rust infected plants, each with a minimum of 6 rust infected leaves and each leaf with a minimum of 100 pustules. Since each newly mature rust pustule contains a mean of 100 spores, the initial inoculum dose per site is up to 6 $x10^5$ spores. Of these spores >85% have the potential to germinate and infect when they land on suitable susceptible tissue, with free water available for a minimum of 8 hours (optimum 15 hours), at an optimum temperature of 15^oC (but with the ability to infect from <4^oC to >25^oC).

The rust source plants were place in dense stands of Himalayan balsam that were between 0.5-1.5 meters in height (3-5 leaf whorl stage), between May and July. They were removed from their pots and planted in the ground in a group, to form a concentrated source of infection, but were not obvious to the casual passer-by, when the site was on public land. Infection on field plants was visible, from 3 weeks after release. In drier soils, it was important to water the rust source plants during the first few weeks while spores are being released from the pustules on the leaves, if there was no rain.

In 2014 the rust was released at three sites in the south of England, and although this was undertaken late in the growing season, the rust was found to spread on to field plants at one of these sites. In 2015 the rust was released at 25 sites: Berkshire (2 sites), Middlesex (1 site), Kent (1 site), Gloucester (1 site), Cornwall (6 sites), West Yorkshire (4 sites), North Yorkshire (2 sites), Northumberland (4 sites), Swansea (2 sites), Carmarthen (1 site) and Ceredigion (1 site). Again, the rust was observed to spread onto field plants at more than half of the sites, but at a low density (few, small pustules) at most of these sites and spread was only a short distance from the place of rust release (1-5 metres).

The results from the 2015 releases showed that it was important not to release the rust too early in the season, as production of the overwintering spore stage (teliospore) was triggered when night temperature dropped regularly below around 10^oC. The cycling, spore stage (urediniospore) spreads the rust between plants and to new weed populations, causing significant damage to the leaves of the plants. The 2016 rust releases were started in mid-June, rather than late May when the initial 2015 releases where made.

The benefit of the approved method of rust release (planting rust infected plants in the field) is that spores are produced on the living plants over a number of weeks, and this increases the opportunity for natural conditions to be favourable for infection of field plants. The down side is that the field plants are challenged with low number of spores and hence the opportunity for a significant build-up of the urediniospores in the weed population is restricted and plant damage and rust overwintering is limited.

Variation in plant susceptibility to the rust

The results of the field releases in 2015 revealed that there was far more genetic diversity in the Himalayan balsam population in the UK than realised. Indeed, a recent publication by Nagy & Korpelainen (2015), suggests multiple introductions of Himalayan balsam in to the UK from different parts of its native range. Not all populations of the plant were found to be susceptible to the strain of the rust that was released from India. The rust is highly selective, and tends to infect genetic types of the plant that originated from the area in the native range where the rust evolved. Fortunately, CABI has a number of rust isolates from different areas in the native range. An on-going molecular study by CABI is currently trying to identify how many biotypes of the weed there are in the UK and from where they originated.

This means that it is critical to check which rust isolate is most suited to release in a particular area, currently CABI has two strains of the rust ready for testing from India and Pakistan.

Protocol for 2017 rust releases

Aims

To achieve establishment of Himalayan balsam rust (*Puccinia komarovii* var. *glanduliferae*) in selected populations of the weed in the UK and reach a level of infection that triggers a rust epiphytotic. This will enable significant damage to occur on the current year's population of the weed, reducing plant seed production and potentially weed density in the following years. Equally important is that high level of spore production will result in high levels of overwintering spore development late in the season (teliospores). These spores remain dormant in the soil leaf litter, germinating in the spring to infect seedlings and enable the rust to complete its lifecycle, and cause a significant reduction in seedling survival.

Testing of plant susceptibility to the rust

Prior to the release of the rust at a site, it is necessary for CABI to determine the susceptibility of the plants to the rust, and establish which of the 2 rust strains is the most pathogenic. Seeds (approximately 30 per site) from each potential rust release need to be sent to CABI, the preceding autumn. If the opportunity to send seed is missed, then recently germinated seedlings can be sent in early spring in the year of release. Seeds need to be collected when mature (i.e. black), air dried for a few days until dark brown, put in an envelope, sent in ridged packaging (e.g. cardboard) and labelled with site details, including Grid Reference.

Site selection

Locate a suitable site for rust release; selection criteria are given below:

- sites that are stable i.e. unlikely to be washed away in the winter;
- have a high natural humidity i.e. near a watercourse;
- not liable to be submerged by water for extended periods during the winter
- have a minimum of ~10m^2 of dense, healthy, actively growing populations of Himalayan balsam;
- open sites or with low shade from trees/shrubs.

Identify and mark with canes or ribbons one (or more) sub-populations of Himalayan balsam, situated within the larger populations, each approximately (3-5m^2) for inoculation with rust. Continue normal Himalayan balsam management techniques outside of the 10m^2 experimental zone.

Rust application

In 2016, a successful application was made to the Pesticide Safety Directorate for an 'Administrative Trial Permit' (ATP) to allow for the application of the rust spores (diluted in water or Talc) to Himalayan balsam plants. A new application methodology has been developed using water, which can be implemented by CABI collaborators, following training. This provides more opportunity to spread the rust in the UK. Rust pathogens are biotrophs, which means that the fungus can only grow and reproduce on its host plant, and so spores cannot be produced in culture. All the spores that will be applied to the Himalayan balsam plants must be harvested from infected plants and stored in a freezer until use. This has been on-going during the winter months, so spores can be applied to young plants at sites around the UK.

Rust spores will be applied on 3 separate occasions during Himalayan balsam growing season: June (when night temperatures are reliability above 10^{o}C), July and August/early September (when night temperatures start to decrease). The same inoculation site can be used each time if no infection is observed or a new site selected for each inoculation. Spore need to be stored in the freezer for July and August inoculations, removed from freezer just before use and take to field site in cool bag with ice blocks.

The spores are first dispersed in water, containing a spore dispersant (surfactant). The microscopic spores are spiny and clump together, so a surfactant (e.g. Tween 80, Polyoxyethylene (20) sorbitan monooleate) is needed to aid dispersal in the water. The suspension is applied using the hand-held spray applicator. Rust spores will be suspended in 250 millilitres of water +Tween 80 (0.05% v/v) at a spore concentration of $1x10^4$ spores ml^{-1}. This will be sufficient to cover approximately 3-5m^2 of Himalayan balsam monoculture, depending on the size of the plants.

The spores need to be in a film of water for a minimum of 8 hours on the lower leaf surface, in order to be able to infect the plant. These conditions are more likely to occur during the night (e.g. dew period) and after rain, when the humidity is high. Therefore apply the spores shortly after rain and in the evening, or late afternoon on a cloudy day, (when ambient temperature is dropping).

The following will be supplied by CABI:

- Full instruction leaflet on application and monitoring of rust
- Hand-held spray applicator
- Surfactant (three doses of Tween 80 in vials) to help spore distribution in water
- Personal protective equipment (PPE); gloves, face mask and goggles – (Please note there is no risk to applicator, PPE is one of the ATP licence requirement, to eliminate risk from spraying in eyes etc.)
- Rust urediniospores in 3 vials, 2 vials to be stored at -20°C (freezer) for July and August applications.
- Data logger for recording temperature and humidity in the rust release plots during the growing season

Rust application and monitoring protocol

- Choose a day when it has recently rained (during the day or night before heavily). Two people will make the job easier.
- Add approximately 250ml of bottled water to the spray bottle and carefully tip one tube of the surfactant into the water. Shake for 10 seconds (frothing will be seen).
- Go to field plot late in the afternoon on an overcast day (or in the evening if sunny) taking the spray bottle containing water + surfactant, one vial of rust spores (in the cool bag with ice blocks) and PPE.
- Mark out the plot where the spores will be applied using the canes supplied, or your preferred method of marking.
- Put on PPE
- Just before spraying, very carefully tip the one vial of the brown rust spores into the water + surfactant. Shake for a few seconds in order to disperse the spores. The water will appear slightly brown. Rinse the spore tube with a few sprays from the spray bottle and tip back into the bottle (since some spores will stick to the tube).

- The rust infects leaves from the lower leaf surface, therefore, you need to spray upwards into the canopy. Cover the lower leaf surfaces so you can see a film of water. After a few sprays, give the suspension a little shake to keep the spores evenly dispersed. The nodes of leaves towards the top of the plants (still maturing) are the most susceptible to infection by the rust.
- Start spraying the lower leaves of the plants, leaning into the centre of the plot taking care not to damage the plants. Once the lower leaves are coated with spores, work up the plants in the plot, starting in the centre again. Try to keep the spray bottle as up right as possible, particularly once a significant amount of the spray has been applied (hence why it is recommended to start spraying the lower leaves first) to ensure the tube remains in in the solution. It will take about 15 minutes of spraying to empty the bottle.
- Rust pustules should be visible erupting on the lower leaf surface about 4 weeks after inoculation. During the summer these will mainly look dark brown (urediniospores), but as autumn arrives and the night temperatures decrease, they will look mainly black (over-wintering teliospores).
- Check plants in the non-inoculated parts of the 10m^2 experimental zone regularly (every 2 weeks) to look for rust spread. If infection is observed, check for infection in nearby populations of Himalayan balsam.
- Once the plants have died back in the autumn, please retrieve the data logger and send to CABI for down loading of the data.
- Check seedlings the following spring for stem (hypocotyl) infection below the large round seed leaves (which also can be infected), when first true leaves are starting to develop.
- Please keep CABI informed of the progress of the work and rust spread; please take and send regular images.
- If rust infection is good; many (>10), large (>1mm) pustules on lots of leaves (>10 leaves of more than half the plants inoculated), then a recording sheet and protocol can be requested from CABI, so the spread of the rust infection can be recorded for analysis.

Rust release sites

Table 1 gives details of all the sites in England and Wales where the rust was released in 2014-16 and where releases are planned for 2017.

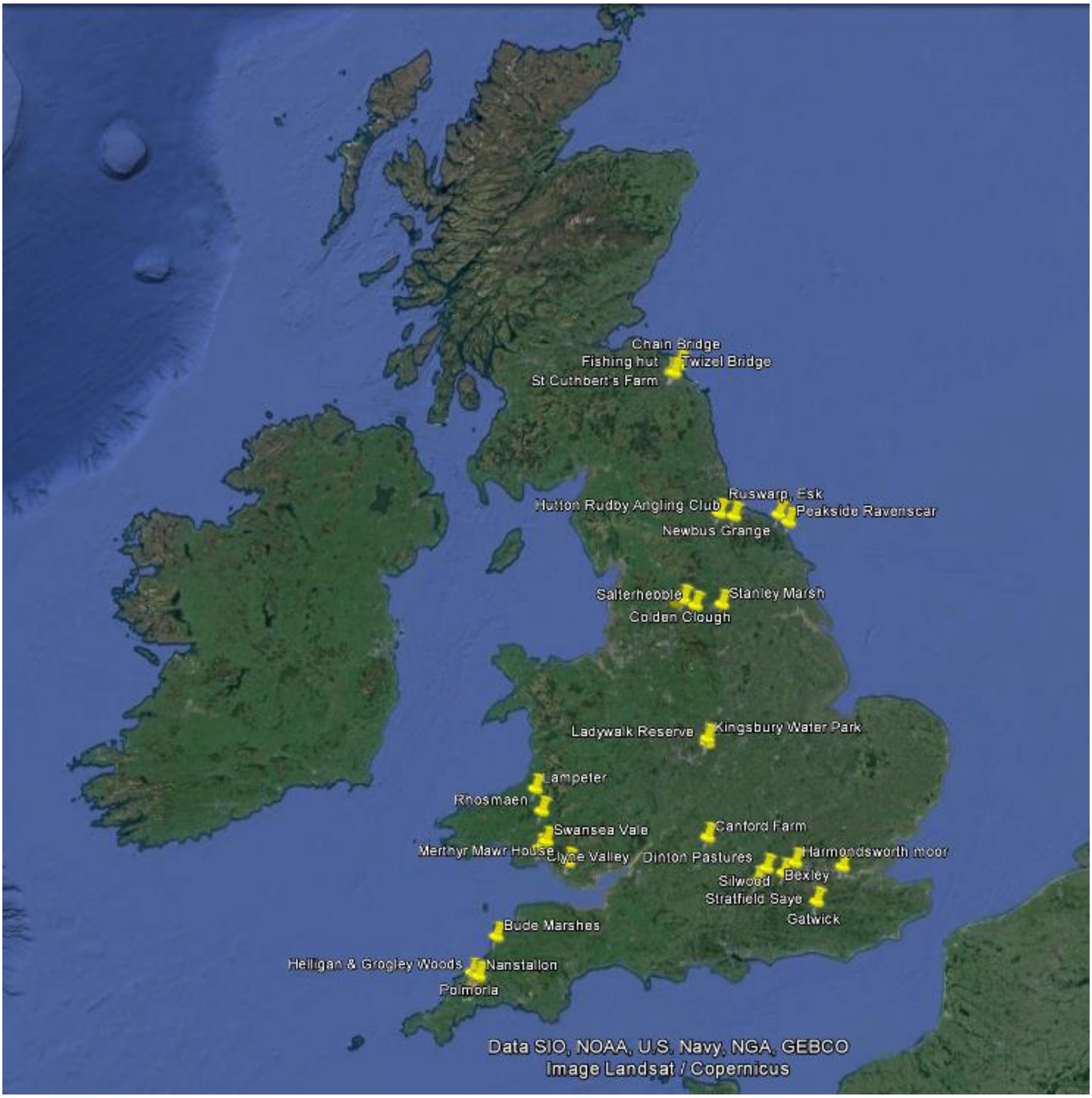
Table 1. Rust release sites England and Wales 2014-2017

Site no.	Site name	Site address	Rust releases (Isolate used[1])
1	Colden Clough	Hebden Bridge, W. Yorkshire	2015 (I), 2017 (P)
2	Gorpley Clough	Todmorden, W. Yorkshire	2015 (I), 2017(I)
3	Salterhebble	Halifax, W. Yorkshire	2015 (I), 2017(I)
4	Stanley Marsh	Wakefield, W. Yorkshire	2015 (I), 2017(I&P)
5	Chain Bridge	Horncliffe (1), Berwick-upon-Tweed, Northumberland	2015 (I), 2017(T)
6	Anglers Bothy	Horncliffe (2), Berwick-upon-Tweed, Northumberland (B)	2015 (I), 2017(T)
7	Gatwick Airport	Crawley, West Sussex	2016 (I), 2017(I)
8	St Cuthbert's farm	Uppsettlington (2), Berwick-upon-Tweed, Northumberland	2015 (I), 2017(T)
9	Twizel Bridge	Uppsettlington (3), Berwick-upon-Tweed,	2015 (I), 2017(I)

		Northumberland	
10	Hall Place Recreation Ground	London Borough of Bexley, London/Kent	2015, 2016 (I); 2017(T)
11	Sunningdale Recreation Ground	Sunningdale Parish Council, Berkshire	2014, 2015, 2016 (I)
12	Silwood Park	Imperial College, Silwood Park, Berkshire	2014, 2015, 2016 (I)
13	Harmondsworth Moor	BA Waterside, Harmondsworth, Middlesex	2015, 2016 (I); 2017(P)
14	C Mill	St Austell, Cornwall	2014, 2015, 2016 (I); 2017(T)
15	C Farm	Gloucestershire	2015 (I), 2017(T)
16	Nantstallon	Boscarne Mill, Cornwall	2015 (I), 2017(T)
17	Polmorla	Wadebridge, Cornwall	2015 (I), 2017(T)
18	Longmeadows	Lanivet, Cornwall	2015 (I), 2017(P)
19	Helligan & Grogley Wood 2	Bodmin, Cornwall	2015 (I), 2017(T)
20	Ruswarp, Esk	Whitby, North Yorkshire	2015 (I), 2017(T)
21	Peakside Ravenscar	Whitby, North Yorkshire	2015 (I), 2017(T)
22	Bude Marshes	Bude, Cornwall	2015 (I), 2017(T)
23	Stratfield Saye	Stratfield Turgis, Hampshire	2016 (I), 2017(T)
24	Swansea Vale Nature Reserve	Swansea Vale, Swansea	2015, 2016 (I), 2017(T)
25	Clyne Valley Country Park	Clyne Valley, Swansea	2015, 2016 (I),
27	Lampeter (River Dulas)	Lampeter, Ceredigion	2015, 2016, 2017 (I)
28	Rhosmaen (River Dulais)	Rhosmaen, Ceredigion	2015, 2016 (I), 2017(P)
29	River Leven	Hutton Rudby, North Yorkshire	2017 (T)
30	Ladywalk Reserve	Tamworth, Staffordshire	2017(T)
31	Kingsbury Water Park	Tamworth, Staffordshire	2017(T)
32	River Ogmore.	Merthyr Mawr, Bridgend, Mid Glamorgan	2017(T)
33	Merthyr Mawr	Candleston Castle, Merthyr Mawr, Bridgend, Mid Glamorgan	2017 (T)

[1] I – Indian; P – Pakistani; T – still to be tested; X – No release planned for 2017

MAP 1. Rust release sites England and Wales 2014-2017



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Appendix.

Himalayan balsam biological control factsheet

Introduction

Himalayan balsam (*Impatiens glandulifera*) is native to the foothills of the Pakistani and Indian Himalayas, where it grows in mixed population of native plants and does not dominate its habitat. It was introduced into the UK as a garden ornamental in 1839, and is now extremely widespread and invasive in the UK and throughout Europe. In its introduced range, Himalayan balsam rapidly forms dense monocultures along river systems (see below), increasing flood risk and outcompeting native species. It produces large numbers of seed (100's per plant) which are dispersed up to 5 metres from the parent plant, by their explosive seed pods. These seeds are easily spread by humans and can also travel through watercourses.

Effective control of this plant is expensive, and can be environmentally damaging, when conventional methods such as herbicides and manual removal are used. Hence, a new approach, classical biological control, which employs the natural enemies of the plants from its native range, was investigated by CABI. This approach has an excellent track record in countries around the globe, when strict scientific procedures are followed.

Biological control

Surveys in the native range revealed a number of natural enemies controlling the plant, and a highly damaging rust pathogen, *Puccinia komarovii* var. *glanduliferae,* was selected for comprehensive assessment of its safety and potential as a biological control agent for Himalayan balsam in the UK (4 years of research). The rust was tested against 74 plant species to ensure its specificity to Himalayan balsam. A Pest Risk Assessment (PRA) which fully detailed the research conducted on the host range, life cycle and ecology of the rust was submitted for official assessment by the Food and Environment Research Agency (FERA) (an Agency of the Department for Environment, Food and Rural Affairs [Defra]) in 2014. The PRA also underwent public consultation, and further evaluation by the European Commission's Standing Committee on Plant Health. Following their feedback, Defra Ministers approved the release of the rust into the wild in England on the 27th July 2014.

The aim of biological control is to reduce the growth and seed production of Himalayan balsam, so it is unable to compete so well with the native plants, or spread so effectively. Thus, Himalayan balsam will not be eradicated, but it is hoped that the rust infection will mean it no longer dominates the habitats where it grows, and thus will allow our native plants to flourish.

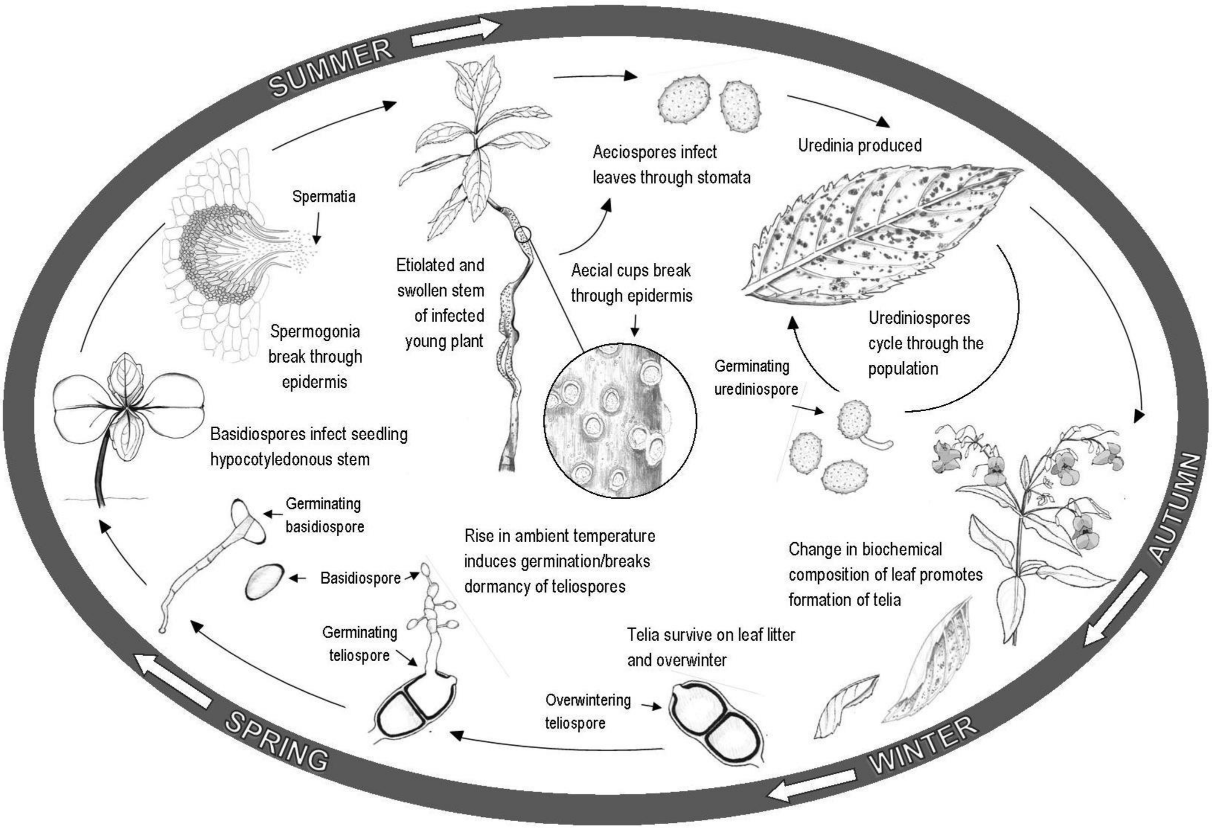
Rust release programme 2014-2016

A nationwide rust release and monitoring programme is currently underway. Initial results look promising: the rust is able to complete its life cycle under UK conditions and is spreading on plants in the field. In 2015 the rust will be released at 25 sites: Berkshire (2 sites), Middlesex (1site), Kent (1 site), Gloucester (1 site), Cornwall (6 sites), West Yorkshire (4 sites), North Yorkshire (2 sites), Northumberland (4 sites), Swansea (2 sites) and Ceredigion (2 sites).

Rust release site at Harmondsworth moor, Middlesex.
Infected plants (within oval) have been planted in a naturally occurring dense stand of Himalayan balsam, in boggy ground and under dappled shade; good conditions for the rust to establish.

Rust life cycle

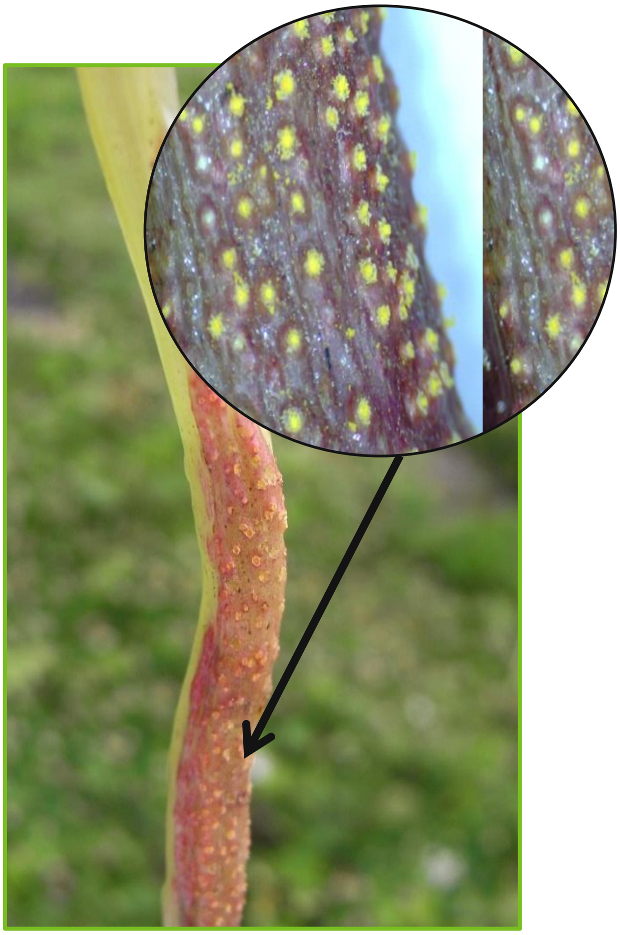
This rust pathogen has a very complicated life cycle, with 5 different spore stages!



What to look for in the field

Spring

The first visible signs of rust infection can be seen during spring (**March – May**) when the stems of small Himalayan balsam seedlings are targeted. Small yellow /orange cups, called aecia, erupt from the surface of the stem below the first leaves (the hypocotyl), causing the stem to elongate, bend and become distorted. The area of infection may also become red. Stems of infected plants are usually longer than seedlings that are not infected.



Symptoms seen on the stems of Himalayan balsam seedlings during spring
Left: bending and distortion of the stem
Right: yellow/orange cups erupting from the stem

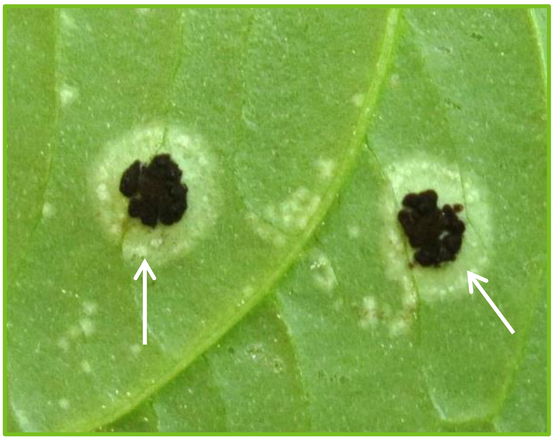
Summer

The most noticeable symptoms can be observed from the end of spring and throughout the summer (**May – September**). During this time, small yellow spots or whitening (known as chlorosis) on the upper leaf surface develops. About a week later, on the lower leaf surface, but associated with these yellow spots, small light brown pustules (uredinia) form. This process repeats itself so patches of chlorosis and the formation of these brown pustules on the leaves will continue throughout the summer. At the beginning of the season, initial signs of infection may be low with only a few pustules forming. However, these spores are readily dispersed by the wind enabling the infection to spread causing symptoms to become more prevalent.

Symptoms seen on infected leaves of Himalayan balsam during the summer months
Left: yellow/white lesions, chlorosis, seen on the upper leaf surface
Right: light brown pustules, uredinia on the lower leaf surface

Autumn

Towards the end of the summer (**August – September**) the light brown pustules on the lower leaf surface, become darker in colour and form dark brown/black pustules (telia). This stage represents the over-wintering stage. To begin with, both the light brown and dark brown pustules may be seen together on the same leaf, and are difficult to tell apart with the naked eye. Infected leaves will naturally fall to the ground where they will remain along with the biocontrol agent over winter and infect the stem again the next spring.



Symptoms seen on infected leaves of Himalayan balsam towards the end of summer/ autumn following a drop in temperature
Left: dark brown/black pustules, telia, on the lower leaf surface
Right: close-up of telia, 'satellite' teliospores (arrows) are starting to develop around the primary telia

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