WEED MANAGEMENT FOR HIMALAYAN BALSAM

IMPLEMENTING A NOVEL WEED MANAGEMENT APPROACH FOR HIMALAYAN BALSAM: PROGRESS ON BIOLOGICAL CONTROL IN THE UK

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Introduction
Himalayan balsam (Impatiens glandulifera) is an annual plant native to the foothills of the Himalayas in India and Pakistan. Since its introduction to the UK as an ornamental plant in 1839, it has gradually spread throughout the country, and parts of Europe. In its invasive range, it inhabits riverine habitats, damp woodlands and waste lands, growing in dense monocultures, outcompeting native plant species (Hulme & Bremner, 2006) and altering the invertebrate community (Tanner et al., 2013). As Europe’s tallest annual plant species, each plant can produce up to 2500 seeds that can be ejected up to 7m away from the parent plant. As the plant dies back in the autumn, there is an increased risk of erosion and dead plant material entering the water course can lead to flooding. Control methods are limited due to its close proximity to water; chemical control is difficult and plants can be inaccessible. Manual removal is common but relies on volunteers; it is time-consuming and control work is required on a catchment scale to prevent recolonisation from upstream. Therefore, since 2006 a consortium of sponsors has funded research into the biological control of this weed. This method considers the introduction of damaging insects, mites or fungal pathogens that have co-evolved with their host plant in their native range. The natural enemies that are selected for risk assessment and potential release have a very limited number of plant species on which they can survive, often only on a single genus or species.

Surveys to look for suitable natural enemies were undertaken from 2006–2010 in the foothills of the Himalayas in Western India and Pakistan throughout the growing season of the plant; and a range of insects and fungal pathogens were found to cause significant damage to I. glandulifera (Tanner et al., 2008). However, the insects were found to have too broad a host-range; being able to feed and complete their life cycle on a number of Impatiens species. A rust fungus Puccinia komarovii var. glanduliferae was observed to infect Himalayan balsam throughout the areas surveyed, causing significant damage to infected plants both at the seedling stage (stem infection, usually leading to plant death) and leaves of maturing plants and, hence, was prioritised for further study. This article discusses the research conducted to date on an isolate of the rust fungus collected in the Kullu Valley, Himachal Pradesh, India (IMI 398718), as a biological control agent for Himalayan balsam, and summarises the results of the first releases of the rust in the UK from 2014–2016 (https://himalayanbalsam.cabi.org).

Renaming the rust
Puccinia komarovii is a macrocyclic (all five spores stages are known: aciiospores, urediniospores, teliospores, basidiospores and spermatia), autoecious (can complete its life cycle on one plant species), rust fungus that was first recorded infecting Impatiens parviflora DC (Piskorz & Klimko, 2006; Coombe, 1956; Sydow & Sydow, 1904). Since this first identification, it has been recorded from seven species of Impatiens, but evidence from field observations, suggested that pathotypes of P. komarovii, specific to different species of Impatiens, may exist. Cross-inoculation studies undertaken at CABI, confirmed this to be the case; the pathotype which infects I. parviflora (but not I. glandulifera) has retained the name P. komarovii and the rust collected on I. glandulifera in the Himalayas, was found not to infect I. parviflora, and was subsequently renamed as a variety, Puccinia komarovii var. glanduliferae RA Tanner, CA Ellison, HC Evans & L Kiss (Tanner et al., 2014).

Host range testing and the test plant list
To assess the host specificity of the rust, native, ornamental and economically important plant species of relevance to Europe were selected and tested for susceptibility in replicated experiments using I. glandulifera as the control. Plant species were selected based on the phylogenetic, centrifugal system, developed by Wasphe (1974). Himalayan balsam belongs to the order Ericales and family Balsaminaceae which consists of just two genera, Impatiens and Hydrocera. The test plant list, drawn up in consultation with experts from the Department...
for Environment Food and Rural Affairs (DEFRA) and the Natural History Museum, London, consisted of 86 entries, 75 species and a further ten varieties. *Impatiens* is horticulturally important and includes popular bedding species such as *I. walleriana* (Busy Lizzie) and *I. hawkeri* (New Guinea hybrid); consequently 31 species on the test plant list were *Impatiens* species.

Of the 75 plant species screened by inoculating with urediniospores, only one non-target species, *I. balsamina*, a non-native ornamental species with low commercial value, was fully susceptible to the rust. In addition, *I. scabrida* was rated as weakly susceptible to the rust, with tiny uredinia (<1mm diameter) forming on the lower leaf surfaces of some of the plants that were inoculated. *I. scabrida* seedlings were also inoculated with aeciospores and basidiospores and were found to be immune to infection. These results show that *I. scabrida* is not a natural host of the rust and that infection observed on this species is an artefact of inoculating under controlled conditions and evidence suggests that infection is unlikely to be observed in the field (Tomley & Evans, 2004).

**Rust life cycle**

Life cycle studies took place in quarantine in the UK to confirm what has been observed in the field; that the rust is macrocyclic and autoecious – all five spore stages are present and occur on one host, Himalayan balsam (Figure 1).

Changes in environmental conditions in spring induce the germination not only of Himalayan balsam seeds, but also of rust teliospores that have overwintered in the leaf litter. The teliospores produce basidiospores that infect the hypocotyl (stem below the seed leaves) of *I. glandulifera* seedlings resulting in the production of spermogonia and later aecia, from cross-fertilisation; their development causing distortion and elongation of the hypocotyl. Infected plants are thus significantly taller than uninfected seedlings, enabling the aeciospores to be more easily dispersed by wind, to infect the young leaves of Himalayan balsam plants (Figure 2).

**Pest risk assessment**

The research data generated on the Indian strain of the rust fungus were put together in a comprehensive pest risk assessment (PRA), in accordance with the FAO Guidelines (IPPC, 2005). The PRA was submitted to DEFRA in 2014 in support of the release of the rust from quarantine for use as a classical biological control agent for *I. glandulifera* in the UK; and published by Tanner et al. (2014 and 2015). Following the acceptance of the PRA, a public consultation process was undertaken, to allow for any objections to be raised by the general public and all issues raised were addressed by CABI (https://himalayanbalsam.cabi.org/frequently-asked-questions/). The PRA was then sent to the EC Standing Committee on Plant Health (SCOPH) for comment, since the rust could potentially reach mainland Europe in natural air currents. Following agreement from the EC and official UK Government Ministerial approval, the rust fungus was released from quarantine in September 2014; this is the first exotic plant pathogen deliberately released as a biological control agent against a weed in the EU.
Field release of the rust

2015

In spring 2015 a nationwide rust release and monitoring programme was initiated. The rust was released at 25 sites: Berkshire (1 site), Surrey (1 site), Middlesex (1 site), Kent (1 site), Gloucestershire (1 site), Cornwall (6 sites), West Yorkshire (4 sites), North Yorkshire (2 sites), Northumberland (4 sites), West Glamorgan (2 sites), Ceredigion (1 site) and Carmarthen (1 site). The method of release is to place rust infected plants (6–10 per release site), within field populations of Himalayan balsam, and then leave the rust to spread naturally, by wind, onto field plants. The rust was found to spread onto field plants at most sites, but this was limited (1–5 metres from the rust source plants). Pustule size was smaller, and urediniospore production within the pustules was reduced, in comparison to infection observed on plants in the field in the Himalayan native range (Figure 3a); and under glasshouse conditions. However, there was variation between sites, with rust spread and pustules size being better at some sites than others (Figure 3b & c).

Climatic data (temperature and humidity) were recorded at each release site and an assessment was made of the impact of different microclimatic conditions, measured at each site, on rust establishment and spread. Rust infection occurs at temperatures between 10°C and 20°C; and when there is water on the leaf surface for at least 8 hours. These conditions tend to occur at night, or early morning during the plant growing season, and this is when most plant pathogens are known to infect. Figure 4 summarises these data, showing relative differences between the sites when conditions were ideal for infection (green bar). The temperature was not limiting, but some sites had low humidity, providing fewer opportunities for rust infection.

The number of field plants that the rust spread onto at each site was also recorded, and the results are presented in Figure 5 for 13 of the sites. The average number of field plants infected at each site was 12%, but the range was 0–52%. The results overall suggest that there is a positive correlation between the level of rust spread onto field plants and the occurrence of optimal conditions for rust infection at a site.

Figure 3. Comparison between rust infection (urediniospores) on Himalayan balsam: a) native Himalayan range, Kullu Valley, India; and invasive range b) Cornwall, England and c) Lampeter, Wales. Arrows indicate satellite pustule formation around primary pustule.

Figure 4. A bar chart showing percentage of the time that there were optimal conditions for rust infection between May and August 2015 at 20 of the release sites, where data were recorded.
In West Yorkshire, for example, spread of the rust was highest at the Gorpley Clough site, where the occurrence of optimal conditions was the highest. At some sites infection levels were poor, such as Tweed B, Northumberland (and Polmora, Cornwall – although not shown in Figure 5), despite optimal environmental conditions. One potential reason for this could be plant resistance, indicating the presence of multiple genotypes of Himalayan balsam in the UK. Indeed, a recent publication by Nagy & Korpelainen (2015), suggests multiple introductions of Himalayan balsam into the UK.

Controlled field experiments conducted in CABI grounds have shown that the rust can complete its life cycle on Himalayan balsam under UK climatic conditions; successfully overwintering and producing all five spore stages. However, the low level of infection in the field resulted in an inadequate production of the overwintering spore stage (teliospores), and hence, no seedling infection was observed in spring 2016 at any of the 25 release sites.

2016

The main aim of the work in 2016 was focused on establishing the rust in the field, at selected sites in southeast England, local to CABI, where the rust could be carefully monitored. The rust was released every two weeks, over the course of the growing season, in order to maximise the opportunity for the rust to establish and reach a level of natural infection that could lead to an epiphytotic. Field conditions were continuously monitored and infection levels recorded in order to establish the best weather conditions, plant growth stage and habitat to release the rust. Funding availability also allowed for the releases in Wales to continue; a bonus, since Lampeter, is where the best field infection was observed in 2015.

The results were initially encouraging, with good field infection observed on young plants in wet open conditions. This followed a very wet spring, which was likely to have aided infection. Pustule size varied as the season progressed and plants matured, although overall there was a decrease in pustule size and number on the leaves. Nevertheless, in August there was a small peak in size and number of pustules at some sites, and this will be compared to the microclimatic conditions that prevailed at the time.

Controlled experiments were also undertaken to investigate factors that may be responsible for the low level field infection and these are discussed below.

**Improving field adaptation of the rust**

A small plot experiment was set-up on CABI grounds to allow natural infection of seedlings from teliospores and the continuation of the rust life cycle throughout the growing season. Trap plants were placed around the infected seedlings in order to become naturally infected with aecia and subsequently uredinia and allow for the build-up of the rust. It was hoped that the genetic variation resulting from the sexual cycle (spermatia and aecia on the seedling hypocotyl stems, see Figure 6) would provide an opportunity for more UK adapted genotypes to be selected over the 2016 growing season. The repeated, natural passing of the rust under UK conditions was to help address the issue of introducing a ‘quarantine adapted’ strain of the rust into the field, and to allow for a period of field adaptation.

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**Figure 5.** A bar chart showing the spread of rust infection (1–5m from the rust source plants) at 13 of the sites that have been analysed in Surrey, West Yorkshire, Wales and Northumberland between May and September 2015.

**Figure 6.** Rust infection of Himalayan balsam seedlings: a) Hypocotyl infected with spermogonia, causing distortion and reddening (arrow), infected seedlings elongate more than the non-infected seedlings; b) aecial development on the stem following cross fertilization of the spermogonia; c) yellow aecial cups erupt from the surface.
We were encouraged initially to see large uredinia developing from aecial infections on the first leaf whorl of field grown seedlings. However, infections on subsequent whorls were small in comparison (Figure 7). This observation has also been made at the field plots.

Variation in susceptibility of Himalayan balsam plants from different sites in England and south Wales to the rust strain from India

Plants were grown from seed collected at 18 sites where the rust was released in England and South Wales in 2015, in order to test their susceptibility to the rust strain that had been collected in India. Plants were maintained outside, treated identically and assessed for variation in infection. The results show significant variation in the susceptibility of individual populations of Himalayan balsam to the rust. There was an increased severity in the purple colouration (anthocyanin production, a known defence response of plants to fungal invasion) on the upper surface of inoculated leaves, on less susceptible populations of plants (Figure 8). It is interesting to note that overall the infection of the different UK plant populations appears to be following the 2015 field results: sites where poor infection was observed in the field are poorly infected in this experiment.

Host specificity testing of new rust isolate from Pakistan

The results of the screening of the 18 populations of Himalayan balsam from across England and Wales, suggested that more than one strain of the rust will be required to control this weed nationally. In a mountainous region such as the Himalayas it is possible that the Himalayan balsam rust has evolved with distinct plant biotypes in isolation and that as such, distinct strains of the rust exist. This would explain the variation observed in the field. A recent molecular study of Himalayan balsam from the UK and mainland Europe has found that this species is likely to have been introduced a number of times, from both Pakistan and India (Nagy & Korpelainen, 2015). Fortunately, additional strains of the rust are live-stored in liquid nitrogen at CABI, collected during the 2006–2010 natural enemy surveys. A strain from Kaghan Valley, Khyber Pakhtunkhwa Province, Pakistan (IMI 505791), was retrieved from liquid nitrogen and successfully established on Himalayan balsam plants from a UK population, in quaran-
tine. Figure 9 shows excellent pathogenicity on one genotype of Himalayan balsam. This strain has been tested on a number of key plant species, closely related to Himalayan balsam, and as expected, this strain appears to have exactly the same level of specificity as the Kullu Valley, Indian strain released in the UK. Permission to release this strain from quarantine has been sought from DEFRA, if successful this strain will be compared with the Indian strain for pathogenicity towards the different UK populations of the plants.

Conclusions

The rust, *P. komarovii var. glanduliferae*, has potential as a classical biological control agent for *I. glanduliferae*, Himalayan balsam, in its invasive range. The rust negatively impacts on the growth of infected plants in two distinct ways: hypocotyl infection at the seedling stage – reducing seedling survival and recruitment to the population; and, then leaf infection of maturing plants – limiting photosynthetic capacity and, hence, reducing the number of seeds produced per plant. The rust was found to spread on field growing plants of Himalayan balsam, and can complete its life cycle under UK climatic conditions. The results of the 2016 research are under analysis, but it is already clear that there is significant genetic variation in the populations of Himalayan balsam in England and Wales. This is reflected in the variation in susceptibility to the strain of the rust from the Kullu Valley, India, which was released in the UK in 2014. A new strain of the rust from Pakistan has been checked for safety, and will be compared to the Indian strain, once released from quarantine.

It is hoped that a full country-wide release programme will resume in 2017; using the evidence gleaned from the 2016 field work, such as a refined rust release strategy, habitat selection and optimum timing for rust release. In addition, a rust stain will be used that has been given the opportunity to acclimatise to UK conditions; and, critically, a potentially more aggressive isolate of the rust from Pakistan could be trialled in some areas.

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References


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