Japanese knotweed is a highly invasive weed that impacts severely on native biodiversity and local infrastructure in its introduced range. Whilst chemicals are currently used to control the weed, this approach is costly and unsustainable. Biological control is an alternative method. The damaging leaf-spot fungus, *Mycosphaerella polygoni-cuspidati*, which attacks the plant in its native range was found not to be suitable as a classical biocontrol agent. However, the pathogen is considered to hold potential as a mycoherbicide. The aim of this project is to undertake proof-of-concept research into a potential mycoherbicide, in collaboration with the private sector.
Japanese knotweed (*Fallopia japonica*) is a highly invasive species in the UK, mainland Europe, North America and parts of Oceania.

Introduced from Japan in the mid-19th century as a prized ornamental, the plant species soon became problematic in its entire exotic range exerting detrimental impacts on both biodiversity and local infrastructures.

Due to its ability to form dense monocultures and to regrow from tiny fragments of rhizome, Japanese knotweed is often governed by special legislation in individual countries, like in the UK where it is illegal to cause it to grow in the wild (Wildlife and Countryside Act 1981).

Currently, the weed can be partially controlled using chemicals however, this is costly and considered unsustainable due to the ease of spread of the invasive, and the increasing restrictions on using herbicides in many countries.

**Classical biological control programmes** targeting Japanese knotweed were initiated in the UK and the USA in 2000, shortly followed by Canada in 2007. From the suite of natural enemies associated with Japanese knotweed in its centre of origin, the psyllid, *Aphalara itadori*, and the fungal leaf-spot pathogen, *Mycosphaerella polygoni-cuspidati*, were selected as having the highest potential as biocontrol agents. The psyllid has already been released as a classical agent in the UK, USA and the Netherlands, whilst the leaf-spot pathogen has been undergoing evaluation as a potential mycoherbicide agent.

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**What we are doing**

The fungal leaf-spot, *Mycosphaerella polygoni-cuspidati*, is a damaging pathogen of *F. japonica* which is common and widespread on its host in the native range.

A thorough evaluation showed that the pathogen is not suitable as a classical biocontrol agent for Japanese knotweed as it can cause restricted disease symptoms on a couple of UK and North American non-target plant species under quarantine greenhouse conditions. However, the pathogen is thought to hold promise as a mycoherbicide due to its genetic and biological properties.

Such a potential mycoherbicide would be based on an isolate of only one mating type out of the two complementary types required by the pathogen for reproduction, persistence and spread in the field. This would allow targeted application while minimizing the risk to non-target species.

To protect this idea, international patent applications held in the name of the Secretary of State for Environment, Food and Rural Affairs, UK (DEFRA) have been filed and published, and a European patent has already been granted.

Building on previous work, the aim of this project is to undertake “proof of concept” research into the potential mycoherbicide in collaboration with the private sector to assess the scope for, and, hopefully, ultimately develop a product which could be applied in much the same way as a herbicide.

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**Results so far**

The *Mycosphaerella* leaf-spot can easily be mass-produced as fungal mycelium in liquid culture, retaining its viability and virulence.

Initial ‘proof of concept’ research, which took place under standardized greenhouse conditions, proved that spray inoculation with such fungal mycelium led to the development of necrotic leaf-spots and could affect the growth habit of treated plants. Japanese knotweed proved to be more susceptible to the isolate used compared to giant knotweed (*Fallopia sachalinensis*) and the hybrid species Bohemian knotweed (*F. x bohemica*). Permission to release the pathogen from quarantine and to undertake experimental field trials was granted by the relevant UK authorities in July 2019.
Four experimental trials to evaluate the pathogen under more natural conditions, and to determine the abiotic factors (ie temperature and humidity) which govern the infection in the field, took place at CABI UK from 2019 to 2021.

The trials showed that the *Mycosphaerella* leaf-spot is able to infect Japanese knotweed under more natural conditions, but that disease development was slower and disease symptoms less pronounced compared to greenhouse trials. Higher ambient humidity was found to lead to more severe disease symptoms while a higher ambient temperature was unfavourable for disease development.

In collaboration with a commercial company, the leaf-spot pathogen underwent a parallel assessment under greenhouse conditions in the Netherlands. Larger-scale fermentation experiments conducted in the Netherlands successfully induced the formation of microsclerotia by the pathogen; these structures can help to increase shelf-life and the delivery of fungal inoculum in the field.

Further information on CABI’s work on Japanese Knotweed and the mycoherbicide can be found [here](https://www.cabi.org/what-we-do/cabi-projects/).

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