

FROM CLASSICAL TO INUNDATIVE CONTROL: *MYCOSPHAERELLA* *POLYGONI-CUSPIDATI* AS A POTENTIAL MYCOHERBICIDE FOR JAPANESE KNOTWEED

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Japanese knotweed (*Fallopia japonica* [Houtt.] Ronse Decr., Polygonaceae), listed as one of the hundred worst invasive alien species (Lowe et al., 2000) has been widely documented to exert detrimental impacts on both the biodiversity and local infrastructures in Europe, North America and parts of Oceania. Native to Japan, *F. japonica* was originally brought to most of its adventive range as an ornamental in the 19th century. However, the plant's ability to form dense monocultures and to regrow from tiny fragments of rhizome has made Japanese knotweed a troublesome invader wherever it has been introduced. Classical biological control (CBC) programs targeting the weed were initiated in the UK and the USA in 2000 and in Canada in 2007. From the suite of natural enemies associated with Japanese knotweed in its center of origin, the psyllid *Aphalara itadori* Shinji (Homoptera: Psyllidae) and the leaf-spot pathogen *Mycosphaerella polygoni-cuspidati* Hara (Mycosphaerellaceae, Ascomycota) were selected for full evaluation as CBC agents (Djeddour et al., 2008). Having undergone the pest risk assessment (PRA) process and a public consultation, the selected strain of the psyllid received ministerial approval for release against Japanese knotweed in the UK in 2010, becoming the first weed CBC agent in the EU. The psyllid was also subsequently released in Canada and has been petitioned for release in the USA.

The *Mycosphaerella* leaf-spot is a damaging pathogen of *F. japonica* and is common and widespread on its host in the native range. Thorough host-specificity assessment of *M. polygoni-cuspidati* under quarantine conditions at CABI UK showed that the pathogen is able to infect and develop fertile spermogonia producing spermatia on the non-target species *Persicaria hydropiper* (L.) Delarbre and *Polygonum maritimum* L., both native to the UK, as well as on *Polygonum glaucum* Nutt., the sister species of *P. maritimum* and a native of North America (Seier et al., 2014). Spermogonia and spermatia, which constitute the first stage in an ascomycete life cycle, developed regularly on inoculated *F. japonica* plants under greenhouse conditions. However, to date all attempts to induce *M. polygoni-cuspidati* to complete its life cycle on its natural host under experimental conditions have failed. This then leaves the question as to whether the pathogen could complete its life cycle on the affected non-target plants unanswered, and thus currently precludes the fungus from use as a CBC agent. Nevertheless, the unique biology of *M. polygoni-cuspidati* lends itself to the potential development as a mycoherbicide. The *Mycosphaerella* leaf-spot is only known in its sexual form and lacks an asexual conidial stage (Kurose et al., 2009) which, in other *Mycosphaerella* species, is the spore type predominantly responsible for repeated infection cycles and disease spread. Furthermore, the pathogen has been shown to be heterothallic (Kurose, 2016), meaning it requires two complementary mating types to complete its life cycle and develop sexual structures and ascospores as the primary and only infective and dispersal propagules in the field. However, akin to a number of other *Mycosphaerella* species, *M. polygoni-cuspidati* has a dual infection mechanism; it is also able to infect through mycelial fragments under experimental conditions (Kurose et al., 2015). Such mycelial infection has been shown to cause the same herbicide-like disease symptoms on Japanese knotweed as seen with ascospores, and this infection route could be exploited by mass producing mycelium fragments of the pathogen in liquid culture for spray application. *Mycosphaerella polygoni-cuspidati* is not able to grow saprophytically and does not persist in its

host by means of systemic infections. Therefore, using a single mating type isolate would prevent the fungus from reproducing, spreading and persisting in the field and allow for targeted spray application against invasive Japanese knotweed stands, especially in urban situations, while minimizing any non-target impacts. This concept has now been protected through UK and International patent applications held in the name of the Secretary of State for Environment, Food and Rural Affairs, UK (DEFRA), and “proof of concept” research has commenced. This research is focusing on improving the shelf-life of mycelial fragments as well as assessing the impact of the *Mycosphaerella* leaf-spot on its host Japanese knotweed.

Mycelial fragments of *M. polygони-cuspidati* produced in liquid medium have a limited shelf-life during which they retain viability and infectivity. Freeze-drying, routinely used to store fungal spores, has a poor track record to preserve fungal mycelium, although some success has been reported for *Claviceps* spp. (Pertot et al., 1997), some mycorrhizal fungi (Tommerup, 1988) and certain basidiomycetes (Singh et al., 2004). Experiments showed that mycelial fragments of the *Mycosphaerella* leaf-spot can retain viability and infectivity during freeze-drying when lyoprotectants, such as sorbitol, inositol or trehalose, are added and combined with 10% skimmed milk. Sorbitol, particularly at higher concentrations of up to 15% (w/v), gave the best results. Reducing the rehydration time of freeze-dried fragments to 0.5 hours was also found to improve mycelial survival rate. However, the highest percentage viability of mycelial fragments after freeze-drying was found to be 27% after one week of storage, but this declined over longer storage periods (assessed up to six weeks after freeze-drying). Consequently, this methodology will need to be optimized to better preserve mycelial fragments as the active ingredient of any future mycoherbicide in order to increase shelf-life.

Impact assessments of the pathogen on Japanese knotweed are currently still limited to quarantine greenhouse conditions. A PRA for the release of a single-mating type isolate of *M. polygони-cuspidati* from quarantine to conduct experimental field trials has been submitted to the respective UK authorities. However, while this documentation is under evaluation, the pathogen is classed and treated as a quarantine organism in the UK. Studies showed that inoculations with mycelial concentrations of the pathogen at 107 fragments/ml led to leaf-drop in inoculated plants while concentrations of 105 to 106 fragments/ml reliably caused disease symptoms in the form of necrotic leaf-spots. However, disease severity was variable and strongly dependent on the age of the leaf-spot culture used for mass production, as well as the post-inoculation ambient relative humidity; fungal cultures older than three months and lower relative humidity impacted negatively on disease expression. Inoculation with *M. polygони-cuspidati* stimulated the production of new shoots linked with an increased length of and number of leaves on these shoots in treated Japanese knotweed plants. This observation can currently only be documented as a trend due to high variability, but it suggests that the pathogen interferes with shoot apical dominance and thereby activates the growth of rhizome buds, a phenomenon also seen in Japanese knotweed plants as a result of cutting or poisoning of aerial shoots (Bashtanova et al., 2009).

It is hoped that an approval of the PRA for the *Mycosphaerella* leaf-spot and permission for its release from quarantine will allow experimental field trials to take place in order to assess the efficacy of the pathogen under field conditions. If mycelial applications of *M. polygони-cuspidati* prove to give good control, and the concept of a mycoherbicide for Japanese knotweed based on a single mating type of the pathogen is to be taken forward, a partnership with the private sector will be crucial and talks with industry regarding this are already in progress.

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