Field release of the rust fungus *Puccinia spegazzinii* to control *Mikania micrantha* in India: protocols and raising awareness

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Summary

Mikania weed, *Mikania micrantha* H.B.K., a perennial plant of neotropical origin, is a major threat to natural and plantation forests and agricultural systems in Asia and the Pacific. In India, it is a serious weed in the south-eastern and north-eastern states. The efficacy of herbicides to control mikania weed is short lived, and manual weeding is labour intensive and expensive. In this context, the rust fungus *Puccinia spegazzinii* de Toni, from Trinidad, shown to be highly specific and damaging to *Mikania*, was assessed for its control. Following a consultation process with the Ministry of Agriculture, Government of India and other local stakeholders, the rust was imported in 2004 into the quarantine facility at the National Bureau of Plant Genetic Resources in New Delhi. After additional host-specificity testing, field release was permitted by the Government of India in 2005. The rust was first released in tea gardens in Assam (north-east India) in October 2005 but did not establish, most likely due to the presence of a biotype of the weed that was partially resistant to the rust pathotype used. In Kerala (south-west India), releases of the rust were initially made in agricultural systems in August 2006, followed by forest sites. These releases are now considered to be successful; the rust has spread and is persisting. This is the first instance where a fungal pathogen has been used as a biocontrol agent against an invasive alien plant in continental Asia. An awareness-raising campaign on the merits of biological control of invasive alien weeds, targeting the general public, farmers, policy makers, forest officials and the scientific community, was undertaken. The range of methods, including engaging the media, publications and demonstrations are discussed.

Keywords: invasive alien species, plantation crops, classical biological control, Kerala, Assam.

Introduction

Mikania micrantha H.B.K. (Asteraceae), a native of tropical and subtropical zones throughout the Americas, is a perennial, fast-growing invasive plant, capable of smothering agroforesty and natural forest ecosystems. It also invades many crops within home gardens and plantation production systems in the tropical moist forest zones of Asia and the Pacific (Waterhouse, 1994; Global Invasive Species Database, 2002). The preferred habitat for growth of mikania weed is open areas with moist soil. It occupies marginal lands, pastures, roadsides, uncultivated areas, degraded forests, plantations and agricultural systems. *M. micrantha* was introduced into the north-eastern part of India during the Second World War for camouflage of airfields and was later used as a ground cover for tea plantations (Parker, 1972). Thence, it has dramatically increased its range within India, spreading to over ten states especially in the north-east and south-west (Sankaran *et al*., 2001).

Mechanical control methods of mikania like sickle weeding, uprooting and digging are labour intensive, expensive and not effective in the longer term. Chemical control based on herbicides, such as glyphosate, and 2,4-D compounds, is practiced in several countries,

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but the efficacy is short term, and vigorous re-growth is observed after a few months of application (Sankaran and Pandalai, 2004). However, the weed was considered to be an ideal candidate for classical biological control using co-evolved natural enemies, since it is rarely a weed in its native range, where natural enemies limit its abundance (Cock *et al*., 2000).

Under a UK-Department for International Development (DfID)-funded project, fungal pathogens were assessed for their biological control potential of *M. micrantha* in India. No local pathogens were found to be suitable in India. However, the rust fungus *Puccinia spegazzinii* de Toni (Evans and Ellison, 2005) was selected from the broad range of coevolved fungal pathogens recorded from the neotropical, native range of the plant (Barreto and Evans, 1995), as a suitable candidate for introduction into India. This rust pathogen causes stem, petiole and leaf infections on *M. micrantha*, and 11 isolates from six countries were evaluated in the CABI Europe-UK quarantine glasshouse. The rust was found to demonstrate intra-species specificity, each pathotype infecting only a selected number of genotypes of its host (Ellison *et al*., 2004).

However, a pathotype from Trinidad (IMI 393067) proved to be virulent against a wide range of Indian populations of the weed, infecting all those tested from the Western Ghats, and hence was selected for further assessment. This pathotype was screened against 65 non-target species and found to be highly specific (infecting a limited number of species in the genus *Mikania*), damaging (infection often leading to plant death) and has a broad environmental tolerance (Ellison *et al*., 2008). After consultation with Indian stakeholders, permission was sought to import and release the pathogen in mikania weed-affected areas in the south-west (Kerala) and north-east (Assam) regions of India. This paper focuses on the processes involved in this and engagement with the public concerning this novel approach to weed control.

Materials and methods

Importation of *P. spegazzinii* into India

Protocols: India has a relatively long history of importing classical biological control agents for the control of invasive alien weeds; however, all the natural enemies have, thus far, been arthropods (Singh, 2001). *P. spegazzinii* was the first pathogen considered by the Indian authorities for classical biological weed control, and in fact, it was a first for mainland Asia. This necessitated consultations between biological control scientists, the Ministry of Agriculture, Government of India, the Indian Council of Agricultural Research, policy makers and other stakeholders to develop and refine the protocols. A dossier on the rust, produced by CABI Europe-UK for The Project Directorate for Biological Control, Bangalore (the nodal point for import of biological control agents into India), following the Food and Agriculture Organization (FAO) Code of Conduct (FAO, 1996; Ellison and Murphy, 2001), was submitted to the Indian Ministry of Agriculture. This document also included permission from the Ministry of Agriculture, Land and Marine Resources of Trinidad and Tobago (where the rust isolate originated) for the use of their genetic resources, following the Convention on Biodiversity (http://www.biodiv.org/). Permission to import the rust into quarantine facility at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi was granted in September 2002.

P. spegazzinii can only survive in living plants; once the infected plant parts are dried, the teliospores are rendered non-viable. In addition, the firmly embedded teliospores do not readily survive scrapping from the host tissue. Thus, the rust had to be shipped to India on the living host plant. However, because high humidity in the shipment box would cause the embedded teliospores to sporulate (produce the infective basidiospore stage), the rust had to be shipped during the period post-inoculation but before the teliospores were fully viable (i.e. 2–10 days after inoculation). The shipment was hand-carried to avoid potential delays that could occur if it was sent as a cargo item. The rust was successfully established in the quarantine facility at the NBPGR in September 2004.

Additional host specificity tests: Following consultation with systematic botanists, mycologists and plant pathologists at Indian Council of Agricultural Research, an additional host-specificity testing list was drawn up, consisting of 74 test species/varieties of plants. Of these, 25 plants were closely related to the genus *Mikania* (members of Asteraceae), and the rest were economically important plants collected from different parts of India. The inoculation procedure is detailed in Evans and Ellison (2005) and assessment procedure in Ellison *et al*. (2004). For each test plant species or variety, eight replicate plants were used.

The screening was completed by April 2005 and a supplementary dossier (Kumar and Rabindra, 2005) was submitted to the Ministry of Agriculture with the application for field release of the rust. In June 2005, the Plant Protection Advisor to the Government of India from the Ministry of Agriculture gave the permit for release of *P. spegazzinii* in four identified areas, two each in Kerala and Assam.

Mikania plants inoculated with *P. spegazzinii* prepared in the quarantine facility of the National Bureau of Plant Genetic Resources were hand carried in polystyrene boxes to Assam Agricultural University in July and September 2005 and to Kerala Forest Research Institute in November 2005 and established in purpose-built facilities. Around 100 rust-infected plants were produced ready for field release at selected sites. The culture conditions under which the field inoculum plants were produced were found to be critical. Plants have to be pest free, therefore they are sprayed with

a general-purpose insecticide at least 1 week before inoculation; young, to ensure that they are growing at their maximum rate; and with abundant meristematic tissue since this is the most rust-susceptible part of the plant.

Field release of *P. spegazzinii* in Assam and Kerala

The release strategy in Assam and Kerala (Table 1) involved placing large earthenware pots containing rust-infected *M. micrantha* plants in strategic positions in infestations of mikania weed. Positions were chosen in humid, slightly shady places, in a dense stand of the weed. Shoots of the plants in the surrounding vegetation were heavily sprayed with a fine mist of water before putting the rust-infected plants in position. Also, as much as possible, the shoots of the surrounding vegetation were pulled underneath the infected leaves, petioles and stems of the source plants. The release sites were regularly monitored, and progress of the rust infection recorded. Specific methodology at the different release sites is detailed below.

Assam: At site one, Experimental Garden for Plantation Crops (EGPC), the rust source plants were set in the ground by excavating a hole 20 cm in diameter and 30 cm deep for each pot, separated by at least 1 m, so the initial field infection could potentially be recorded separately for each inoculum pot. At site two, Cinnamara Tea Estate (CTE), the pots containing the rust-source plants were hung in a dense stand of mikania weed at the level of the tea table (flat top to rows of tea bushes where leaves are plucked) at about 75 cm height, in a shady place suspended from a bamboo pole with rope (Table 1). The mikania leaves were sparse at ground level due to shading by the tea bushes. All the release sites were sprayed with water twice a day for 15 days, except on rainy days.

Kerala: Each pot containing three rust-infected *M. micrantha* plants were placed on the soil surface within a defined 2 × 2 m quadrat separated from the next quadrat by 3 m; this potentially would allow the spread of each rust infection to be recorded separately for more than one generation. The total number of leaves, petioles and stems infected by the rust was determined for each site, at each date.

Table 1. Details of the *Puccinia spegazzinii* de Toni releases in the field in Assam and Kerala (India) during 2005–2006.

Site	Site details	Release dates	Average temperature and relative humidity[a]	Inoculum source	Ecosystem type
Assam					
Site 1	Experimental garden for plantation crops, Assam Agricultural University	a. Early October 2005 b. April 2006 c. June 2006	a. 20–30°C, 85–90% b. Not recorded c. Not recorded	Six pots each release separated by at least 1 m	Mikania weed monoculture
Site 2	Cinnamora Tea Estate	a. Early November 2005 b. April 2006 c. June 2006	a. 20–30°C, 85–90% b. Not recorded c. Not recorded	Two groups of three pots separated by 4 m each release	Tea plantation (*Camellia sinensis* [L.] O. Kuntze) heavily infested with mikania weed
Kerala					
Site 1	Echippara, Trichur Forest Division	a. 24 August 2006 b. 25 September 2006	a. & b. 23.3–29.6°C, 70–100%	a. 11 pots b. Three pots (10 m from release a.)	Agricultural system with mixed cropping of coconut (*Cocos nucifera* L.) and areca nut (*Areca catechu* L.). On the banks of a perennial stream with dense canopy
Site 2	Palappilly, Thrissur Forest Division	25 September 2006	23–28.7°C, 80–100%	Three pots	Degraded moist deciduous forest
Site 3	Peechi, Thrissur Forest Division-Kerala Forest Research Institute (KFRI) campus	a. 19 September 2006 b. 30 October 2006	See Table 3	Six pots each release	Degraded moist deciduous forest

[a] During 15 day inoculation period.

Raising awareness: An important part of the mikania weed classical biological control programme involved an awareness-raising campaign on the benefits of biological control amongst the communities where the rust was planned to be released, as well as the government policy makers, forest officials and scientists. At Kerala Forest Research Institute, the opinion of farmers concerning the use of host-specific natural enemies to control invasive alien weeds rather than chemicals was initially sought via farmer questionnaires and meetings. This was followed by demonstrations and exhibitions aimed at the agricultural and forestry extension services and students from universities and schools. A pre-rust-release workshop was held at Kerala Forest Research Institute and a post-release workshop in Assam Agricultural University to educate all stakeholders on the usefulness of biocontrol agents in controlling invasive weeds.

The media was also engaged: local newspapers in Kerala published articles on the release of the rust depicting the rust as a welcome solution to the weed problem; CABI Europe submitted press releases in the UK and India, resulting in popular articles being published in the press and radio interviews; Kerala Forest Research Institute in collaboration with the Audiovisual Research Centre, University of Calicut, produced two documentary films aimed at the general public. The first '*Weeds: the Biological Invaders*' was telecast all over India through the National Television Network. The second, focusing on biological control of weeds, is currently being edited prior to broadcast.

Publications have included a 'popular-style' book aimed at policy makers in the developing world; '*Invasive Alien Plants: Problems and Solutions*' (in press, CABI-Europe); and local-language brochure for Kerala farmers on the sustainable management of invasive alien weeds.

Results and discussion

Importation of *P. spegazzinii* into India

Additional host specificity tests: None of the 74 plant species inoculated was infected by *P. spegazzinii*, showing that the pathogen was highly host specific. Mild chlorotic flecks were observed on a few top leaves of four cultivars of sunflower, but the leaves recovered from the symptoms and there was no sporulation of the rust. The non-susceptibility of sunflower was also ascertained through histopathological studies (Ellison *et al*., 2008). All the inoculated sunflower plants showed normal growth and flowering, confirming the host specificity results from CABI Europe-UK and establishing that *P. spegazzinii* can safely be used as a classical biological control agent in India.

Field release of *P. spegazzinii* in Assam and Kerala

Assam: At site one, the Experimental Garden for Plantation Crops, infection on field *M. micrantha* plants was observed 12 days after the release of the rust. Rust pustules were observed on leaves, petioles and stems of the surrounding mikania vegetation. However, the pustules were smaller than those normally observed on fully susceptible plants. The number of leaves infected ranged from 8 to 33, and maximum number of pustules developed on leaves ranged from 5 to 32. The number of stems and petioles infected was low (one to three). Following the first release, rainfall was continuous for 14 days, after which the common mikania leaf spot pathogen, *Cercospora mikaniacola* F. Stevens, became abundant on leaves. This sudden high level of *Cercospora* caused early senescence of the rust-affected leaves, hence slowing the spread of the rust infection. However, stem infections were not affected by *Cercospora*, so rust infection was able to progress in the field with the inoculum released from the infected stems. The disease progression was noted until January 2006, after which no progress was observed due to the non-conducive environmental conditions (high temperature and low humidity). Release of the fungus in April 2006 also resulted in good infection in the field, but the disease spread only a short distance from the inoculum source (30 cm in 9 weeks). Unfortunately, dry conditions following the June release prevented the rust from spreading.

At site 2, CTE, the rust infected the *M. micrantha* plants surrounding the inoculum source and spread further than at site one (1 m). As with site one, the pustules were small. However, progress of the rust infection was curtailed as the environmental conditions became non-conducive for natural spread of the rust. A similar result was found for the 2006 inoculations as at site one. The first inoculation at both sites was undertaken late in the wet season, when the conditions suitable for rust infection were already deteriorating. However, infection and spread was still achieved, but (at the chosen release sites) the rust was unable to survive the dry season. The 2006 inoculation did not lead to a significant level of infection and spread of the rust. This relatively disappointing result has been attributed mainly to the presence of a semi-resistant biotype of mikania weed present at the release sites, demonstrated by the small pustule size.

The evaluation of the pathogenicity of pathotypes of the rust against biotypes of the weed was reported by Ellison *et al*. (2004). This showed the presence of biotypes of mikania in Assam that were semi-resistant to the rust pathotype from Trinidad and that the Peruvian pathotype should be released in Assam as well. However, it was decided to proceed initially with importing the Trinidad pathotype into India, which was fully screened and is fully pathogenic to all biotypes tested

in Kerala (the region targeted during the first phase of the project, when original rust selection work was undertaken) and most of those from Assam.

Kerala: At all sites, the initial symptoms of the disease on the field *M. micrantha* plants were noticed a week after release of the rust. The results are summarized in Table 2. Similar results were observed at all plots inoculated in August and September although with varying degrees of disease severity and a maximum distance of spread of 1.5 m away from the rust-source plant. However, by October, the environmental conditions became non-conducive for spread of the rust, and levels of infection gradually declined (Table 3). Inoculations carried out in late October led to low levels of infection. By December, no rust infection was observed in the field.

The results, in general, indicate good spread of the rust from the source plant to field population of mikania weed in Kerala and Assam. Even though the field inoculations were carried out late in the wet season in Kerala, there was still also good spread within the field population until late October. Laboratory studies with the rust have shown that in the Western Ghats, optimum conditions conducive to rust infection (see 'Introduction') are likely to occur from June to September, although the temperature can go above optimum (up to 33°C) during August and September. However, beyond this period (until April or May), the maximum atmospheric temperature rises to over 40°C, and minimum relative humidity goes down to 30%. The conditions are more or less similar in Assam where the hotter conditions may extend until June. During this summer period, mikania weed tends to die back in open areas, although in areas with perennial standing water and along permanent streams, plants continue to grow and maintain leaves. Hence, over most of the mikania-infested areas, the rust will not be able to perpetuate. Evidence from the native range of *M. micrantha* and from glasshouse studies suggest that the rust will survive in living stems as cankers in open areas and on all aerial parts of plants surviving by permanent water. These 'rust refuges' could act as the inoculum source to initiate the rust epidemic as the rains begin and the mikania weed starts to reinvade.

Raising awareness of the use of CBC

The result of a farmer survey showed that over 90% were willing to try the biocontrol agent in their farm. The Government of India adjudged the film '*Weeds: the Biological Invaders*' as the best documentary film on humanity, environment and human rights, in 2004.

Communication activities were undertaken to create awareness among the general public, forest officials, scientists and policy makers. The Tea Research Institute at Valparai, Tamil Nadu and plantation owners within Kerala approached KFRI on the possible use of the biocontrol agent to control mikania weed in their farms and plantations. Overall, this campaign showed that it is possible to cultivate a positive thinking on the use of biocontrol agent against invasive weeds in India.

Conclusions

In Kerala in 2007, the aim is to significantly increase the frequency and quantity of inoculations of the rust

Table 2. Field infection of *Puccinia spegazzinii* de Toni on *Mikania micrantha* H.B.K. in Kerala, India.

Progress of the disease in the field	**Site 1**						**Site 2**		**Site 3**			
Dates of field release	24 August 2006				25 September 2006		25 September 2006		19 September 2006		30 October 2006	
Days after release	25	54	75	100	22	40	20	70	20	37	17	36
No. of leaves infected	67	27	10	1	43	0	24	1	82	0	6	0
No. of pustules per leaf	1–3	1–2	1–2	1	1–3	0	1–2	1	1–10	0	1–5	0
No. of petioles infected	7	0	0	0	0	0	2	0	19	0	0	0
No. of stems infected	1	0	0	0	0	0	0	0	4	0	0	0

Table 3. Air temperature and relative humidity at Peechi (Kerala, India) during August–December 2006.

Month	Air Temperature °C[a]		Relative humidity %[a]	
	Minimum	Maximum	Minimum	Maximum
August	21.7–24.8 (23.3)	24.4–33.2 (29.6)	52.0–95.7 (70.1)	100 (100)
September	22.0–25.1 (23.1)	24.1–32.8 (28.7)	60.0–98.7 (79.7)	100 (100)
October	22.1–25.8 (23.4)	26.3–44.0 (35.8)	38.9–62.6 (51.2)	90–100 (99.3)
November	22.1–25.1 (23.6)	29.1–42.0 (37.7)	45.0–59.0 (50.8)	88–100 (99.0)
December	18.3–25.4 (22.5)	33.5–40.4 (36.6)	31.3–54.6 (41.1)	76–100 (88.6)

[a] Mean value in parentheses.

in the field during the season most favourable to its spread (June to August), in order to build up the rust concentrations. As with the first releases in 2006, the selected areas will be those which encourage optimum rust propagation, e.g. cooler sites under shade or along the banks of perennial streams. It is suggested that once there is a critical concentration of the rust in an area, the infection will enter an epidemic phase.

Work is continuing in Assam to identify release sites with populations of mikania weed that are fully susceptible to the rust, where new releases can be made. In addition, the screening of the pathotype of *P. spegazzinii*, collected in Peru against a few selected plant species closely related to *M. micrantha* at CABI, has suggested that its selectivity, outside of its host species, is identical to the Trinidad strain. The Peruvian pathotype was subsequently (2006) imported into quarantine at National Bureau of Plant Genetic Resources, New Delhi, and additional confirmatory host-specificity screening is near completion. Permission to release this isolate in the field in Assam is being sought.

Awareness-raising activities will continue and will be combined with a rust-distribution programme by farmers and foresters, supported by the extension services, once optimum rust release strategies have been established.

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