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Evaluation of Classical Biological Control of *Mikania micrantha* with *Puccinia spegazzinii*

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In collaboration with
CABI Bioscience Europe-UK, Project Directorate of Biological Control, Bangalore,
Assam Agricultural University, Jorhat and
National Bureau of Plant Genetic Resources, New Delhi

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Contents

Abstract

Mikania micrantha (mikania), a neotropical invasive plant, is a major threat to natural and plantation forests and agricultural systems in Asia and the Pacific. In India, its infestation is a serious environmental and economical issue especially in the north-eastern and south-western states. Mechanical and chemical control methods of the weed are labour intensive, expensive and effective only in the short-term. In this context, a survey was conducted in the native range of the weed to identify natural enemies for use in classical biological control. Of the plant pathogens identified during the survey, *Puccinia spegazzinii*, a microcyclic rust, collected from Trinidad, was shown to be highly specific and damaging to the Indian population of mikania. After a preliminary pest risk analysis conducted in the CABI Europe-UK, the pathogen was imported into the quarantine facility of the National Bureau of Plant Genetic Resources (NBPGR), New Delhi in August 2004. It was subjected to additional host specificity tests against 74 plant species in the NBPGR facility. Since the rust was proved to be highly host specific, the Govt. of India issued permission to release it as a biocontrol agent in selected mikania infested localities in Assam and Kerala. The rust was hand-carried to Kerala in November 2005 and multiplied and maintained in the glass-house at KFRI. It was released in selected mikania infested agricultural systems and forest sites in the State during August-October 2006. The releases were successful in the sense that the rust had spread to the native population of mikania in all the sites. However, the rust persisted on the field population of the weed only till December 2006 - until the environmental conditions were suitable for the disease spread. Low inoculum load and inappropriate time of release are considered to be the main reasons for the failure in survival of the rust in the field beyond December 2006. Fresh releases will have to be done taking these aspects into consideration. Since the releases of the rust in Papua New Guinea and Taiwan were successful in controlling the population of the weed, it is believed that the future releases in Kerala may be successful provided a high load of inoculum is applied in the field during the south-west monsoon (June-July).

Introduction

Mikania micrantha Kunth (Asteraceae) is a fast growing perennial vine with a native range with in Central and South America. It is treated as one among 100 of the world's worst invaders in the Global Invasive Species Database. The plant is widespread in a number of countries in the moist tropical zones of Asia and the Pacific and its exotic range is still expanding (Waterhouse 1994; Zhang *et al.*, 2004). In its invasive range, mikania is commonly known as "mile-a- minute weed" which denotes its extremely fast growth rate (eight to nine centimeters in 24 h) (Choudhury, 1972). It was introduced in the north-eastern parts of India during the Second World War for camouflage of airfields and was later used as a ground cover in tea plantations (Parker, 1972). Thence, it has dramatically increased its range within India, spreading over to a dozen states especially in the north-east and south-west (Sankaran *et al.*, 2001, Gogoi, 2001). The plant is rarely a weed in its native range where natural enemies exert a significant pressure on the occurrence and abundance of the species (Cock *et al.*, 2000)

In Kerala, *M. micrantha* (mikania) was first recorded from a rubber plantation at Kottayam in 1968 (Nair, 1988). In its native range, the species has a cryptic riparian habit growing along riverbanks and amongst reed-like vegetation around standing water (Barreto and Evans, 1995). However, in the introduced ranges it invades forest borders and clearings, roadsides, railway tracks, pastures, forest plantations, agricultural areas, agro-forestry systems, open lands, disturbed areas and banks of rivers and streams (Sankaran *et al*, 2001). Wherever invaded, mikania smothers, penetrates crown and pulls over plants. Thus, the plant causes significant reduction in growth and productivity of agricultural and plantation crops including rubber, cashew, citrus, teak, eucalypt, pineapple, coconut and plantain (Fig. 1-6). It is also a big threat to natural forests affecting native biodiversity. The vine makes harvesting of agricultural crops and bamboo and reeds in natural forests difficult due to its creeping and twining habit. Aerial parts of mikania dry up in summer wherever water is not available which

poses a serious fire hazard. Its adverse impact on crops is also through the release of allelopathic substances into the soil. The weed is currently widespread in Kerala impacting heavily on natural forests, forest plantations and agricultural areas (Sankaran and Pandalai, 2004).

The socio-economic studies conducted on home garden farming systems in the Western Ghats showed that mikania has an impact on production costs and income in all sizes of holdings (Sankaran *et al.*, 2001). In general, weeds form the greatest constraint to cultivation and weeding mikania accounted for 10-20% of the total weeding costs in agricultural systems in Kerala. Farmers are compelled to employ more laborers as a result of vigorous growth of the weed. In summary, mikania has been shown to have a significant negative impact in agricultural and agro-forestry systems and forest plantations. The life of tribal people living in natural forests in Kerala was also affected since mikania infestation frustrates their attempts to collect non-wood forest produce from the forests (Sankaran *et al.*, 2001).

Mechanical control methods of mikania such as sickle weeding, uprooting and digging are labour intensive, costly and ineffective in the longer term. Herbicidal applications using glyphosate, triclopyr, diuron and 2,4-D compounds are practiced in several countries but the efficacy is short-term and vigorous re-growth is observed after a couple of months (Sankaran and Pandalai, 2004). However, the weed was considered as 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 restrict its growth and spread (Cock *et al.*, 2000). Classical biological control (CBC) offers an effective and environmentally safe method for the control of alien invasive weeds and has a proven track record (Barton, 2004; Julien and Griffiths, 1998). Molecular studies on world-wide populations of mikania have shown that there is only a narrow genetic base of the weed in tropical Asia (Ellison and Murphy, 2001). This improves the chances of successful CBC throughout the exotic populations, with a few genotypes of a eco-evolved natural enemy (Ellison *et al.*, 2004).

Though several pathogens affecting local populations of mikania were recorded from India, none of these showed potential for biological control (Sreenivasan and Sankaran, 2001). Hence, fungal pathogens from the neo-tropical native range of the weed were assessed for their potential to be used in CBC (Evans and Ellison, 2005). Of these, the rust fungus *Puccinia speagazzinii* de Toni was selected from a broad range of co-evolved natural enemies as a suitable candidate for introduction into India (Barreto and Evans, 1995). This pathogen is able to infect leaves, petioles and stems of the plant causing necrosis, cankering and often leading to plant death restricting the growth and spread of the plant in the native ranges. Eleven isolates of the pathogen from six countries (Argentina, Brazil, Costa Rica, Ecuador, Peru and Trinidad and Tobago) were evaluated for pathogenicity towards a range of populations of mikania collected from its native (6 countries) and exotic ranges (8 countries) in the CABI Europe-UK quarantine glasshouse. This study showed that *P. spegazzinii* has intra-species specificity, each pathotype infecting only a selected number of genotypes of its host (Ellison *et al.*, 2004).

Of the eleven isolates tested, a pathotype of *P. spegazzinii* 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. The Trinidad isolate was found to be more damaging on the Indian populations of the weed, with frequent plant deaths, than on the host biotype from which it was originally isolated (Ellison and Murphy, 2001). This strain was selected for further testing and screened for host specificity using the centrifugal phylogenetic testing sequence against 65 non-target species (Wapshere, 1974). The strain was found to be highly host specific and damaging to the population especially from the Western Ghats (Ellison, 2001). In this situation, it was decided to import and release this pathogen in south-west and north-east India after obtaining necessary permission from the Govt. of India. It was expected that, if released in the moist tropics, the rust would establish and spread and will exert a significant impact on the abundance and spread of mikania populations within a few growing seasons. And, in the long term, the growth and spread of the weed maybe reduced and it will no longer pose a threat to different ecosystems.

The project deals with the importation of the pathogen into India, testing of its host specificity and release of the pathogen in Kerala. The main objectives of the study were 1) confirming the host specificity of the pathogen, 2) its multiplication on the local population of mikania under glass house conditions and maintenance of the inoculum, 3) release of the pathogen in the weed infested areas and 4) assessing the impact of releases.

Classical biological control of mikania - a prelude to importation of *P. spegazzinii* into India

Classical bio-control

Classical biological control involves importation and release of efficient co-evolved natural enemies from the homeland of the target weed with the aim of reducing its competitive ability and hence achieving control in the invaded areas. It is possible that small numbers of the natural enemy succeed in establishing a population when they are released. Thus, CBC is an environmentally benign, cost effective, sustainable and safe method of weed control. There are several success stories of CBC with plant pathogens (Harris, 1991). To cite a couple of examples of rust fungi as CBC agents, which is relevant here, *Puccinia chondrillina* was released in Australia in 1971 from Europe for the successful control of the weed *Chondrilla juncea* (Cullen and Hasan, 1988). Likewise, *Puccinia xanthii* was introduced into Australia to control *Xanthium occidentale*. Chippendale (1995) reported that three years after the release of *P. xanthii*, there was a visible impact of the pathogen on the *Xanthium* population in Australia. The economic study on the benefits of CBC of *Xanthium* showed that in less than 10 years the net benefit was close to A$ 17 million. Thus, CBC also proves to be an economically viable option for weed control.

Selection of the bio-control agent

In the first attempt on bio-control of mikania, an insect *Liothrips mikaniae* collected from Trinidad was released in Solomon Islands in 1988 and in Malaysia in 1990. However, the agent failed to establish in the field (Cock *et al.*, 2000). It was found that ant

predation of the nymphal stage of the insect was the main reason for the failure in establishment. In an attempt to locate suitable pathogens for bio-control of *Mikania micrantha*, Evans (1987) conducted a survey in the native ranges of the weed and brought out a comprehensive account of its mycobiota. Later, Barreto and Evans (1995) analyzed the potential of the pathogens identified on mikania for use in bio-control. The taxonomy of the rust fungi infecting mikania was reported by Evans and Ellison (2005). These studies were followed by glass-house evaluation of the potential of the rust fungi identified from the native range of miknaia to act as biocontrol agents against various populations of the weed. It resulted in the selection of *P. spegazzinii* as the prime candidate for introduction into the invasive range of the weed in India (Ellison, 2001). As discussed earlier, a pest risk analysis of the pathogen was conducted in the UK on 65 non-target plant species before it was decided to release it into India (Ellison *et al.*, 2008).

Molecular characterization on mikania population world-wide

Since mikania exhibits phenotypic plasticity within populations, it was necessary to understand the degree of genetic variation within the weed in the exotic range before introducing bio-control agents. Forty-two populations of *M. micrantha* collected from its native and exotic ranges and eight other species of *Mikania* were included in this study (Ellison and Murphy, 2001). The genetic variability of the weed species was assessed through AFLP. The dendrograms drawn from the populations showed that the isolates from India, Malaysia and Sri Lanka cluster together with a similarity value of 87% and this group then clusters with the group from Costa Rica at about 84% and this group then links to the isolates from Mexico at 83%. In summary, it appears that the Indian population may have originated from the Central America. The populations from India appeared relatively homogenous genetically although the grouping suggest that there may have been two separate introductions of the weed into north-east and south-west parts of India.

The bio-control agent – Puccinia spegazzinii

Puccinia spegazzinii (Uredinales) is a microcyclic (reduced number of spore stages in the lifecycle), autoecious (completes its life cycle on one host species) rust which produces only teliospores and basidiospores in its life cycle. Spermagonia, aecia and uredinia are unknown in the field (Arthur, 1922; Baretto and Evans, 1995). The yellowish to dark brown teliospores are embedded in the host tissue in distinct raised sori which are 2-6 mm in diameter. These occur on the dorsal side of leaves and over the entire circumference of petioles and stems where they often merge to form elongated pustules. Under high humidity, basidiospores are produced and liberated from teliospores. Unlike other rusts, teliospores are not released from the sori. The basidiospores infect the young meristamatic tissue of the host giving rise to telia and teliospores; older plant tissue is less susceptible (Ellison and Murphy, 2001). The pathogen causes necrosis of leaves and cankers on stem and petioles leading to mortality of plants if the infection is severe. The pathogen can survive in stem cankers for long periods which help it to tide over unfavorable conditions for infection and multiplication.

Puccinia spegazzinii has only been recorded from the Neotropics. Literature survey indicate the distribution of the rust on various species of *Mikania* from southern USA through Mexico to northern Argentina. However, the surveys carried out by CABI Bioscience Europe-UK revealed its occurrence only in Argentina, Brazil, Cost Rica, Ecuador, Trinidad and Tobago and southern Peru; it was not found in Mexico (Ellison and Murphy, 2001).

The pathotype of *P. spegazzinii* (collected from Trinidad- IMI 393067) selected for introduction into India was found to have a very narrow host range, being capable of infecting only a limited number of species within the genus *Mikania*. Since it has a broad environmental tolerance, this pathotype was considered suitable for release in the Western Ghats. The risk of the pathogen infecting other species of *Mikania* is not an issue since there are no native species of *Mikania* in India. Moreover, there are no records of the rust fungi infecting any other plant species in the indigenous range of the

weed. The rusts are inherently stable genetically and so the chances of mutation and the pathogen affecting other non-target plants are minimal. Besides, such pathogens are also able to adapt to the genetic changes in the host. Hence, the development of resistance in the host to the pathogen is highly unlikely. There are also no evidences to show that the rust spores are toxic to either humans or animals.

Climatic conditions conducive for infection

Studies conducted by CABI Bioscience Europe-UK has shown that there is a significant effect of temperature on infection by the rust with more infection achieved between 15-25^{0}C with an optimum near 17^{0}C. The pathogen is able to infect even at 12^{0}C. The dew period requirement was 8 h with maximum infection achieved after 14 h. This relatively short requirement of free water on the leaf surface is ideal for a tropical pathogen, allowing infection to occur overnight, when dew forms, rather than being dependent on rainfall (Ellison *et al.*, 2008). Basidiospores were produced and liberated from the teliospores embedded in the host tissue. Under glass house conditions, basidiospores were released after 2 h under high humidity conditions (> 90%) and continued at least for a 24 h period. Plants placed under inoculum that had been in the dew chamber for 24 h still became infected when subjected to an additional 24 h dew period. After 12 h, a bloom of basidia and basidiospors will be clearly visible over the entire surface of the telium (Ellison and Murphy, 2001).

Materials and methods

Importation of Puccinia spegazzinii

India has a long history of importing CBC agents for control of weeds. However, all natural enemies imported so far have been arthropods (Singh, 2001). *P. spegazzinii* was the first pathogen considered for import to control an invasive alien weed in continental Asia. To enable import, a dossier on the rust and its potential as a bio-control agent against mikania was produced by CABI Europe-UK on behalf of the Project Directorate of Biological Control, Bangalore (the nodal agency for import of biological control agents into India) following the FAO code of conduct (FAO, 1996; Ellison and Murphy,

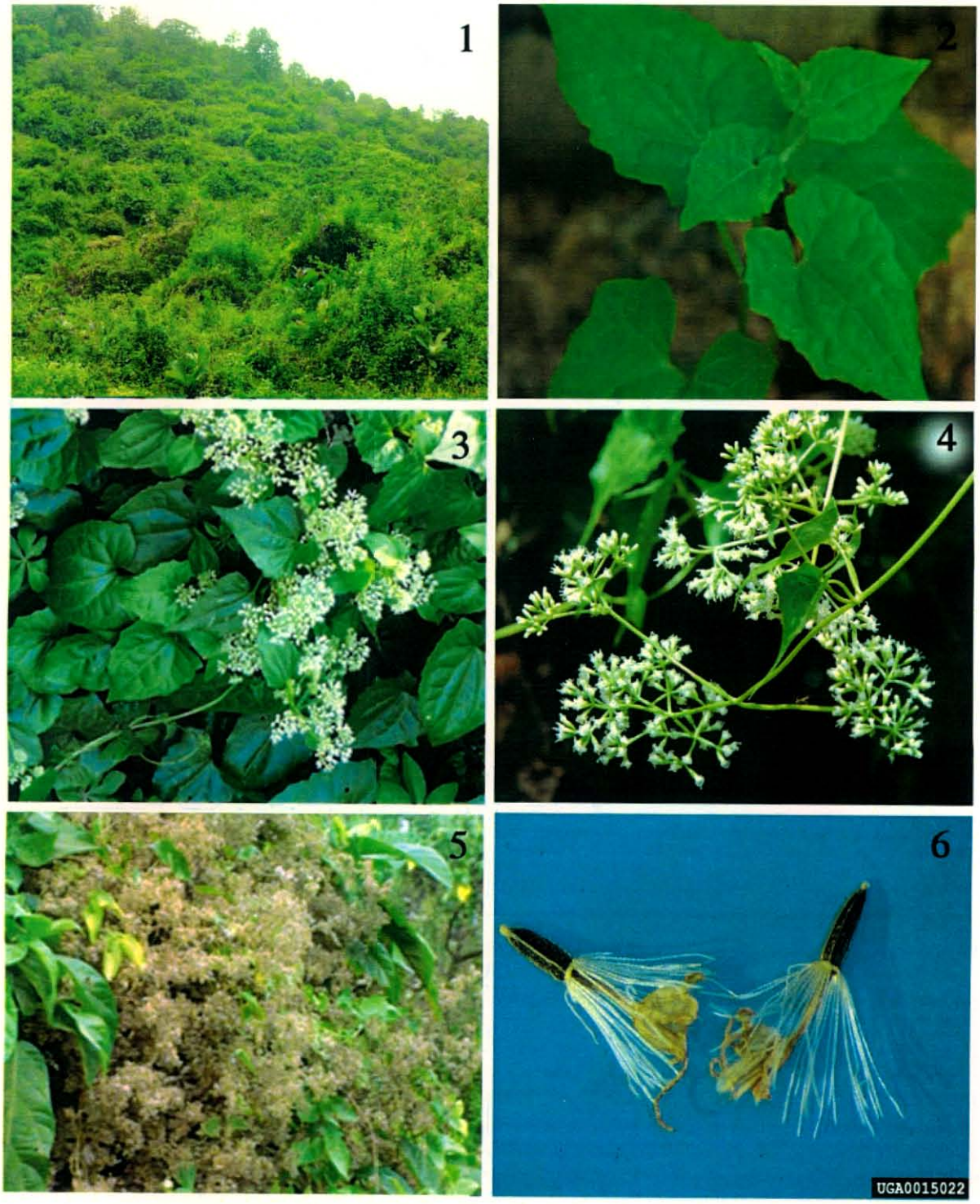

Fig 1. Mikania infestation in a teak plantation in Vazhachal, Thrissur; Fig. 2. Seedlings of mikania; Fig 3. Leaves and flowers of mikania; Fig 4. A close-up view of flowers; Fig 5. Mikania bush with mature seeds; Fig. 6. Close-up view of seeds.

2001). This document was submitted to the Ministry of Agriculture, Govt. of India for permission to import the rust. The dossier also included permission from Govt. of Trinidad and Tobago (where the rust isolate originated) for use of their genetic resources following the Convention on Biodiversity. After due consultations and discussions, permission to import the rust into the quarantine facility of National Bureau of Plant Genetic Resources (NBPGR), New Delhi was granted by the Govt. of India (Permit No. 33/2004 in PQ Form 13; issued on 3 August 2004).

Puccinia spegazzinii is an obligate parasite and hence it can only survive on living plants. Once the plant is dead, the teliospores become non-viable. This necessitated import of the pathogen on the living host plant. Since the teliospores may start producing the short-lived basidiospores during transit (because of high humidity in the shipment box), the rust was shipped during the post-inoculation period before the teliospores are fully mature (2-10 days after inoculation). To avoid delay during shipment, the infected plants were hand-carried by placing them in safety boxes. The plants were maintained in the quarantine facility at NBPGR and observed for symptom development.

Since *P. spegazzinii* is attacked by a number of natural enemies in the field and glass house, adequate precautions were taken to ensure that the rust is free of any natural enemies during import. This was achieved by culturing the rust in a separate quarantine CT room for at least 3 generations before shipment. It was also essential that the mikania plants, on which the rust is transported, are pest-free.

Host plants

Collections of mikania received from Assam and Kerala were maintained at the NBPGR glass-house in plastic pots containing garden soil amended with organic and inorganic fertilizers. Plants were multiplied through cuttings (roots were readily produced at the leaf nodes once pegged in moist soil) and maintained at 18± 1^{0}C until inoculation. Plants were trimmed at periodic intervals to produce fresh meristamatic growth in bushes which was ideal for infection by the rust. Adequate care was taken to ensure that the plants do not harbor any insect or mites.

Inoculation

Puccinia spegazzinii produces basidiospores under high humidity from cushions of teliospores that are embedded in the plant tissue. The teliospores are not released and hence the inoculum used in the experiment was composed of infected leaf, petiole and stem with mature telia. For inoculation, mikania/other test plants (bushy plants with fresh meristamatic growth) were first transferred to a purpose built humidity chamber maintained at 100% humidity and 18-20^0 C. These plants were sprayed with a fine mist of de-ionized water and the inoculum was spread over a grill like tray kept 5 cm above the test plants in such a way that the pustules of the rust faced the healthy plants kept on the lower rack. The inoculum was removed from the humidity chamber after 12 hrs. The inoculated plants were transferred from the humidity chamber after 24 hrs and maintained in the glass-house. Suitable controls without the application of inoculum were also maintained.

Multiplication and maintenance of the rust inoculum

In most cases, chlorotic spots on the leaves of the inoculated plants will develop after 7-8 days of inoculation. Viable telia are produced from these chlorotic spots within 15 days and the telia mature in 18-20 days. The telia will be ready to use as inoculum after this period. The incoulum is multiplied and maintained on mikania plants through this cycled process.

Additional host specificity tests using the rust fungus

Following consultations with experts at the Indian Agricultural Research Institute and discussions among scientists attached to the research organizations involved in this collaborative project, *viz.*, CABI Bioscience, Europe-UK, Project Directorate of Biological Control, Bangalore, Assam Agricultural University, Jorhat and Kerala Forest Research Institue (KFRI), Peechi it was decided to conduct additional host specificity tests at the quarantine facility at NBPGR before the biocontrol agent is released in Assam and Kerala. Of the 74 plants that were selected for screening, 25 were closely related to *Mikania micrantha* and belonged to the family Asteraceae (Table 1). Seeds, seedlings and

cuttings of the test plants were procured from New Delhi and the states of Assam, Karnataka and Kerala, and grown in the quarantine facility at the NBPGR maintained at 18± 1^{0}C. As with the host plant, it was ensured that all test plants were young, healthy and with a good number developing shoots. The pesticide Imidacloprid was used at regular intervals to protect the plants from sap sucking insects.

The test plants were inoculated with *P. spegazzinii* as described earlier. In each case, eight replicate plants were inoculated. The 48 h dew period provided after inoculation ensured every opportunity for infection to develop on test plants. Mikania plants inoculated along with the test plants served as susceptible checks. The plants were checked for disease symptoms for about six weeks. As is mandatory for all quarantine experiments, all the used-up inocula, test plants and related materials were autoclaved and incinerated.

Permission for release of the rust fungus

The additional host specificity tests were completed in April 2005 and a supplementary dossier was submitted to the Ministry of Agriculture, Govt. of India (Kumar and Ravindra, 2005) with the application for field release of the rust. Permission was accorded by the Plant Protection Advisor, Govt. of India for field release of the pathogen in selected mikania infested areas in Kerala and Assam in June 2005.

Release of the rust fungus in Kerala

Rust infected mikania plants were hand-carried in polystyrene boxes to Kerala Forest Research Institute in November 2005. The rust was inoculated on to mikania plants collected from different localities in Kerala and maintained in the KFRI glass-house. The inoculation was carried out in a purpose-built humidity chamber under conditions discussed above. The inoculated plants were transferred to the glass-house benches after 24 h. The plants were observed for development of symptoms. Fresh batches of plants were inoculated at monthly intervals for bulking-up and maintaining the inoculum.

Three sites were identified in Thrissur district for release of the rust *viz.*, 1) Echippara, an agricultural system with mixed cropping of coconut and areca nut, near the Chimmini dam; 2) Palappilly, part of a degraded moist deciduous forest in the KFRI field research centre campus; and 3) Peechi- part of a degraded moist deciduous forest in the KFRI main campus (Table 2).

The release strategy of the rust involved placing large earthenware pots with rust infected mikania plants (after 18 days of inoculation) in strategic positions in infestations of the mikania weed. Positions were chosen in slightly shady places in a dense stand of the weed. Shoots of the mikania plants in the surroundings were sprayed with a fine mist of water before putting the rust infected plants in position. Also, the shoots of the mikania growth were pulled underneath the infected leaves, petioles and stems of the potted source plants. At each site, pots containing infected mikania plants (three plants in each pot) were placed within a defined 2x2m quadrat separated from the next quadrat by 3m which will allow recording spread of the rust for more than one generation. Three to eleven pots were placed per site based on the intensity of infestation at each. The release sites were regularly monitored and the total number of leaves, petioles and stems infected by the rust determined for each site at different intervals.

Raising awareness

An important part of the mikania bio-control program was to create awareness on the benefits of the biological control of the weed amongst various stakeholders such as farmers, forest officials and scientists. The opinion of farmers was sought through questionnaires and meetings prior to the release. A pre-rust-release workshop was held in KFRI which involved farmers, plantation owners and forest officials. Also, the benefits of releasing the bio-control agent and its genetic stability were discussed in a meeting of the senior forest officials held in the Forest HQ, Thiruvananthapuram. The local news papers published articles on the release of the rust depicting the rust as a welcome solution to the weed problem.

Fig 7. Purpose built humidity chamber for inoculation of rust in KFRI glass-house; Fig. 8. Collections of mikania plants for inoculation in KFRI glass-house; Fig 9. Mikania plants after inoculation; Figs. 10-12. Symptoms of rust infection in the glass-house.

Results and discussion

Importation of the bio-control agent

The rust was successfully established in the quarantine facility at the NBPGR in New Delhi in September 2004. It was maintained in the glasshouse by periodic inoculation (approx. every six weeks) on fresh mikania plants. Inoculum originated from these stock plants were used for host specificity tests. The rust was successfully established in the KFRI glasshouse in November 2005 from the source plants hand-carried from NBPGR (Fig. 7 - 12).

Symptoms of the rust disease observed in the glass-house at NBPGR

Symptoms of the disease were first visible after six days of inoculation as chlorotic spots on leaves, petioles and stems. The spots enlarged and sori of embedded teliospores were first evident erupting on the dorsal side of leaves and on petioles and stems after about 12 days of inoculation. The teliospores became matured and produced basidiospores in 18 days. However, they remained viable for several weeks, especially those on petioles and stems, depending on the conditions in the glass-house. The teliospores on the leaves are noted to lose viability more quickly. The viability of the basidiospores becomes unreliable after four weeks of inoculation. The infection led to necrosis, canker formation and gradual wilting of plants.

Mikania populations collected from two different localities in Kerala *viz.*, Vazhachal in Thrissur district and Shoranur in Palakkad district were found to be highly susceptible to the rust compared to collections from other localities. Likewise, plant collections from 15 different localities in Assam *viz.*, Jorhat, Nagajanka, Tinsukia, Kokrajhar, Diphu, Darrang, Tezpur, Sibsagar, Lakhimpur, Silapather, Nagaon, Borpeta, Nalbari, Silchar and Titabar were also susceptible to the rust.

Studies on the host range of the rust

None of the 74 plant species inoculated with *P. spegazzinii* was affected by the pathogen showing that it was highly host specific (Table 1). All the mikania plants inoculated

along with the test plants got infected. Mild chlorotic flecks were observed on a few top leaves of four cultivars of sunflower but the leaves recovered from the symptoms and the rust did not produce teliospores. Ellison *et al.* (2008) have established the non-susceptibility of sunflower to *P. spegazzinii* through histo-pathological studies conducted in the UK. In summary, the host-specificity studies conducted at NBPGR confirmed the host-specificity of the rust pathogen to mikania and thus establishing that it can be safely used as a classical biological control agent against the plant.

Symptoms of the rust disease on mikania observed in the glass-house at KFRI

Similar to the disease development observed in the glass-house at NBPGR, chlorotic spots were visible after 6-7 days (of inoculation) on leaves, petioles and stems of all inoculated plants. Teliospores were produced after 10-11 days of inoculation and the teliospores produced basidiospores in 17-18 days.

Field release of the bio-control agent in Kerala

At all release sites in Kerala, the initial symptoms of the disease in the field population of mikania were noticed a week after the release of the rust (Fig. 13 - 18). The results are summarized in Table 3. Similar results were recorded from all the plots inoculated in August and September although with varying degrees of disease severity. The maximum distance of spread was 1.5 m away from the source plant observed at Echippara. However, by October, the environmental conditions became warmer and less humid (Table 4) and levels of infection gradually declined. Inoculations carried out in late October resulted in only low levels of infection. By the first week of December 2006, no rust infection could be located in the field.

Overall, the results indicated good spread of the rust from the source plants to the field population of mikania in all the release sites. Though the releases were made late in the wet season (during August-October), there was still a good spread within the field population till mid October. The optimum conditions for rust infection, as evidenced by laboratory studies, occur during south-west monsoon (June–August) in Kerala although

the temperature during August-September can go above optimum (up to 33^{0}C). However, beyond this period (until April-May), the maximum atmospheric temperature rises above 33^{0}C and the minimum relative humidity will be around 40-50%. During the summer period, mikania tends to dry-up in open areas, although in areas with perennial standing water and along permanent streams, plants continue to grow and maintain leaves. However, in most of the other areas the plant will dry-up and the rust will not be able to survive.

Evidence from the native range of mikania suggests that the rust will survive on living stems as cankers in open areas and on all aerial parts of plants surviving close to perennial water sources. These 'rust refugees' could act as the inoculum source to initiate the rust epidemic as the rains begin and the mikania starts to re-invade. However, periodic visits to the rust released areas for the past six years (from June 2007 to August 2013) did not show presence of the rust on the field population. Reports from Papua New Guinea and Taiwan where the rust was released in 2008 and 2009, respectively, indicate that the rust has spread far and wide in the native population of the weed and is surviving. It also had a significant impact on the growth and spread of the weed in these countries (Ellison, 2012 - personal communication).

Guidelines for future work

Results of rust release from Papua New Guinea (Day *et al.*, 2013) and Taiwan (Ellison, 2012 - personal communication) indicate the strong possibility of survival of the rust in the field in Kerala exerting an impact on the population of mikania. An evaluation of the release strategy adopted in Kerala reveals that the load of the inoculum released may not have been sufficient enough to cause a severe infestation in the field. It is thus apparent that the low level of infection and the resultant poor population of the fungus contributed to its failure to survive the harsh climatic conditions during November - December in Kerala. The time of release was also not ideal. The releases should have been ideally done in June-July during the south-west monsoon when the climatic conditions are suitable for the multiplication and fast spread of the fungus in the field.

Also, the release sites should be chosen carefully so as to encourage optimum rust propagation - cooler sites under shade or along the banks of perennial streams. It is evident from the results recorded elsewhere that once there is a critical concentration of the rust in the area, the infection will enter into an epidemic phase. All these factors will have to be taken on board while fresh releases of the rust are attempted in Kerala.

Outcomes and conclusions

1. A host-specific, co-evolved natural enemy viz., *Puccinia spegazzinii*, which controls growth of *Mikania micratha* in its native range, was located from Trinidad after rigorous trials on virulence and host specificity carried out at the CABI Bioscience Europe- UK.

2. The fungus was imported into India and established in the quarantine facility at the National Bureau of Plant Genetic Resources, New Delhi.

3. Host specificity of the fungus was tested against 74 species of plants which included those related to mikania (family Asteraceae) and several economically important plants. These trials indicated that the rust is highly host specific.

4. Based on the release permit, the rust infected mikania plants were hand carried to Kerala and multiplied and maintained in the KFRI glass-house.

5. The rust was released in 3 sites in Kerala. It got spread to the field population by 7 days and further spread within the population was also observed.

6. The rust could not survive beyond December 2006 in the field population. Observations on the survival of the rust as 'refugees' were continued until August 2013 but infection could not be located at any of the release sites.

7. Low inoculum load and inappropriate time of release could have been the main reasons for the failure in survival of the rust in the field beyond December 2006.

8. Fresh releases during the south-west monsoon (June-July) using a heavy load of inoculum may help survival of the pathogen in the field and subsequent control of the weed population.

Figs. 13-14. Field release of the rust; Figs. 15-16. Symptoms of the rust infection on field population of mikania at Echippara; Fig 17. At Palappilly and Fig 18. At Peechi.

Table 1. Results of host-specificity tests using *P.spegazzinii* at NBPGR, New Delhi

S.No.	Scientific name	Common name	Family	Cultivar	Source/ place of collection	Rust symptoms
A. Plant species closely related to *M.micrantha*						
1	*Ageratum houstonianum* Mill.	Floss flower, mist flower	Asteraceae (Tribe: Eupatorieae)	-	Sunder Nursery, New Delhi	X
2	*Artemisia annua* L.	Sweet sagewort, Wormwood	Asteraceae (Tribe: Anthemideae)	EC 202429	NBPGR Regional Station, Bhowali	X
3	*Aster chinensis* L.	China aster	Asteraceae (Tribe: Astereae)	-	Sunder Nursery, New Delhi	X
4	*Bellis perennis* L.	Daisy	Asteraceae (Tribe: Astereae)	-	Do	X
5	*Brachycome iberidifolla* Benth.	Swan river daisy	Asteraceae (Tribe: Asterceae)	-	Do	X
6*	*Calendula officinalis* L.	Calendula	Asteraceae (Tribe: Calenduleae)	-	NBPGR, New Delhi	X
7*	*Catharamus tinctorius* L.	Safflower	Asteraceae (Tribe: Cynareae)	-	Bangalore, Karnataka	X
8	*Centaurea cyanus* L.	Cornflower, Bachelor's-button	Asteraceae (Tribe: Cynareae)	Frosty mix	Namdhari Seeds Bidadi, Karnataka	X
9*	*Chromolaena odorata* (L.) R.M. King & H. Robinson	Siam weed, Chromolaena	Asteraceae (Tribe: Eupatorieae)	-	NBPGR, New Delhi	X
10*	*Chrysanthemum carinatum* Schousb.	Tricolor chrysanthemum	Asteraceae (Tribe: Anthemideae)	-	NBPGR, New Delhi	X
11	*Cosmos bipinnatus* Cav.	Cosmos	Asteraceae (Tribe: Heliantheae)	-	PDBC, Bangalore	X
12	*Dimorphotheca sinuata* DC.	Cape-marigold	Asteraceae (Tribe: Calenduleae)	-	Sunder Nursery, New Delhi	X

13	*Eupatorium adenophorum*Speng.	Crofton weed	Asteraceae (Tribe: Eupatorieae)	-	Ootacamund, Tamil Nadu	X
14	*Gazania rigens* R. Bt.	-	Asteraceae (Tribe: Arctotideae)	-	Sunder Nursery, New Delhi	X
15*	*Gerbera jamesonii* Bolus ex Hook. F.	Transvaal daisy, Barberton daisy	Asteraceae (Tribe: Mutisieae)	-	Do	X
16	*Guizotia abyssincia* Cass.	Niger-seed, Rantil	Asteraceae (Tribe: Heliantheae)	-	Bangalore, Karnataka	X
17*	*Helianthus annuus* L.	Sunflower	Asteraceae (Tribe: Heliantheae)	AHT-16	AAU, Jorhat, Assam	Mild chlorotic flecks
				AHT-17		X
				IH-673		Mild chlorotic flecks
				IH-662		X
				CO-2	Tamil Nadu	X
				Morden		Mild chlorotic flecks
				Swarna Hybrid		X
				CO-4 (TNAUSUF-7)		X
				TCSH-1 (TNAU)		Mild chlorotic flecks
18	*Matricaria aurea* Boiss	-	Asteraceae (Tribe: Anthemideae)	-	Sunder Nursery, New Delhi	X

19*	*Parthenium hysterophorus* L.	Congress weed	Asteraceae (Tribe: Anthemideae)	-	NBPGR, New Delhi	X
20	*Solidago canadensis* L.	Golden rod	Asteraceae (Tribe: Astereae)	-	PDBC, Bangalore	X
21	*Sonchus arvensis* L.	Field sowthistle	Asteraceae (Tribe: Lactuceae)	-	NBPGR, New Delhi	X
22	*Tagetes erecta* L.	Big marigold, Aztec marigold	Asteraceae (Tribe: Helenieae)	African marigold (Tall)	Do	X
23	*Tagetes tenuifolia* Cav.	Striped marigold	Asteraceae (Tribe: Helenieae)	Single signet		X
24	*Tithonia diversifolia* (Hemsl.) Gray	Mexican sunflower, tree marigold	Asteraceae (Tribe: Heliantheae)	-	Ganganagar, Bangalore,	X
25	*Vernonia anthelmintica* (L.) Willd.	-	Asteraceae (Tribe: Vernonieae)	-	NBPGR, New Delhi	X

B. Other economically important plant species						
26	*Lobelia erinus* L.	-	Campanula-ceae	Crystal palace	Sunder Nursery, New Delhi	X
27	*Ochlandra travancorica* (Bedd.) Benth. Ex Gable.	Elephant grass, reed	Gramineae	-	AAU, Jorhat, Assam	X
28*	*Oryza sativa* L.	Paddy, rice	Gramineae	-	National Seeds Corporation (NSC) Ltd., New Delhi	X
				NDRK 5026-R	Genetics Division, IARI, New Delhi	X
29	*Pennisetum typhoides* (Burm.f.) Stapf. & C.E. Hubb.	Pearl millet	Gramineae	HHB-117	NBPGR, New Delhi	X

30	*Triticum aestivum* L.	Wheat	Gramineae	PDW-343	Genetics Division IARI	X
31	*Sorghum vulgare* Pers.	Sorghum	Gramineae	GV UP CHARI-2	NBPGR, New Delhi	X
32*	*Zea mays* L.	Maize	Gramineae	Lakshmi		X
33	*Saccharum officinarum* L.	Sugarcane	Gramineae	CO-1148	AAU, Jorhat, Assam	X
34	*Vigna unguiculata* (L.) Walp.	Cowpea	Leguminosae	Pusa Phalguni	NBPGR, New Delhi	X
35*	*Cocos nucifera* L.	Coconut	Palmae	Bengal Selection	AAU, Jorhat, Assam	X
36	*Areca catechu* L.	Betel-nut palm, Arecanut	Palmae	Mangala	Kerala Agri. University (KAU), Thrissur, Kerala	X
37	*Cinnamomum zeylanicum* Blume.	Cinnamon	Lauraceae	IISR Navasaree		X
38	*Syzygium aromaticum* (L.) Merr.&Perry	Clove	Myrtaceae	-		X
39	*Piper betle* L.	Betel-pepper, Betel vine	Pipereaceae	-	KAU, Thrissur, Kerala	X
40*	*Theobroma cacao* L.	Coca, Cacao	Sterculiaceae	CCRP-1		X
41	*Piper nigrum* L.	Black pepper	Piperaceae	Panniyur-1		X
42*	*Coffea arabica* L.	Arabian coffee	Rubiaceae	Kaveri		X
43*	*Camellia sinensis* (L.) O. Kuntze	Tea	Theaceae	TV 23	AAU, Jorhat, Assam	X
44	*Musa paradisiaca* L.	Banana	Musaceae	-	AAU, Jorhat, Assam	X
45*	*Tectona grandis* L.	Teak	Verbenaceae	-	KFRI, Kerala	X
46	*Bambusa arundinacea* (Retz.) Willd.	Thorny bamboo, Spiny bamboo	Bambusaceae			X
47	*Mangifera indica* L.	Mango	Anacardiaceae	Mallika	New Delhi	X
48	*Artocarpus heterophyllus* Lamk.	Jack tree, Jack fruit	Moraceae	-	AAU, Jorhat, Assam	X

49	*Ananas comosus* (L.) Merr	Pineapple	Bromeliaceae	Mauritius	KAU, Thrissur, Kerala	X
50	*Zingiber officinale* Rosc.	Ginger	Zingibereaceae	-	New Delhi	X
51	*Elettaria cardamomum* Maton	Cardamom	Zingibereaceae	CCS-1	Cardamom Research Centre, Appangala Karnataka	X
52	*Anacardium occidentale* L.	Cashew	Anacardiaceae	Vengurla	Bangalore, Karnataka	X
53*	*Arachis hypogaea* L.	Peanut, groundnut	Papilionaceae	TG-45	NBPGR, New Delhi	X
54	*Corchorus capsularis* L.	Jute, White jute	Tiliaceae	JRC-212	AAU, Jorhat, Assam	X
				JRO-524		X
55	*Gossypium hirsutum* L.	Upland cotton	Malvaceae	MECH-162 (Non-Bt)	Maharashtra Hybrid Seeds Co.	X
				MECH-162 (Bt)		X
56	*Gossypium arboreum* L.	Desi cotton	Malvaceae	Karbi	AAU, Jorhat, Assam	X
57	*Sesamum indicum* L.	Sesame, gingelly	Pedaliaceae	-		X
58	*Dioscorea bulbifera* L.	Potato yam	Dioscoreaceae	Gajendra		X
59	*Myristica fragrans* Houtt.	Nutmeg	Myristicaceae	IISR Viswashree	KAU, Thrissur, Kerala	X
60	*Brassica nigra* (L.) Koch	Black mustard	Cruciferae	RK-01-03	NBPGR, New Delhi	X
61	*Coronopus didymus* (L.)	Lesser swinecress	Cruciferae	-		X

62	*Matthiola incana* (L.) Ait.	Tenweeks stock	Cruciferae	-	Sunder Nursery, New Delhi	X
63*	*Raphanus sativas* L.	Radish	Cruciferae	Pusa desi	NSC Ltd., New Delhi	X
64	*Capsicum annuum* L.	Chilli, red pepper	Solanaceae	Pusa Hyper-2		X
65	*Nicotiana tabacum* L.	Tobacco	Solanaceae	-	NBPGR, New Delhi	X
66*	*Solanum melongena* L.	Brinjal	Solanaceae	PK	NSC Ltd., New Delhi	X
67	*Ricinus communis* L.	Castor	Euphorbiacea e	DCH 519	NBPGR, New Delhi	X
68	*Viola tricolor* L.	Pansy, heart's ease	Violaceae		Sunder Nursery, New Delhi	X
69	*Tropaeolum majus* L.	Garden nasturtium	Tropaeolaceae			X
70	*Antirrhinum majus L.*	Snap-dragon	Scrophulari-aceae	-		X
71	*Linaria bipartita* Willd.	Toad flax	Scrophulari-aceae	-		X
72	*Phlox drummondii* Hook.	Drumm-ond phlox, annual phlox	Polemonia-ceae	-		X
73	*Dianthus* sp.	Dianthus	Caryophyll-aceae	-		X
74*	*Linum usitatissimun* L.	Linseed, flax	Linaceae	RLC 81	NBPGR, New Delhi	X

Note: X = No symptoms.
*Plant species tested during the first Phase also and found non- susceptible to *P. spegazzinii.*

Table 2. Details of the *Puccinia spegazzinii* de Toni releases in Kerala

Site	Site details	Release dates	Average temp. and RH	Inoculum source	Ecosystem type
1	Echippara, Thrissur Forest Division	a. 24 Aug. 2006 b. 25 Sept 2006	23.3- 29.6^{0}C 70-100%	a. 11 pots b. Three pots (10 m away from the first release area)	Agricultural system with mixed cropping of coconut and areaca nut (*Areca catechu* L.) - on the banks of a perennial stream with dense canopy
2	Palappilly, Thrissur Forest Division	25 Sept. 2006	23-28.7^{0}C 80-100%	Three pots	Degraded moist-deciduous forest
3	Peechi, KFRI main campus	a. 19 Sept. 2006 b. 30 Oct. 2006	See Table 3	Six pots at each release	Degraded moist-deciduous forest

Table 3. Field infection of *Puccinia spegazzinii* on *Mikania micrantha* in Kerala

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 4. Atmospheric temperature and relative humidity at Peechi during Aug. – Dec. 2006

Month	Air Temperature °C		Relative humidity %*	
	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)

* Mean value in parentheses

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