CROP PROTECTION PROGRAMME

Classical Biological Control of *Mikania micrantha* with *Puccinia spegazzinii*: Implementation Phase

R8228 (ZA0539)

FINAL TECHNICAL REPORT

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CABI Bioscience (A Division of CAB International)

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R8228 Crop Protection Programme

Executive Summary

Mikania micrantha H.B. K. (Asteraceae), or mile-a-minute weed, is a neotropical invasive plant in many countries within the tropical moist forest zones of Southeast Asia. This perennial vine can smother agro-forestry and natural forest ecosystems, as well as many crops within homegarden and plantation production systems. The project purpose was to implement a classical biological control (CBC) strategy for this weed using a rust fungus (*Puccinia spegazzinii*) from Trinidad, identified and assessed under Phase I (R6735), in Southwest and Northeast India. The project involved 4 Indian organisations; Kerala Forest Research Institutes (KFRI); Project Directorate of Biological Control (PDBC), Indian Council of Agricultural Research (ICAR); Assam Agricultural University (AAU) and National Bureau of Plant Genetic Resources (NBPGR), and CABI Bioscience, UK.

The project consisted of nine main research areas, given below with their outputs:

1. *Studies on the long-term live storage of pathotypes of candidate rust CBC agents.* Techniques were developed using liquid nitrogen, for storage of 10 pathotypes of *P. spegazzinii*, and two 'reserve' candidate rust species. These will act as voucher specimens.
2. *Introduction into quarantine in India of the rust for additional host specificity screening.* A total of 74 plant species were screened and none of them, except *Mikania* could be infected with *P. spegazzinii*.
3. *Release of the rust into the environment in Assam and Kerala*. The release permit was issued on 27th June 2005. Rust infected plants were transported from quarantine at NBPGR, to AAU in July, and to KFRI in November, 2005.
4. *Development of rust propagation techniques.* In Assam and Kerala purpose built rust propagation units were constructed and used to successfully establish the rust and develop mass production techniques.
5. *Investigation of optimum rust field release strategy.* The rust was released and established in the field in Assam, at two sites in tea plantations, using two different techniques. The rust will be released in Kerala in February 2006.
6. *Impact assessment of* Mikania *on tea production in Assam*. *Mikania* caused significant yield loss of tea and increased labour costs. Farmers ranked *Mikania* as the major production constraint.
7. *Assessment of the density of* Mikania *in permanent sample plots in Assam*. Two plots in tea were established as the sites for the initial release of the rust in Assam. Quantitative sampling of the *Mikania* in these plots, pre-release of the rust, was conducted at fortnightly intervals for 9 months. Changes in the phenology of *Mikania* over the growing season were quantified.
8. *Implementation of publicity and information campaign on CBC of* Mikania. An International publicity campaign was implemented though news releases, popular articles, radio interviews and scientific papers and bulletins. The techniques that will be used in the implementation of a local information campaign were fully developed.
9. *Stakeholders' workshop on* Mikania *problem in Nepal.* This additional activity established the need for a CBC implementation project in Nepal.

DfID's development goals focus on sustainable technology for the improvement of the livelihoods of poor people. CBC (through the introduction of exotic fungal pathogens) is a self-perpetuating and thus long-term and sustainable management option for the *Mikania* problem in Asia. This technology is appropriate for resource poor farmers, since it requires no financial or time inputs from them in order to be implemented or sustained, and will potentially significantly reduce the impact of this weed on RNR systems.

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ACRONYMS

AAU – Assam Agricultural University

CBC – Classical biological control

CQF – Containment–cum-Quarantine facility

EU – European Union

ICAR – Indian Council for Agricultural Research

IUCN – The World Conservation Union

KAU – Kerala Agricultural University

KFD – Kerala State Forestry Department

KFRI – Kerala Forest Research Institute

MoA – Ministry of Agriculture

NATP - National Agricultural Technology Programme

NAIP - National Agricultural Innovation Programme

NBPGR – National Bureau of Plant Genetic Resources

PDBC – Project Directorate of Biological Control

RPU – Rust Propagation Unit

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Background

Mikania micrantha (Asteraceae) or mile-a-minute weed is a neotropical invasive plant that can smother both agro-forestry and natural forest ecosystems, as well as many crops within homegarden and plantation production systems in the tropical moist forest zones of Southeast Asia; tea and plantain are particularly severely affected (Holm *et al.*, 1977; Waterhouse, 1994). It has been an on-going problem since the 1940s in north-east India, but its importance has escalated in recent decades due to large-scale degradation of natural forests, from which stronghold *Mikania* can invade the tea gardens. This has led to a massive increase in herbicide use in the tea gardens, which is proving to be detrimental to the marketing of the tea crop, particularly in the EU, due to herbicide residues. *Mikania* arrived more recently in the Western Ghats where it now poses a threat to natural and home garden ecosystems throughout the region and beyond. Current control focuses on cultural (slashing) and chemical (herbicides) methods, but are expensive, ineffective, not sustainable, and can be environmentally damaging (Sen Sarma & Mishra, 1986; Muniappan & Viraktamath, 1993; Palit, 1981).

DfID funded (through NRI CPP) a collaborative project between two Indian organisations; Kerala Forest Research Institute (KFRI), and Assam Agricultural University (AAU) and CABI Bioscience, UK from 1996 to 2000 (R6735) to investigate an IPM approach for the control of *Mikania* in the Western Ghats and Assam. At the end of this first phase, the Project Directorate of Biological Control (PDBC), Indian Council of Agricultural Research (ICAR), entered into the project framework, as the nodal point for biological control in India. In Phase I the work included: mapping the distribution and monitoring the spread of the weed; assessing its socio-economic impact on homegarden subsistence agriculture; and evaluating fungal pathogens as biological control agents (mycoherbicides and classical introductions). The KFRI input into this programme is published in Sankaran *et al.* (2001a), and the classical biological control (CBC) initiative undertaken by CABI, is summarised in Ellison (2001b).

Surveys in India have revealed no indigenous natural enemies that could be used effectively to control *Mikania*. However, the weed was considered to be an ideal candidate for CBC using co-evolved natural enemies, since it is rarely a weed in its native range in the Americas, where natural enemies are seen to exert a significant pressure on the occurrence and abundance of the species (Parker, 1972; Cock *et al.* 2000). World-wide populations of the weed were characterised using amplified fragment length polymorphism (AFLP) technique of DNA fingerprinting under this phase of the programme. This work showed that the weed has a relatively narrow genetic base in tropical Asia, whereas in its native range it is highly variable. This confirms the evidence in the literature of a small number of deliberate introductions of the plant (perhaps two in India), as a cover crop and also as airfield camouflage during World War II (Barbora, A.C., pers. comm.; Wirjahardja, 1976). This improves the chances of successful CBC throughout the exotic range, using a single or limited number of natural enemies.

An evaluation was undertaken of the broad range of fungal pathogens that have been recorded on *M. micrantha* from its neotropical native range (Barreto & Evans, 1995). From this evaluation, three autoecious, microcyclic rust species were selected for further assessment, *Dietelia portoricensis, D. mesoamericana* and *Puccinia spegazzinii* (Uredinales). From these results, the last named species was considered to be the prime candidate for introduction into southern India as a classical biological control agent. It is a common and damaging pathogen on *M. micrantha* in the Neotropics, but is not found in the exotic range of the weed. Eleven isolates of the rust, from six countries (Brazil, Costa Rica, Argentina, Trinidad and Tobago, Peru and Ecuador) were evaluated in the CABI Bioscience UK quarantine glasshouse. The pathogen was found to demonstrate intraspecies specificity,

each isolate only infecting a selected number of genotypes of its host. However, one pathotype from Trinidad (W1761, IMI 393067) proved to be virulent against a wide range of Indian isolates of the weed, infecting all those tested from the Western Ghats, and hence was selected for intensive screening. This pathotype was shown to be totally specific to *M. micrantha* (55 non-target species had been tested, including many important crop species in India), as well as highly damaging (leaf, petiole and stem infections leading to cankering and whole plant death). In addition, the rust has a broad environmental tolerance (able to infect after less than 10 hours of dew, at temperatures ranging between 15 and 25^{o}C). It is an obligate biotroph, surviving only on living plant material: if infect plant material is dried, the rust is rendered non-viable.

Dietelia mesoamericana, collected on *M. micrantha* from Mexico, was also maintained in the CABI Bioscience quarantine glasshouse, as the 'reserve' candidate. A basic investigation found *D. mesoamericana* to be equally as damaging to its host as *P. spegazzinii*, and has only been recorded from *M. micrantha*. The Trinidadian isolate of *P. spegazzinii* was found to be capable of infecting almost all the collections of *M. micrantha* from the Old World to which it was challenged. For the focus area of the first phase of the programme, the Western Ghats, all target populations screened (10), were fully susceptible. Seven plant collections were screened from the north-east of India, and three of these from Assam (Grampani, and two from Kaziranga) were found not to be fully susceptible to W1761 (reduced pustule size). However, *D. mesoamericana* was found to fully infect all three of the semi-susceptible collections and hence, could be considered as an additional CBC agent in north-east India.

Although a suite of natural enemies may be required to achieve significant control of some alien invasive weeds, the extensive field observations within the native range of *Mikania* as well as glasshouse investigations, suggest that *P. spegazzinii* could prove to be the 'silver-bullet' for the suppression of the weed throughout the moist forest regions in Asia. It is predicted that, if released in these regions, the rust would establish and spread rapidly under the prevailing environmental conditions, and should unilaterally exert a significant effect on the abundance and spread of the *Mikania* populations within a few growing seasons. In the long term, it is predicted that the growth and fecundity of *Mikania* would be severely reduced over a significant part of its range. The weed, thus, should no longer pose a threat to the agricultural economy of the infested regions, and would contribute towards poverty alleviation of subsistence farmers, by increasing crop yields and/or reducing time spent on weeding. Where the weed is currently controlled by herbicides, there would also be a significant reduction in the potential environmental damage caused by these chemicals and associated user health risks. Equally important, the rust would perform a significant role in the conservation of biodiversity of natural forest ecosystems by reducing the impact of this invasive alien weed in these habitats.

This first phase of the programme culminated in a workshop that was held at KFRI; 'Alien Weeds in Moist Tropical Zones, Banes and Benefits' (2-4 November 1999). The workshop was attended by local and regional scientists involved in the control of invasive weeds, and recommendations included the following:

> 'Development of an implementation phase involving farmer validation of the integrated weed management programme including classical biological control and herbicides being researched by KFRI and Indian ICAR. In particular, an application should be made for the introduction of the *M. micrantha* – specific exotic rust fungus *Puccinia spegazzinii*.

A dossier was produced by CABI Bioscience, for the Indian collaborators, containing detailed data on the information summarised above concerning the rust *P. spegazzinii* (Ellison & Murphy, 2001). This was submitted to the relevant quarantine authorities in India, and

permission to import the rust into quarantine was applied for by PDBC, Bangalore. At this time, PDBC were in the process of building an on-site quarantine facility, but it was looking unlikely to be completed in time for the rust screening work need under Phase II. A contingency plan was agreed at a National Agricultural Technology Programme (NATP) funded quarantine workshop undertaken in May 2002 at PDBC (technical expertise supplied by CABI Bioscience). One of the participants, Dr. R.K. Khetarpal from National Bureau of Plant Genetic Resources (NBPGR), New Delhi, offered their (pathogen secure) National Containment-cum-Quarantine Facility (CQF) for Transgenic Planting Material, for the undertaking of the rust screening work.

Before this project could commence, it was necessary that an import permit be obtained from the Head, Division of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi. This was issued in October 2002, and was to be the first time in Indian history that a pathogen had been imported for the control of a weed. Unfortunately, despite the project funding from NRI being released at this time, the start of the project in India was delayed for a further 6 months awaiting clearance from ICAR. Therefore it was not possible to hold the Inception Workshop until June 2003.

Project Purpose

The project's specific purpose, based on the CPP purpose is:

> 'Implementation of a classical biological control strategy using a rust fungus (*Puccinia spegazzinii*) for the invasive alien, perennial weed *Mikania micrantha,* in tree crop, ago-forestry and small-holder farming systems in the moist tropical regions of southwest and northeast India.'

The economic significance of *Mikania* in India was quantified during Phase I (R 6735) (Murphy, Ellison and Sankaran, 1999). The socioeconomic studies conducted on homegarden farming systems in the Western Ghats region showed that *Mikania* has an impact on production costs and income of all sizes of holdings. In general, weeds form the greatest constraint to cultivation and weeding *Mikania* accounted 10-20% of the total weeding costs. An increasing number of farmers have to employ more labourers as a result of the vigorous growth of this weed. These studies also showed that *Mikania* has a significant negative impact in forest plantation production systems and for tribal communities living the natural evergreen forest areas in Kerala. In north-eastern India, *Mikania* is a major constraint to tea production, and prevents growers from changing to organic systems. The Output-to-Purpose Review under R6735 demonstrated the demand and likely impact of the programme.

Specific demand for the project outputs comes from three sources:

a. Homegarden farmers in the Western Ghats. This group of farmers is looking for ways to reduce weeding costs. Most of the farmers spoken to supported the concept of biological control.
b. Recommendations of stakeholders from workshop held at the end of R 6735. The stakeholders included State and National Agricultural research and extension organisations (Sankaran *et al.* 2001).
c. Request from ICAR, HQ, Natural Resource Management to address the problem of *Mikania* in the north-eastern States.

Research Activities

1. Biocontrol agents prepared for release in India:

1.1 Inception workshop (undertaken at the same time as activities 3.1 and 4.1). The Inception workshop held at NBPGR, and attended by all key collaborators.

Figure 1. Project collaborators outside NBPGR quarantine, New Delhi. Left to right: P. S. Kumar (PDBC), R. P. Bhuyan and K. C. Puzari (AAU) and K.V. Sankaran (KFRI)

The workshop included the following sessions:

- Inauguration session attended by Dr. G. Kalloo, Deputy Director General (Crop Science and Horticulture) and Dr. O.P. Dubey, Assistant Director General (Plant Protection).
- Project updates from KFRI, AAU, PDBC and CABI.
- Discussion on rust shipment and establishment in the quarantine unit.
- Work plan for Phase II.
- Additional host specificity testing.
- Plenary session.

1.2 Rust culture prepared and shipped to India. *Puccinia spegazzinii* (ex Trinidad, IMI 393067) was prepared for shipment to India at CABI Bioscience Quarantine Unit, Ascot, UK. The rust can only survive on living plants, once the infected plant part are dried the rust is rendered non-viable. The preparation was undertaken in a separate dedicated CT room, to ensure that the host plants remained free from pests and the rust from hyperparasites. The plants were grown from cuttings, which that had been treated with a general insecticide (0.025% imidacloprid and 0.05% methiocard), prior to entering the CT room. The cuttings were grown in sterile vermiculite or perlite, with added nutrients. The rust was cultured through three generations in the CT room on these plants, the previous generation being removed before symptoms showed on the newly inoculated plants, to eliminate the chance of any potential rust hyperparasites being passed-on.

The rust teliospores release their basidiospores under conditions of high humidity, as would occur during transit to India. Therefore, plants were shipped during the 2-10 day period after inoculation and before teliospores are fully viable. Rust inoculated plants were removed

from their pots, the roots carefully washed in water to removed all the growth media, and then wrapped in wet tissue, and the roots placed in a small plastic bag, tied loosely around the stem. The whole plants was then placed in a large self sealing plastic bag, the root bag was attached with tape to the side of the large bag to prevent movement during transit, and the large bag inflated and sealed to form a protective bubble around the aerial part of the plant. A number of these 'units' were placed in plastic boxes (so no movement was possible) and the boxes put in a polystyrene shipment box. Frozen ice blocks were wrapped in absorbent paper, and placed in the shipment box, gaps around the boxes and ice blocks were filled with polystyrene chips, to ensure no movement within the shipment. Appropriate labelling, including Indian import licence, addresses, contents etc were be attached to the outside of the box.

Puccinia spegazzinii was shipped to NBPGR, New Delhi (Figure 2), rather than PDBC, Bangalore because, as anticipated, the quarantine unit at PDBC was not completed in time. The plants can survive for at least 5 days in the shipment box, but to reduce the very real risk of customs holding-up the shipment and so resulting in plant death, the shipment was hand carried to India. Plant propagation material was prepared in advance and necessary equipment previously purchased (dew chamber was shipped to India from a UK supplier). The first shipment was taken at the time of the Inception workshop (June 2003). This was 3 months behind schedule, because of delays in obtaining clearance by ICAR to commence the project. Unfortunately, due to problems establishing the rust at NBPGR a further three shipments in September 2003, January 2004 and September 2004 were required before the host specificity testing could commence. During this time regular project meeting were held to discuss the reason for the lack of success and the way forward. Detailed notes were taken at these meetings and sent to the UK for comment and advice.

During this standardization process involving the establishment of the rust at NBPGR, the authority involved with the issuing of the import permits changed. With the ratification of the Indian Plant Quarantine Order (2003), a new application (dated 16 June 2004 in PQ Form 12) was submitted to the Plant Protection Advisor (PPA), Government of India, and approval (Permit No. 33/2004 in PQ Form 13; date of issue: 3 August 2004; valid up to: 2 February 2005; Blue/ violet label in PQ Form 14) was obtained for importing the fourth consignment of the rust into quarantine at NBPGR.

Figure 2. NBPGR Quarantine Unit, New Delhi. Note back-up generator outside unit. *Inset*: P.S. Kumar (PDBC) receiving first rust shipment from UK.

1.3 Completion of additional host specificity screening. ICAR set-up a committee to oversee the host specificity tests. Members included: Dr. O.P. Dubey, Prof. Rabindra (PDBC), Dr. E. Roshini Nayar, Principal Scientist (Economic Botany), and Dr. C.A. Ellison (CABI). A list of additional plant species and varieties that needed to be tested with the rust before it could be released into the environment was drawn-up in consultation with all the project collaborators and approved by the ICAR committee. A total of 74 plant species were included, 18 of which had already been tested during the original screening (55 species in total) at CABI, UK. Of these 74 species, 25 were from the same family as *M. micrantha,* and the rest were unrelated plant species, but of economic importance within the regions where the rust was potentially to be released.

Receiving and establishing P. spegazzinii

On 2 September 2004, the fourth rust consignment was received from CABI Bioscience, UK Centre (Ascot) at New Delhi. Three small plastic boxes containing 5 *Mikania* plants ('Peechi' ecotype inoculated with the rust isolate W1761 on 28 August 2004) formed the shipment. The plants were bare-rooted and did not show any symptoms on arrival. On the same day planting of all the 5 plants was done in individual big white plastic pots inside Bay 4 of the CQF, which had been kept clean. All the 5 plants from the UK produced good symptoms and telia formation was observed. All the host-specificity studies were carried out with the inoculum established from this fourth consignment. The rust was passed through *Mikania*, especially Kerala (Thrissur) ecotype, many times during 2004-2005. The pathogen is being maintained without any hyperparasites in the CQF.

Rust maintenance and inoculation procedure

Puccinia spegazzinii was maintained on living *Mikania* plants, by re-inoculation, approximately every six weeks, onto fresh plants, which were given a standard 24 hour dew period.

Puccinia spegazzinii produces basidiospores under high humidity conditions from cushions of teliospores that are embedded in the plant tissue. The teliospores are not released, and hence, the inoculum used in the experimental work was composed of infected leaf, petiole and/or stem. Two types of inoculation procedures were compared before selecting the suitable method. In the first method, test plants were sprayed with a fine mist of deionized water, and the inoculum suspended about 5 cm above the test plants using a thread attached to the top of bamboo supports. The plants were then placed for 24 hours in a dew chamber (Mercia Scientific, UK) set at 100% humidity and 20 ºC (Figure 3). Usually the inoculum was removed after 12/24 hours (varied). Although the start of basidiospore release occurs after 3 hours at high humidity, this process is clearly visible to the naked eye after 12 hours, as a white bloom over the brown teliospore surface. All inoculum was assessed at this stage for viability, and any test plants with poorly germinated inoculum over them were discarded. Inoculated plants were observed daily for the development of symptoms. In the second method, which was finally selected for conducting the host-specificity tests, the inoculum was spread over the grill-like tray just above the plants to be inoculated in such a manner that the pustules (for example, on the lower surface of the leaves) faced the healthy plants kept on the lower rack.

Within each test run, susceptible control plants were included and a 48-hour dew period used. Plants were monitored for twice the time taken for full symptom to develop on the control plants (approximately six weeks). The prolonged dew period ensured that every opportunity was given for infection of the test plants, and the extended monitoring time ensured that any delayed or latent disease expression would be picked-up. For each test

plant species, eight replicates were used. Plants were assessed using the scale below developed in Phase I of the project.

Pathogenicity score for the evaluation of Puccinia spegazzinii

0 No macroscopic symptoms

1 Necrotic or chlorotic spots on inoculated leaves - no sporulation

2 Abnormal infection site: chlorotic patches on leaves with very low teliospore production around edges of chlorosis.

3 Abnormal infection site: pustules reduced in size (<4mm) or with low teliospore production in relation to compatible host-pathogen interaction. Lower than compatible infection sites on petioles and stems.

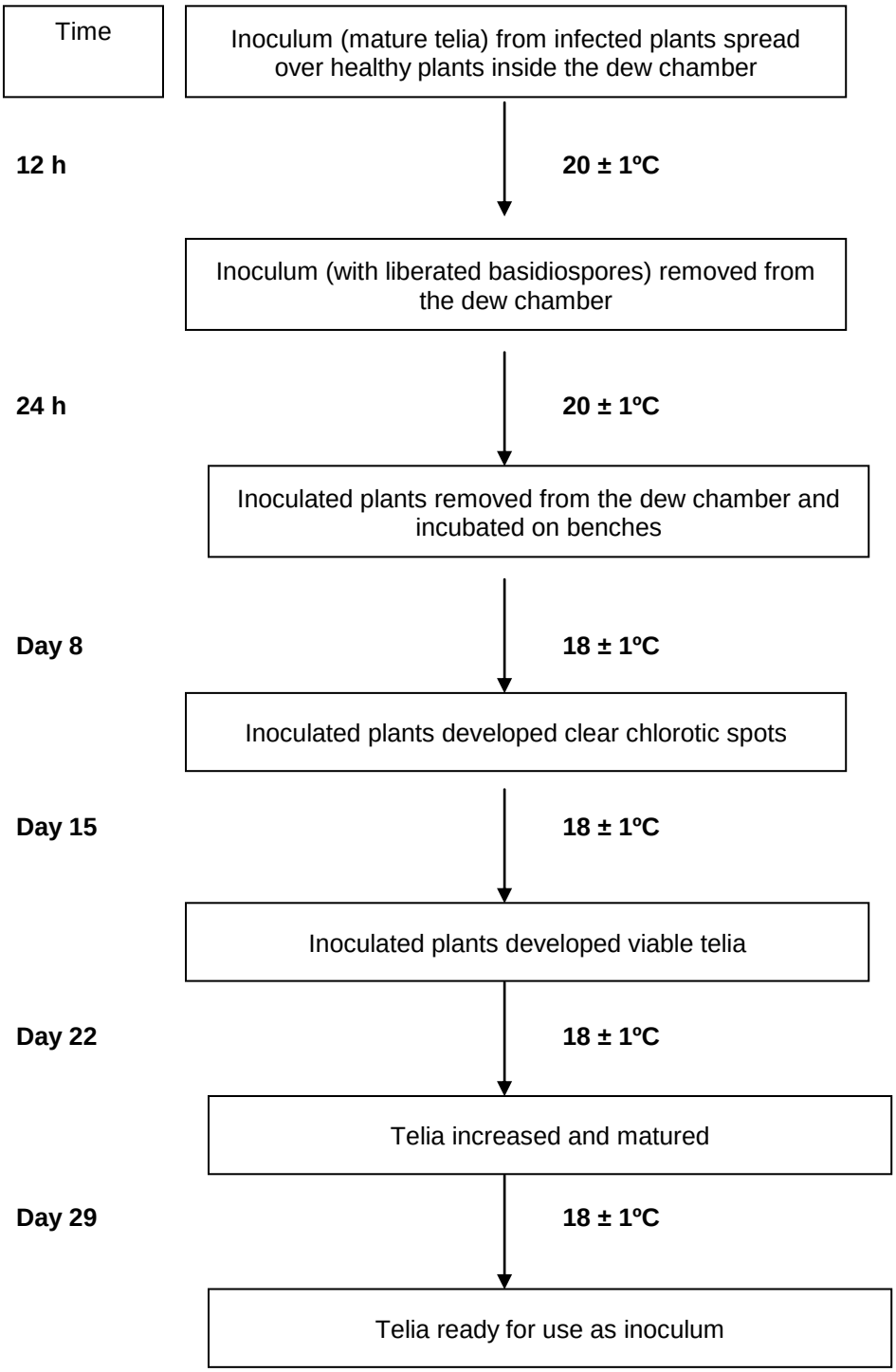
4 Fully compatible host-pathogen interaction. Normal pustule formation (4-7mm diameter) on leaves petioles and stems (Figure 4).

Figure 3. Host specificity tests at NBPGR, P.S. Kumar setting-up test run (left). Note dew chamber in the background, used for undertaking rust inoculations.
Figure 4. *Mikania* infected with the rust CBC agent, showing a fully compatible score 4. Note stem, petiole and leaf infection.

The stepwise procedure for inoculation of host plants and the sequence of events in the formation of mature telia in susceptible *Mikania* plants are indicated in Figure 5.

The screening was completed by April 2005 and release permit applied for to the Indian Plant Protection Advisor to the Government, Directorate of Plant Protection, Quarantine and Storage Department of Agriculture and Cooperation, Ministry of Agriculture (MoA).

Figure 5. Procedure for inoculation of *Puccinia spegazzinii*



1.4 Bulking-up of rust. Adequate numbers of *Mikania* plants inoculated with *P. spegazzinii* were prepared for distribution to release sites at AAU, Assam and KFRI, Kerala, once permission for the field release had been issued.

1.5 Rust storage studies. A study was undertaken at CABI Bioscience to find a suitable method for the long-term live-storage, of each pathotype of the three *Mikania* rust fungi, using liquid nitrogen (*Puccinia spegazzinii, Dietelia portoricensis* and *D. mesoamericana*). The main aim was to deposit voucher specimens in the CABI Culture Collection, for potential future reference. This would allow regulatory authorities to compare genetic, physiological and anatomical attributes with strains released into the environment, should this ever be required. In addition, it provides a back up in case of difficulty with field establishment. It is also important to maintain all the rust strains under evaluation in CABI Bioscience quarantine, since they may be of value to control additional invasive biotypes of the weed in the future. The work was published in *Cryoletters*: 'Development of a Cryopreservation Protocol for the Microcyclic Rust-Fungus *Puccinia Spegazzinii*' (see 6.2 Ryan & Ellison, 2002).

Rusts are biotrophic fungi and, therefore, can only be maintained in association with the plant host making long-term preservation laborious and expensive, and with a risk of genetic change of an isolate or even complete loss. Since rusts are not culturable *in vitro*, they are not amenable to traditional methods of cryopreservation. In addition, unlike many rust fungi, which liberate powdery, relatively thick-walled urediniospores that are readily cryopreserved "dry", *P. spegazzinii* (and the two species of *Dietelia*) only produces teliospores that are embedded in the host tissue and delicate basidiospores. Neither of these two spores types are acquiescent to direct plunge freezing.

A search of the literature failed to find reports of any preservation procedures for recalcitrant microcyclic rust fungi and this is not surprising as these rusts cannot be maintained *in vitro*. For most fungi, samples are preserved from propagules taken from *in vitro* culture as these can be relatively concentrated and easy to manipulate and are most are able to withstand the stresses that occur during the cooling and thawing stages of the cryopreservation procedure (Ryan *et al.,* 2000). However, various stages of this rust life cycle have the potential to be successfully preserved. These include the site of the *in situ* infection of the fungus with leaf, stem or petiole tissue; the teliospore stage or the basidiospore stage. The aim of this investigation was to develop suitable cryopreservation protocols for 10 isolates of *P. spegazzinii,* and one each of the two species of *Dietelia,* pathogenic to *Mikania* and to test the resultant viability by either the ability of the teliospores to produce further spore structures (basidiospores) and, in some cases, to initiate infection in the host plant.

Rust isolate selection and maintenance

Rust isolates were maintained on living *Mikania* plants (ex Peechi, Thrissur District, Kerala Province, India) grown in a 50:50 mixture of general-purpose peat based potting compost and JI number 2 soil based compost. This genetic type of the weed was found to be fully susceptible to all isolated of all the three rusts. Plants were kept in a quarantine glasshouse chamber (CABI Bioscience, Ascot, UK) with an air-conditioning unit set at 22 +/-5^{o}C, with a 12 hour -light/-dark cycle (metal halide, full spectrum, light intensity ranging from 8,000 to 13,000 Lux, depending on the time of year and weather conditions). Plants were inoculated by suspending mature, rust infected plant material (leaf, petiole and stem), 2-6 cm above the plants, using plastic coated wire ties attached to the top of plastic plant supports, after spraying the plants with a fine layer of distilled water. The teliospores, embedded in the host tissue, produce and release basidiospores under high humidity conditions, which are capable on infecting the new host tissue (meristematic tissue is the most susceptible to

infection by the basidiospores). Plants were placed in a dew chamber set at 100% humidity (Mercia Scientific, UK), at 20^{o}C for 24 hours to allow for this process to occur. This long length of dew period was used to ensure that the test plants had every opportunity for becoming infected with rust. Teliospores are mature approximately 18 days after inoculation. All rusted plant material used in this study was from plants that had been inoculated 18 to 28 days prior to use.

Cryopreservation of teliospores and basidiospores

Basidiospores were collected in sterile distilled water by attachment of infected plant tissue (stem, petiole, leaf) to the under-side of a 50mm diam. glass Petri dish using petroleum jelly, which was placed over a watch glass containing 1ml of sterile distilled water. Each "combi" was incubated for 4 hours at 20^{o}C to allow liberation of basidiospores from the infected material into the distilled water. Teliospores were collected by 'scratching' infection sites with a sharp scalpel blade over a Petri dish (50mm diam.) containing a 10% glycerol (aq) cryoprotective solution. Aliquots (500μl) of either teliospore or basidiospore suspension were transferred to sterile cryovials (1.5ml System 100, Nalgene, NY, USA), and cooled in a controlled rate cooler.

In-situ *cryopreservation*

Infected plant material was collected and characterised. Fleshiness (i.e. the moisture content of the plant material) was graded on a 1 to 5 scale (1 very dry material to 5 very moist material). Infection was graded as minimal, average and severe. Infection sites were separated from the host material; petiole and stem specimens were cut into 20mm long sections and leaf specimens were cut into *ca.* 15mm^{2} squares. Specimens were then collected into sterile cryovials and transferred to a controlled rate cooler.

Controlled rate cooling and cryopreservation

Samples were cooled in a Kryo 10 Series II controlled rate cooler (Planer Products Ltd., Sunbury, UK) at either 1, 5, 10 or 30^{o}C min^{-1}. For comparison, some samples were plunged directly into liquid nitrogen. Sample were stored in the liquid nitrogen vapour phase for at least 7 days and then thawed by direct immersion of each cryovial in a water bath maintained at 36^{o}C for *ca.* 2 minutes. Viability studies were undertaken immediately post-thaw.

Viability assessment

Viability was assessed by observation of teliospores and basidiospores under a light microscope. Basidiospores were germinated by suspending leaf/stem/petiole material over a watchglass in a humid chamber. Teliospores were considered viable if they produced a basidium, sterigma and basidiospores. Basidiospores were considered viable if they produced a germ-tube.

Pathogenicity testing

Pathogenicity testing was undertaken using the standard inoculation procedure given above. Although, due to its small size and flaccidity, the inoculum was suspended over the *Mikania* plants by attaching to Petri dishes on sticks, using petroleum jelly. All cryopreserved material used, was thawed less than one hour prior to suspension over the plants. In addition, basidiospores, produced by suspending the material over distilled water in a Petri dish for 4 hours, were placed directly on to plant shoots by: a) touching the shoot with the germinated teliospores, and b) removing the teliospores with forceps and placing them on a shoot.

2. Inoculative strategy developed:

2.1 Rust infected plants hand-carried to Kerala. Rust infected plants were hand-carried by Usha Dev from NBPGR to Kerala Forestry Research Institute in November 2005, and inoculated on to plants originating from Kerala, in the glasshouse, ready for release in the Western Ghats. At this time, CABI personnel were also present at KFRI. This was a great opportunity for collaborators to share experiences in handling and propagation of the rust, and for training of KFRI technical staff. Under this project a misting facility for inoculation had been constructed, similar to that at AAU.

Figure 6. Kerala Forest Research Institute (KFRI) rust propagation facility. Usha Dev (NBPGR) delivering rust consignment (upper left); outside view of facility (lower left); K.V. Sankaran showing purpose built mist chamber for rust inoculation (upper right); *Mikania* plants ready for inoculation and then field release (lower right).

2.2 Inoculative strategy implemented in Kerala.

The release permit issued by the MoA in June 2005 (Appendix 1) stated that the rust could be released at two sites in the Western Ghats. These sites were in protected area, in state owned forest sites, within the previously established, permanent sample plots:

a) Vazhachal, Thrissur Forest Division (1 Hectare)

b) Kottapara, Malayattoor Forest Division(I Hectare)

Figure 7. Teak plantations, 10 years old. Designated rust release site at Vazhachal, Kerala (left); note teak trees in foreground and going up the hill, stunted or killed by *Mikania* infestation. Teak trees where *Mikania* was successfully manually controlled (right).

Unfortunately, the release of the pathogen in the natural forest areas did not proceed according to schedule. This was because further clearance had to be obtained from Kerala State Forestry Department (KFD) by KFRI, and permission was not forthcoming. The KFD officials required wider consultations before a permit was issued for release. Dr. J.K. Sharma, KFRI Director, took up the matter through the Kerala State Council for Science, Technology and Environment and a positive response has now been received from the KFD.

The pathogen has been multiplied and is being maintained in the KFRI glasshouse facility. Major release programs are anticipated as soon as the southwest monsoon begins (June-July 2006).

3. Inundative strategy developed:

3.1 CABI consultancy to Assam. Sean Murphy and Carol Ellison from CABI Bioscience undertook a consultancy to Assam, during the same visit as the Inception Workshop, in June 2003 to:

- Establish the work programme with Assam Agricultural University;
- Advise on the construction of the rust mass production unit;
- See the Mikania problem in the field (both in agro-ecosystems, forestry and Kasiranga National Park);
- Look at the potential sites for the permanent plots.

3.2 Investigation of plant/pathogen propagation methodology. A rust propagation unit (RPU) was constructed at AAU consisting of a shade house with an attached mist inoculation chamber (Figure 8). Prior to the arrival of the rust, *Mikania* plant propagation methodology was investigated. Since September 2005, after the arrival of the rust, techniques for mass production of rust infected plants has been investigated.

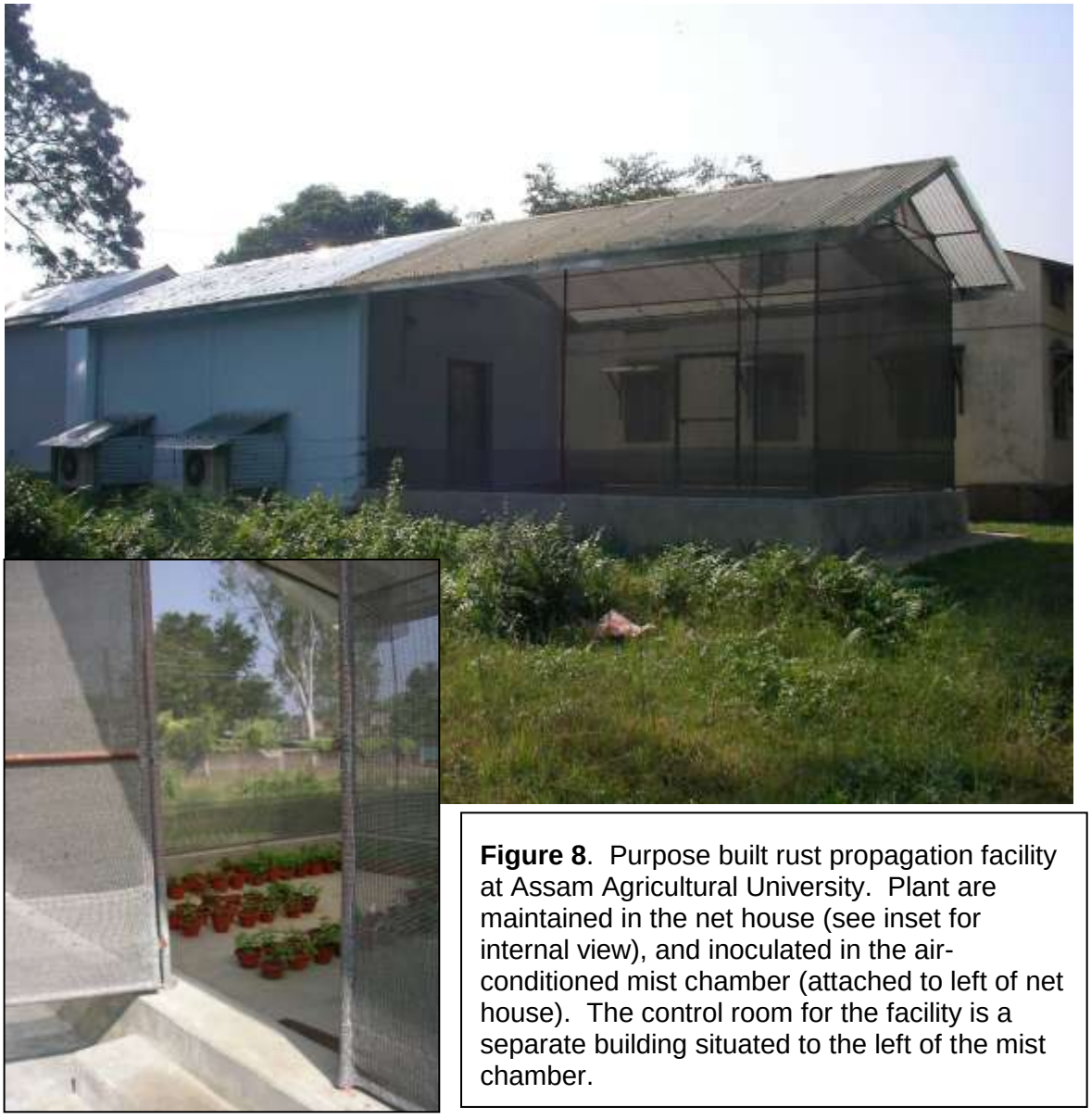


Figure 8. Purpose built rust propagation facility at Assam Agricultural University. Plant are maintained in the net house (see inset for internal view), and inoculated in the air-conditioned mist chamber (attached to left of net house). The control room for the facility is a separate building situated to the left of the mist chamber.

3.3 Inoculated plants hand-carried to Assam. Three consignments of rust infected plants were brought from the NBPGR New Delhi, to AAU in Assam. The rust was inoculated onto pre-grown *Mikania* plants already established in the RPU.

Rust consignment 1: The first consignment of rust infected plants were taken by road to AAU in July 2005 and consisted of 26 infected plants. The rust was inoculated on to 30 plants originating from Borbheta, Jorhat, Assam, in the purpose-built RPU, using the standard inoculation technique, reported in 1.3 above. After inoculation, the plants were maintained in the net house (35% agro-shade net) attached to the mist chamber (35.5oC +/- 2oC maximum and 29+/- minimum).

Rust consignment 2: The second consignment was transported by air in September 2005 and consisted of eight plants. The rust was inoculated on to plants originating from Borbheta, Jorhat, Assam (24 plants) and Kerala (6 plants). No genotypes from Kerala that had previously been tested (FTR Phase I), showed resistance to the Trinidad isolate of the rust. The inoculated plants were maintained in an air-conditioned (AC) chamber, set at 25oC with a 12-hour light/dark cycle, during symptom development.

Rust consignment 3: The third consignment was transport by air and these plants used to undertake the second field inoculation at CTE (see 3.4 below).

3.4 Inundative strategy implemented in Assam.

Field release of P. spegazzinii

The release was made in two different sites in Jorhat, Assam on two different dates:

a) Experimental Garden for Plantation Crops, AAU (EGPC), 1st week of October 2005.
b) Cinnamora Tea Estate (CTE), 1st week of November 2005.

a) EGPC. The release at EGPC was undertaken using six pots of *Mikania* infected with the rust: three from Assam and three from Kerala. These infected plants were selected from those plants inoculated with rust consignment 2 (see 3.3 and Table 8).

The plants were set in the ground, in a shady place, in a dense stand of *Mikania*, by digging a pit 20 cm diameter and 30 cm depth for each pot, separated by between 1 and 3.75m. These were watered twice daily in the morning and evening. Growing shoots of the plants in the surrounding vegetation were, as much as possible, pulled underneath the infected leaves, stems and petioles of the source plants. The inoculated site was sprayed heavily with a fine mist of distilled water twice a day (morning and evening) for 15 days, except on rainy days. The inoculation was done just after rainfall (118 mm). Average temperature recorded during the 15-day period was 20-30^{0}C with a RH of 85-90%. The release site was regularly monitored and the rust progress recorded.

b) CTE. The release at CTE was carried out with rust infected *Mikania* plants from Kerala only. The plants were potted in earthen pots (25 cm diam.) containing 5 kg of cow dung and field soil (50:50). It was necessary to use a modified inoculation strategy used at EGPC, because the *Mikania* leaves were very sparse at ground level, so the plants could not be set in the ground. Instead, infected plants were hung in a dense stand of *Mikania* at the level of tea table in a shady place, suspended from a bamboo pole with rope (Figure 9). Two groups of 3 plants were set up, approximately 3-5 metres apart. The release site was maintained the same as a).

Figure 9. Rust release site at Cinnamora Tea Estate. Pots with rust infected plants hanging in tea/*Mikania* canopy (left); tea estate worker spraying rust site with water in the evening to encourage infection by increasing the humidity (right).

3.5 Inoculative strategy implemented in Assam. An inoculative release programme will also be undertaken in the forested regions, as is planned for the Western Ghats, but this is now not within the scope of this project.

4. Farmer and forest department information campaign implemented:

4.1 CABI consultancy to KFRI and Assam to discuss farmer campaign. Over the course of the project, a series of meetings (under this project and while CABI project staff were with collaborators on other funded business) were held between CABI personnel and project collaborators form KFRI, AAU and PDBC to discuss and develop an information campaign, about the implementation of CBC, for farmers and foresters. Under this project, a progress meeting was held at NBPGR New Delhi, in November 2004 and was attended by most key project collaborators. This meeting covered all aspects of the progress of the project including the farmer campaign.

4.2 Workshops undertaken in Assam and Kerala.

Assam: A workshop was undertaken at AAU in Assam in November 2005, for the training of extension workers in agriculture and forestry and students, in the concepts and principles of biological control of weeds (see banner below). Also, to inform these groups about the *Mikania* project and the practicalities of the management approach.

Participants from the workshop also went out to the CTE to see the rust in the field. A final project meeting was held between CABI, AAU and PDBC to discuss the continuation of the work.

Kerala: KFRI in Kerala, since there were additional problems that need to be resolved before the rust could be released in the field (see 2.2 for details), it was decided to commute the intended workshop into a stakeholders meeting for KFD Officials and representatives from Kerala Agricultural University (KAU). This was held in November 2005, just prior to the Assam workshop.

4.3 Educational material produced & campaign implemented: Following the issuing of the release permit by the MoA, ICAR attached a subsequent condition, that the release of the rust could not be publicised amongst planters, farmers and forestry workers until field efficacy had been established. This, together with the delays in the initiation of the project and the subsequent delays caused by the time take to establish the rust at NBPGR, has meant that the farmer information campaign could not be implemented within the time frame of the project. However, relevant educational material is being developed for these groups, and will be produced in local languages both in Kerala and Assam. As soon as the rust is fully established in the field and its impact is visible, the campaign will be implemented in reas where rust is released.

5. Impact studies:

5.1 Selected sites regularly monitored; impact studies initiated in both Kerala and Assam. Rust impact studies could not be initiated within the scope of this project. However, rust monitoring procedure and protocols were discussed in depth between collaborators, and protocols established.

In Kerala, the monitoring of the level of *Mikania* infestation, in the permanent sample plots in the forest sites, had been undertaken in Phase I, and repeated during the time between the two phases of the project. However in Assam, the level of *Mikania* infestation in the permanent sample plots at the release sites (EGPC and CTE) were assessed under this Phase II, and are give below.

Permanent *Mikania* sample plot monitoring in Assam

The fixed plot monitoring was conducted from April, to December 2004 at two sites: Cinnamora Tea Estate, Hatigarh Division, Jorhat (CTE) and the Experimental Garden for Plantation Crops, Assam Agricultural University, Jorhat (EGPC). Plot areas of 1.0 ha and 0.2 ha in the first and second sites respectively were chosen for the monitoring. Both plots were chosen from areas with a high density of *Mikania* plant and were selected in consultation with the tea estate managers.

Each plot was divided into two blocks: in one the *Mikania* infestation was left to run its natural course while in the other the weed infestation was kept weed free (the latter was for the impact study and further details are given later).

The monitoring of the annual outbreak of the *Mikania* infestation was started just at the onset of monsoon, i.e., mid April and was continued up to the flowering period (in December; this was done every 15 days). The abundance of *Mikania* at each interval and in each block was measured as follows: Fifty $1m^2$ quadrates were chosen at random in each plot; the number of individual *Mikania* plants in the quadrate was then counted; from this, the total number of plants per ha was estimated from the mean. These were assigned a grade number (Table 1) to provide an index of abundance.

Table 1. Severity scale for quantification of level of infestation with *Mikania* under tea ecosystem

Level (Grade)	*Nos. of stalks of plants per ha*	
	Original	**Modified**
Absent (-)	0	0
Negligible (0)	1-10	1 – 200
Scattered (1)	10-25	201 – 400
Low (2)	25-50	401 – 600
Moderate (3)	50-75	601 – 800
Medium (4)	75-100	801 – 1000
High (5)	Above 100	Above 1000

Climate data was also recorded as follows: maximum and minimum temperatures, morning and evening relative humidity (RH), rainfall, and bright sunshine hours (BSSH). A multiple regression analysis was made of the *Mikania* densities in the plots versus these variables to identify any associations.

Impact from the fixed plot sampling

Details of the selection of the plots and the blocks at the two sites were given above. In the weed free blocks, all management practices were kept similar to that of a well-managed commercial tea estate. Weeding was by sickling, cheeling (using a cheel hoe) or forking (using a fork hoe). In terms of labour, the total cost of weeding per ha, taking four rounds in a season was estimated this was done for each of the sites. Yield of tea was estimated by comparing the production of made tea obtained from the mean data of the *Mikania* free and the *Mikania* infested areas. Yield data of tea was taken as total green leaves plucked during the year. Made tea (kg / ha) was estimated by dividing the total yield of green leaf with a conversion factor 4.77 assuming an average recovery of 21%.

5.2 Inundative release monitored through farmer surveys. This activity, to be undertaken in Assam, can only take place once the inundative rust release strategy has been fully implemented, which will not be until after the end of this project funding period.

5.3 Economic assessment initiated. Pre-release farmer surveys to establish the cost of controlling *Mikania* were conducted in Kerala during Phase I of this project. These results will be used to compare to the impact of the weed post-release, in future years. Both the directors of KFRI and AAU have agreed that the monitoring and impact work will continue under the support of each institute.

In Assam, a pre-release farmer survey has been undertaken under this Phase II of the programme, and is described below.

Distribution, agricultural importance and impact of *Mikania* in the tea gardens of Assam

Information about the distribution of *Mikania* in the northeastern States of India is patchy and largely only available in unpublished reports. Most of information is also qualitative in nature and it is difficult to understand the true impact of *Mikania* on crop production and on natural ecosystems.

Studies were conducted in Assam to understand more comprehensively the geographical distribution and abundance of *Mikania* in this state, and also to determine the impact of *Mikania* on tea production. The methods used are based on those of Sankaran *et al.* (1999); these authors developed robust survey techniques for *Mikania* in the Western Ghats.

Assam state is divided into six major agro-climatic zones. Tea is mostly produced in three of these: Upper Brahmaputra Valley (UBVZ); North Bank Plain (NBPZ); and, Barak Valley (BVZ). The climatic and environmental parameters of theses are given in Table 2.

Table 2. Climate and environmental parameters of the major tea-growing zones of Assam

PARAMETERS	TEA GROWING ZONE		
	Upper Brahmaputra Valley	**North Bank Plain**	**Barak Valley**
Climate	Hot humid	Per humid characterized by mild summer and winter	Humid
Annual temperature	>22°C	>23°C	>22°C
Annual precipitation	1600-2550 mm	-	2,277 mm
Potential evapo-transpiration	1000-1450 mm	-	1,219 mm
Soil type	Very deep, moderately well-drained, medium to light texture	Shallow to deep imperfectly well-drained, slightly loamy to loamy skeletal	Well to excessively drained, deep loamy to fine textured

Tea holdings vary in size across these zones. Zone-specific data on holding size is not available but data for the whole of Assam (Table 3) gives an indication of the different types.

Table 3. Area under tea plantations in Assam

Tea Gardens	Number of gardens	Area (ha)
Small tea growers	> 38393	> 56,871.00
Corporate Sectors	16	6981.30
Company/Estates	>700	>508000.00

Distribution and agricultural importance

The geographical survey was conducted in total 85 tea gardens situated in the three different agro-climatic zones of Assam (Table 4).

Table 4. Surveyed area for *Mikania* infestation in Assam in different zones

Agro climatic zone	Number of Tea garden surveyed
Upper Brahmaputra Valley Zone (UBVZ)	35
Lower Brahmaputra Valley Zone (LBVZ)	-
Central Brahmaputra Valley Zone (CBVZ)	-
North Bank Plain Zone (NBPZ)	36
Hill Zone (HZ)	-
Barak Valley Zone (BVZ)	14
Total	**85**

The 85 teagardens were selected as follows: Initially 320 survey questionnaires covering garden type and problems with *Mikania* etc. (see Appendix 2 – further details about this questionnaire are given under the impact studies) were distributed to tea gardens of all holding sizes in these zones; these gardens were chosen at random. Of those gardens that responded, 50% were selected for the survey. Of the 85 gardens, 33 were small tea growers (STG), three were corporate sector and rest 49 gardens were Estate sector (Table 5); a map showing the locations of the gardens is shown in Figure 10. STG are registered tea gardens of Small Tea growers Advisory Programme (STAP) with a maximum area of 10.12 ha under cultivation. Corporate sectors are under the Assam Tea Corporation, Government of Assam, while the Estate gardens are under Company sectors. The survey was made during the peak growth time of *Mikania* (July to October, 2005). Each garden was visited once during this period (Figure 11).

Figure 10. Location of Surveyed Gardens

Figure 11. Project scientists discuss *Mikania* problem and attitudes to CBC with tea grower in Assam.

Table 5. Surveyed gardens for *Mikania* infestation in Assam (different districts)

Zone	District	No. of tea growers			Total
		STG	Corporate Sectors	Estate /Company	
Upper Brahmaputra Valley Zone	Jorhat	6	3	10	19
	Sibsagar	2	-	3	5
	Dibrugarh	3	-	3	6
	Tinsukia	2	-	3	5
North Bank Plain Zone	Lakhimpur	10	-	4	14
	Sonitpur	6	-	8	14
	Darrang	4	-	4	8
Barak Valley Zone	Silchar	-	-	10	10
	Hailakandi	-	-	2	2
	Karimganj	-	-	2	2
Total		**33**	**3**	**49**	**85**

The density of Mikania plants in each garden was estimated as follows: in each garden three areas of 1 ha each infested with Mikania were selected at random (if available) and the mean number of plants/ha were calculated from 50 quadrates of $1m^2$ taken from each area. The mean of these three areas was then calculated. These were assigned a grade number (see Table 1. in 5.1 above) to provide an index of abundance (Sankaran et. al., 1999, modified by Puzari et al., 2004)

Impact studies from the geographical survey

Studies were conducted to measure the impact of *Mikania* on tea production in Assam. This was done by collecting further information from the tea gardens selected for the survey studies outlined above. Impact was measured as:

- Cost of cultivation of tea – by measuring the extra labour needed.
- Loss of yield of tea.

These parameters were measured from further information collected during the two survey studies. A comparison could then be made between the farm situation and under experimental conditions, as described in 5.1 above. The experimental study allowed the actual monetary costs to be estimated.

In the questionnaires (see Appendix 2), questions were included about the costs of cultivation of tea and the major constraints and the labour needed for weeding *Mikania*; farmers were asked to compare their experiences from areas in their farms with and without *Mikania*.

For yield loss the following two methods were used. The first was on the basis of information's given by the respondents for entire garden. The second was by comparing the production of made tea obtained from the mean data of three *Mikania* free and the three *Mikania* infested areas of same tea age, same variety grown and same production practice. Yield data of tea was taken as total green leaves plucked during the year. Made tea (kg /

ha) was estimated by dividing the total yield of green leaf with a conversion factor 4.77 assuming an average recovery of 21%.

Data from all size holdings for each of the three agro-climatic zones were pooled.

5.4 CABI consultancies in years 3 and 4. See 4.1 and 4.2

6. Results publicised with and adopted by stakeholders.

This project has been very well publicised, through scientific publications, media reports, local (Kerala) TV programme, popular articles, press releases, local and UK press reports.

6.1 Public awareness campaign implemented (see 4.3)
The local poster and leaflet campaign aimed at farmers and forestry workers had to be postponed due to government restrictions, but this aspect will be implemented once the rust is established in the field (see 4.3 above). However, an extensive international media campaign was successfully implemented.

6.2 Preparation of scientific report and papers.

Normal NRI'nt reporting procedures (quarterly and annual reports) were adhered to during the course of this project and CABI internal back-to-office reports were prepared after each overseas trip. In addition, a number of reports and scientific papers were prepared.

6.3 Long-term dissemination. The long-term dissemination of the results from this project can be considered from three perspectives:

a) Achievements during the implementation of the project in the development of Indian government policy;

b) Interactions with ICAR concerning development of projects for invasive alien species control;

c) Short project R8502, funded by NRI'nt to help broadened the long-term dissemination outputs from this project.

Additional Activities

First National Stakeholders' Workshop, Kathmandu, Nepal. During the course of this project a number of people from Nepal had contacted CABI and KFRI concerning the growing *Mikania* invasion in Nepal; Dr. Hem Sagar Baral, at the time working for Bird Conservation Nepal was the key contact. Dr. Baral, now working for Himalayan Nature [HN], was able to establish a link with IUCN, Kathmandu, and using their venue, free of charge, organised a *Mikania* workshop. This took place on 25th November 2004 and interested stakeholders from agriculture, forestry, conservation and tourism were invited to discuss the *Mikania* problem in their country. CABI (through the travel budget of R8228) was able to provide a small amount of funding to hold this meeting (lunch, refreshment and cost of printing the proceedings from the meeting). This workshop was organised to coincide with the time that the NRI'nt-*Mikania* project meeting was held in New Delhi (see 4.1), hence enabling S. Murphy and C. Ellison to attend, in order to present the current status of the *Mikania* biological control project in India.

Figure 12. Participants at the first national workshop for stakeholders on *Mikania* weed invasion in Nepal.

The meeting was well attended by all relevant sectors, and was unusual in that such interactions between these groups are rare (Figure 12). In addition to the organisers (CABI and HN), there were representatives from Central Department of Botany; Bird Conservation Nepal; Department for National Parks and Wildlife Conservation; Department of Plant resources; Department of Forest Livelihoods and Forestry Programme (Terai); IUCN Nepal; Nepal Agricultural Research Council; King Mahendra Trust for Nature Conservation; and Federation of Community Forest User Groups in Nepal (FECOFUN).

Outputs

1. Biocontrol agents prepared for release in India:

1.1 Inception workshop. A proceeding of this workshop was produced: 'Inception Workshop on the collaborative project "Classical Biological Control of *Mikania micrantha* with *Puccinia spegazzinii*—implementation phase" held from June 27-28, 2003 at NBPGR, New Delhi'. The following conclusions were drawn-up during the Plenary Session;

a) The technical programme of the project as decided during the Workshop must be implemented in right earnest by all the partners.
b) Dr. Usha Dev, Pathologist, Plant Quarantine Division, NBPGR should be co-opted as Co-Principal Investagator in the project. Action should be initiated by the Project Director, PDBC, Bangalore.
c) Funds should be made available to all the partners well in time by CABI-UK. The funds earmarked for quarantine facility should be transferred to NBPGR. In case, the funds earmarked for quarantine work are not found to be sufficient, the same may be managed from the overall savings out of the project funds. Action to be initiated by PDBC/NBPGR.
d) The venue of the future meetings/workshops should be rotated appropriately. The renowned pathologists of the country should be invited to participate in the Workshops**.**
e) The issues relating to the security and sensitivity must be addressed by all concerned in right earnest.

f) Project Director, PDBC should visit each center once in 6 months for monitoring the progress and constraints, if any.
g) The cost towards hiring of the power back up generator to be met out of the project funds.
h) Extra care should be exercised at the time of establishment of the infected plants in the transgenic containment facility. Two pathologists should be present upon arrival of the rust shipment from the UK (Dr. Kumar and either Dr. Sankaran or Dr. Puzari). The protocol give above must be strictly adhered to.
i) The recruitment of the sanctioned staff must be put in place immediately.
j) Mr. Ram K. Dwivedi, Manager, CABI-India, will coordinate the various activities of the project as required from time to time.
k) The protocols for issue of permit for release of rust in India for field-testing by the partners should be ensured and the requisite formalities completed as per guidelines on the subject.

1.2 Rust culture prepared & shipped to India. The plants arrived in India in good shape from all four hand-carried shipments. All the plants in the first shipment died within a week, it was thought to be possibly because of a failure in the back-up generator, after a power cut, and hence over-heating of the chamber. This was the first project to be undertaken at the new NBPGR facilities and hence, teething problems were unsurprising. The second and third shipment in January and September 2003 established, but the rust died out, this would appear to be due to a number of compounding factors:

- The staff trained in handling the rust during Phase I from KFRI, PDBC and AAU, could only be present intermittently at NBPGR, (due to other project commitments at their institutes.
- In-house temporary rust inoculation facilities were inadequate for the precise requirements needed to establish the rust. The specialised dew chamber (sent from the UK) arrived between shipments two and three.
- Even though training had been received, the technical expertise required to establish and maintain the rust took time to develop and for protocols to be established.

This process, in essence, one of Indian project staff taking ownership of the implementation of the project, can be considered to be a major achievement of the work. The utilization of fungal pathogens for the CBC of invasive alien weeds in a new technology; not just for India, but for Continental Asia. This project has successfully opened the door to this weed management approach, for which many weed-pathogen targets are waiting to be exploited. Indeed, PDBC have already successfully attained funding to import a rust pathogen for the control of *Parthenium hysterophorus*.

The four and final rust shipment arrived 28th August 2004; was successfully established, and hence, the screening could commence. At this stage the project progress in India had been delayed by nearly 1.5 years.

1.3 Completion of additional host specificity screening. The host specificity testing is given in Appendix 3. In summary, none of the 74 plant species tested in the additional host-specificity testing could be infected with *P. spegazzinii*. All the *Mikania* control plants included in each test run were severely infected with the rust. In the case of sunflower, mild chlorosis was observed on a few top leaves that were directly under the heavy inoculum inside the dew chamber. However, the leaves recovered during further incubation and were observed until senescence had occurred, in order to check for latent infection. It was a hypersensitive resistance response to high inoculum load, there was no telia formation, or in other words, true infection by the rust did not happen. This phenomenon was observed in only 4 cultivars out of the 9 different cultivars screened. All the inoculated sunflower plants showed normal growth and flowering, further confirming their non-susceptibility to *P. spegazzinii*. In the screening undertaken at CABI Bioscience, UK, during Phase I of the

project, mild chlorosis had also been seen on sunflower, but there also was no true infection of the plants.

On 27th June 2005 the Plant Protection Advisor to the Government of India gave permission for a 'limited' field release of the rust (See Appendix 1). All collaborators understood that a limited release of a pathogen is not realistic, once released there is minimal, probably no chance, of retrieving the rust. Nevertheless, this was the type of permit issued and it was under this that the release was carried-out.

1.4 Bulking up of rust. The rust infected plants were ready at NBPGR to be transported to Assam and Kerala by July 2005.

1.5 Rust storage studies.

Direct cryopreservation of teliospores and basidiospores

Cryopreservation of teliospores and basidiospores was not successful. No spores were viable after controlled rate cooling at -1, 5, 10 & 30^{o}C min^{-1} and after plunge cooling. The addition of a cryoprotectant (10% glycerol) to each sample had no effect.

In-situ cryopreservation

Success with *in situ* cryopreservation techniques was initially mixed. Freezing of infected leaves (either wrapped in aluminium foil or cut and placed in a cryovial) was not successful, even with the application of different cooling rates. This was because water became displaced form the leaf tissue immediately after thawing and saturated the infection sites. Cryopreservation of infected petiole and stem tissue at cooling rates of -5, -10, -30^{o}C min^{-1} and by plunge cooling was not successful. However, most replicates were viable after application of a cooling rate of -1^{o}C min^{-1} (summarised in Table 6). Replicates were considered viable if the teliospores produced sterigmata and basidiospores, although the extent and degree of germination varied. For example, both petiole and stem replicates of isolate W 2020 exhibited very limited production of sterigmata and few basidispores whereas both petiole and stem replicates of isolate W1868 exhibited very high production of sterigma with many basidispores.

Pathogenicity testing

Host plant infection was not achieved with any of the three techniques employed with any of the isolates tested. However, although primary systemic infection was not induced, some limited signs of host-pathogen interaction were observed on some of the test plants, in the form of brown flecks (hypersensitive response) on the leaves.

Table 6. Isolate designation / histories and summary of viability after *in situ* controlled rate cooling (-1^{o}C min^{-1}) cryopreservation of stem and petiole tissue

ID	Isolate	Geography Country (Region)	Type	Description (see below)	Viable
Puccinia spegazzinii					
1	W1958	Ecuador (Lita, Imbabura Province, western Ecuador.)	Stem	F:4 I:M	NO
			Petiole	F:5 I:S	YES
			Stem	F:2 I:M	YES
			Petiole	F:4 I:A	YES
2	W1693	Brazil (San Miguel do Anta, Viçosa, Minas Gerais)	Stem	F:1 I:S	YES
			Petiole	F:3 I:S	NO
			Stem	N/A	YES
			Petiole	N/A	YES
3	W1690	Brazil (Victoriano Velosa, Prados Rd., nr. Tiradentes, Minas Gerais)	Stem	F:5 I:A	YES
			Petiole	F:5 I:A	YES
			Stem	F:2 I:S	YES
			Petiole	F:3 I:S	YES
4	W1868t	Costa Rica (Rio de Madre de Dios, Siquirres-Guápiles [Km 47], Limón. Alt. 160m)	Stem	F:5 I:A/S	YES
			Petiole	F:4 I:A/S	YES
			Stem	F:2 I:A	YES
			Petiole	F:3 I:A	YES
5	W2020	Argentina (Sendero Macucu, Rio Iguassu)	Stem	F:1 I:A	YES
			Petiole	F:2/3 I:A	YES
			Stem	F:2 I:M	YES
			Petiole	F:1 I:M	YES
6	W1694	Brazil (Maripó, Minas Gerais)	Stem	F:4 I:A	YES
			Petiole	F:3 I:A	YES
			Stem	N/A	YES
7	W1692	Brazil (Rio Guaxulo do sul, nr. Mariana, Minas Gerais)	Stem	F2 I:S	YES
			Petiole	F2 I:S	YES
			Stem	F2 I:S	YES
			Petiole	F:2 I:A	YES
8	W1761	Trinidad (Parrylands oilfield, Guapo, South-west Trinidad)	Stem	F:2 I:A	YES
			Petiole	F:2 I:A	YES
			Stem	F:4 I:S	YES
			Petiole	F:1 I:M	YES
9	W1949	Trinidad (Mount Catherine [Mount Pleasant range], Chaguaramas, North-west Trinidad)	Stem	F4 I:A	YES
			Petiole	F4 I:A	YES
10	W1960	Ecuador (Rio Pucuño, Loreto-Pangayacu Road, Napo Province, eastern Ecuador)	Petiole	F5 I:S	YES
			Stem	F2 I:A	YES
Dietelia portoriscensis					
11	W1868t	Costa Rica	Petiole	F5 I: S	YES
			Stem	-	YES
Detelia mesoamericana					
12	W1868a	Mexico	Petiole	F5 I:M	YES
			Petiole	F4 I:S	YES

Isolates 3,4,5,6 & 7 were tested for pathogenicity. N/A= not analysed. **F=** Fleshiness (1 very dry material to 5 very moist material). **I**= Infection (minimal, average and severe)

Discussion

Non-culturable microorganisms, especially microcyclic rust fungi, are notoriously difficult to cryopreserve. Importantly, this is the first report of a method for cryopreservation of *Puccinia spegazzinii* and indeed any rust with an imbedded spore stage. However, there are a number of limitations both to the method and the extent of the future use of cryopreserved specimens. Both of the methods that employed traditional techniques of cryopreservation for fungi were not successful. The collection and cryopreservation of teliospores did not produce any viable material post-preservation, this was probably because of the effects of condensation moisture, which inhibits the ability of the teliospores to produce basidiospores rather than the direct effect of cryopreservation *per se*. However, the failure of the method for the cryopreservation of basidiospores was probably a result of the concentration effects that occur during cooling and thawing as no basidiospores exhibited any ability to germinate post cryopreservation. Due to poor initial results and logistical constraints, neither of these methods were pursued, a particular technical difficulty with these method was in obtaining enough spores to cryopreserve and the time taken to achieve this.

The *in situ* method was relatively simple and very successful, with most isolates and replicates surviving cryopreservation. Viability was indicated by analysis of the ability of teliospores to produce sterigma and basidiospores, but plant infection was not achieved using the post-cryopreserved produced basidiospores. This may have been due to either, the inability of the basidiospores to reach the host, or that they had lost their pathogenicity. In the former case, basidiospores may have been prevented from being released, by being trapped in a water film. There is evidence that basidia can grow through a thin water film, to enable the release of basidiospores into the air (Shaw, 1991). However, microscope observation of the tissue showed extensive release of the internal water, that a basidium would be unlikely to penetrate. It cannot be pre-determined how long after thawing this will take place. In some cases, films of yeasts were observed to grow on the material and this too may have negatively influenced the germination of the teliospores. Cryopreservation is known to affect the virulence of some pathogens (Hajek *et al.,* 1995), and it may be that the cooling and thawing procedure affected the ability of the basidiospores to induce pathogenesis either because the spores have become physically damaged or their physiological integrity has become compromised. This is substantiated by the observed hypersensitive reaction, suggesting that the basidiospore penetration process had been terminated by the host response. The basidiospores and germinating teliospores placed directly onto plant shoots did not result in infection, this also adds evidence to the argument, particularly since infection has previously been achieved by spraying a suspension of freshly produced basidiospores onto a plant (C.A. Ellison, unpublished data).

Despite the material not retaining pathogenicity, the success in obtaining viable material post-preservation cannot be underestimated. Preserved material can be used as reference material for morphological and anatomical investigations. In addition, fresh material (basidiospores) can be obtained from the cryopreserved material to allow molecular investigations to be undertaken, for example genetic fingerprinting and analysis of fungal gene function (Ryan *et al.,* 2001). Perhaps more importantly for biocontrol strains, it provides a permanent reference for strains released into the environment and may satisfy the legal prerequisites of patent legislation should it be required.

A number of parameters with the *in situ* cryopreservation method cannot be easily controlled such as the differences in water content "fleshiness" of the host material and the severity of the rust infection, because there are too many variables to manipulate. The presence of other contaminating opportunistic microorganisms especially yeasts is also a problem because sterility would be very difficult to achieve in glasshouse experiments. "Fleshiness" and the severity of infection were noted prior to cryopreservation but no direct correlation between these parameters and the resultant viability was evident. Nevertheless, after

infected leaves were resuscitated from cryopreservation water was displaced from the leaf tissue, saturating the infection pustules, and reducing the ability of the teliospores to germinate. The stem and petiole tissue was generally much drier, and moisture did not appear to be such an over riding problem post preservation. Therefore 'drier' material would be selected in any future experiment. The application of different cooling rates had a marked effect on the viability of the samples. Faster cooling rates proved to be lethal, but the application of a slower cooling rate of $-1^{o}C\ min^{-1}$ was successful. This corresponds with previous research, which suggests that slower cooling rates that prevent the formation of intracellular ice are generally suitable for most fungi including the Basidiomycotina.

In summary, great progress has been made in the development of a protocol for the *in situ* cryopreservation of recalcitrant plant pathogens; the techniques developed could be adapted for other rust and obligate fungi. However, additional research is required to corroborate the findings that the basidiospores may have lost their pathogenicity during the preservation process. Technology transfer between disciplines is essential when working with specialist groups of microorgansisms, in this example a competent plant pathologist was required to verify the viability of the organisms**.** Alternative cryopreservation techniques may work for cryopreservation of recalcitrant rust fungi but will require extensive development. Potential methods could involve the preservation of rust-infected meristematic tissue that could be preserved using plant cell culture cryopreservation techniques and the application of desiccants to cryovials during cooling and thawing.

The results showed that an *in situ* cryopreservation technique was the only method identified as having any potential for the long-term cryopreservation of the 10 *P. spegazzinii* isolates and 2 *Dietelia* species that were tested. Material from either petiole or stem tissue remained viable after cryopreservation determined by the ability of the preserved material to produce basidiospores. However, despite great progress being made in developing an optimal cryopreservation technique, infection of the host plant by the basidiospores produced from thawed, previously cryopreserved teliospores, embedded in leaf petioles, was not achieved. However, living voucher specimens were accessed into the CABI Reference Collection.

2. Inoculative strategy developed:

2.1 Rust infected plants hand-carried to Kerala. The rust was successfully transferred on to plants at KFRI in the glasshouse.

2.2 Inoculative strategy implemented in Kerala. Due the delays in the implementation of each stage of the project, as outlined in the Background and the delays in getting clearance from KFD, the rust was not released in the field in Kerala within the time scale of this project. Although, the rust is not being held in quarantine at KFRI. In December 2005, following additional lobbying by the director of KFRI, Dr. J.K. Sharma, permission to release the rust was considered positively by the KFD. It is hoped that a formal permission to release the pathogen will be issued very soon pending a meeting of the senior forest officials. Though the next few months (pre-monsoon) may not be a very suitable time for release, KFRI has decided to release the pathogen, on a trial basis in the two designated areas, as soon as the clearance is received. However, the main release will have to wait until the rains start in June 2006.

The aim with the main release is to use the established network of forestry personnel, extension workers and farmer cooperatives to distribute pot grown rust infect plants within the infested region, produced in the rust propagation unit at KFRI. To this means the project has initially been given a 6-month extension to June 2006 by Dr. J.K. Sharma.

3. Inundative strategy developed:

3.1 CABI consultancy to Assam.

- **Establish the work programme with Assam Agricultural University**. The following work programme at AAU was agreed: record the density of *Mikania* in the permanent sample plots in tea, undertake a farmer survey to establish the impact of *Mikania* on their crops and construct and test the rust mass propagation unit. Plant sampling techniques and methods used in undertaking farmer surveys (e.g. types of questionnaires) were discussed and protocols established, in collaboration with KFRI and based on those used at KFRI during Phase I.
- **Advise on the construction of the rust mass production unit**. No suitable facilities for producing rust infected plants for mass release were available at AAU. A plan for the construction of the rust propagation unit was discussed and agreed (see 3.2).
- **See the *Mikania* problem in the field (both in agro-ecosystems, forestry and Kaziranga National Park).** A field trip was undertaken to look at *Mikania* weed infestations in tea (Cinamara Tea Estate, Tocklai Tea Research Association, Instructional-cum-Research Farm), sugarcane (Buralicksion Sugarcane Research Station) and forestry (Institute of Rain & Moist Deciduous Forest Research), Gibbon Forest Reserve and three small tea growers' gardens of Jorhat. Infestations are dramatic in all these production systems. A note was given to project collaborators during the visit by a tea farmer (with about 1hectre of tea) describing his perception of the *Mikania* problem in tea (Appendix 4).

  Kaziranga National Park is a World Heritage Site, and the last stronghold of the one-horned rhino. It is being invaded by the invasive alien species *Mikania micrantha* and *Mimosa invisa*, which are threatening the food sources of the large herbivores in the park. A meeting was held with the Forestry Department, interest was shown in pursuing the issues further with CABI and our Indian collaborators.

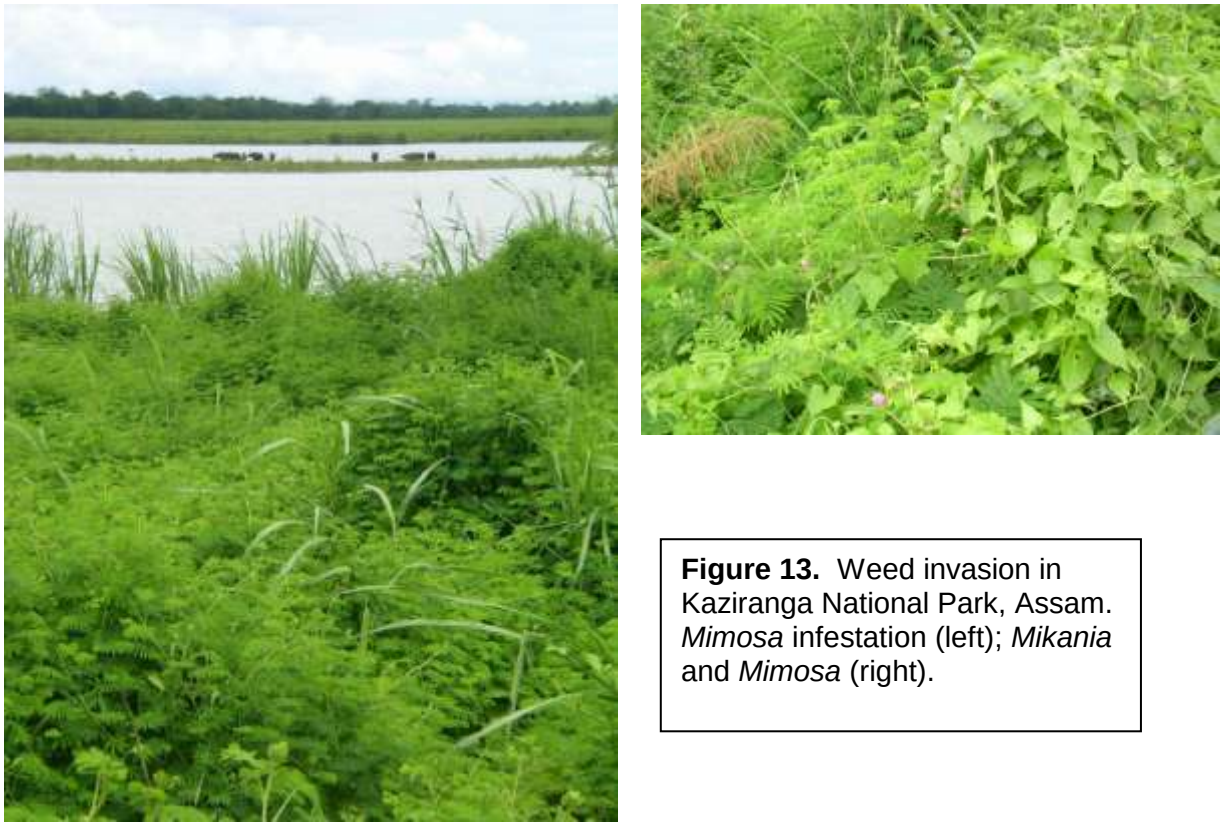


Figure 13. Weed invasion in Kaziranga National Park, Assam. *Mimosa* infestation (left); *Mikania* and *Mimosa* (right).

- **Look at the potential sites for the permanent plots**. Excellent sites for the release of the rust, within dense infestations of *Mikania*, were observed at Cinamara Tea Estate. The *Mikania* was seen to survive the dry season in the drainage ditches between the tea lawns, and in the forests surrounding the tea estate. It was commented by the manager, that the *Mikania* came out of the forests and ditches like a 'green tidal wave' as soon as the rains started (Anon, 2003 – see 6.2 below). The concept of inundative releases of the rust was discussed in detail with collaborators, and a draft protocol established. Large number of rust infected plants would be produced in the RPU at AAU, just as the rains started. These would be transported and placed at intervals (optimum density to be established) within the new *Mikania* plant growth. This would create an early season epiphytotic of the rust, and if successful would slow the progress of the weed, perhaps to below economic threshold. Overtime, once the rust is fully established in the ecosystems, it may no longer prove necessary to implement this inundative approach.

Figure 14. *Mikania* infestation in tea, Assam. Note *Mikania* whips above the tea lawn, which interferes with plucking.

3.2 Investigation of plant/pathogen propagation methodology. The rust propagation unit (RPU) was completed according to schedule, but techniques for mass production of rust infected plants were only able to commenced in July 2005, after the arrival of the rust at AAU. Problems arose in propagation of the rust, due to the high temperatures that are maintained in the net house attached to the RPU (see 3.3 below). This required adjustment to be investigated including the following:

- Provision of a post-inoculation chamber, with plant-growth lights, of size 3m X 4m within the RPU for housing the inoculated plants, during rust symptom development. This will provide for gradual transition between the low temperature mist room (17-20^{o}C) and the ambient external temperature.
- Providing 50% agro shade net on the net house, that can be easily retracted when not required.
- Providing a rotating stand fan in the net house.
- Provision of a small dew chamber for maintaining the rust within the RPU.

3.3 Inoculated plants hand-carried to Assam

Rust consignment 1: Nearly half of the 26 rust infected plants in the first consignment failed to survive. The rust could not be established on the 30 Assam plants onto which it was inoculated. Only chlorosis was observed on 18 of the inoculated plants, no pustules developed after 30 days of observation. This was thought possibly to be because of the high, constant temperatures in the net house where the inoculated plants were kept during pustule formation. In the native range of the rust (and in the *Mikania* habitat in the field in the invasive range), these temperatures are not unusual during the day, but the night temperature is much lower (>25^{o}C). The diurnal temperature variation was not experienced in the net house, and could have been responsible for the lack of symptom development. There was also the possibility that the Assam plants that the rust had been inoculated onto were in fact resistant to the rust. The results reported in Phase I of the project did show that there was at least one genotype of *Mikania* in Assam that was semi-resistant (only small pustules developed) to the pathotype of the rust from Trinidad.

Rust consignment 2: All of the eight plants in the second consignment survived. Table 7 gives the results of the inoculation from the consignment 2 plants onto plants grown at AAU. Kerala plants were found to be more susceptible to the rust than Assam Plants. All the six Kerala plants became infected by the fungus and attained the maturity stage with pathogenicity score of 4 (fully susceptible) using the standard infection sale given in 1.3 above. Only three Assam plants out of the 24 inoculated showed the same symptoms, but these pustules were reduced in size giving a pathogenicity score of 3. In the infected Kerala plants, a maximum of 37.5% of the leaves were found with matured pustules but for Assam plants it was 15.79%. Number of infected stems and petioles were also found to be higher in Kerala plants than that of Assam plants.

Table 7. Infection of *Mikania* plants grown in net house in Assam, from rust consignment 2

Plants	Total number of leaves/plant	Infected leaves/plants	Average number of pustules	Stem infection (number)	Infected petioles (number)
Kerala	38	12 (31.58)	8.77	2	4
Kerala	48	18 (37.50)	19.13	4	5
Kerala	41	11 (26.83)	6.14	3	4
Kerala	30	10 (33.33)	9.89	4	5
Kerala	35	2 (5.71)	8.54	3	3
Kerala	37	3 (8.11)	6.53	2	1
Assam	32	1 (3.13)	4.00	1	-
Assam	38	6 (15.79)	1.33	1	3
Assam	46	1 (2.17)	2.00	1	1

Data in the parentheses are percentage of leaves infection

The three most infected plants from Kerala (top three in Table 8), and the only three infected plants from Assam were as the source plants for the first field release at EGPC (see 3.4 below).

Rust consignment 3: All plants from this consignment survived and were used for the field inoculation at CTE (see 3.4 below).

3.4 Inundative strategy implemented in Assam.

Field release of P. spegazzinii

a) EGPC. Infection of the field *Mikania* plants was observed 12 days after release, from the inoculum source plants originating from Kerala. No infection of the field plants was observed from the inoculum source plants originating from Borbheta, Jorhat, Assam. Rust pustules were observed on leaves, petioles and stems of the surrounding *Mikania* vegetation, Table 8 shows the results of this first release.

Table 8. Initial rust infection of field plants at EGPC after the first release of *Puccinia spegazzinii*

Sources plants with rust inoculum	Number of leaves infected	Maximum number of pustules/leaf	Number of stems infected	Number of petioles infected
Kerala	33	32	2	1
Kerala	6	5	-	-
Kerala	8	12	3	1
Assam	-	-	-	-
Assam	-	-	-	-
Assam	-	-	-	-

Following the first release, rainfall was continuous for 14 days, after which the common *Mikania* leaf spot pathogen *Cercospora mikanicola* became abundant on all the field plants plants. This pathogen is known not to have a significant impact on the *Mikania,* since it only causes damage to mature leaves. However, it is on the mature leaves that the rust mainly sporulates (having infected the meristematic tissue), so this sudden high level of *C. mikanicola* caused early senescence of the rust infected leaves, hence slowing the spread of the rust infection. Fortunately, stem infections were not affect by the leaf spot pathogen, so the rust infection was able to progress in the field with the inoculum released from the infected stems. Table 9 shows that on a rust infected leaf a maximum of 39 spots of *C. mikanicola* were observed against a maximum 26 spot of the former.

b) CTE. Infection of the field *Mikania* plants was observed 15 days after release, around all of the three original source plants. Table 10 shows the extent of the new infection in the field *Mikania* plants.

The rust was initially released at the end of the main rains (April-October), and so although the rust established in the permanent sample plots (by daily hand-spraying with water at CTE) the subsequent environmental conditions were not conducive to the natural spread of the rust. By January 2006, the rust had only spread within I meter of the initial infected plants put out in the field. In addition, it was apparent the infection on Assam plants in the field was not a fully-susceptible response (see Figure 15). It was already known (FTR I) that in Assam (but not in Kerala) semi-resistant genotypes of *Mikania* were present, and the need to release an additional pathotype of the rust in Assam, had been established at the end of Phase I. However, it had been decided by all collaborators that it was best to complete the work with the Trinidad pathotype first. Once all the procedures were in place in India, for the import of fungal weed biological control agents, an application could be made to import a

new pathotype for Assam. It was considered that to request two pathotypes, at the same time, would only confused issues, and be counter-productive. However, it was disappointing that the first release site in Assam was within a semi-resistant population.

Table 9. Dynamics of *Cercospora mikanicola* and *Puccinia* spegazzinii infection in field condition

Leaves	Number of pustules of *P. spegazzinii*	Number of pustules of *C. mikanicola*
1	5	20
2	4	12
3	6	11
4	1	8
5	18	28
6	22	33
7	24	32
8	26	39
9	12	33
10	21	39

Table 10. Initial rust infection of field plants at CTE after the first release of *Puccinia spegazzinii*

Sources plants with rust inoculum	Number of infected leaves	Maximum number of pustules/leaf	Number of stems infected	Number of petioles infected
Kerala	15	20	6	4
Kerala	14	18	5	3
Kerala	12	17	4	3
Average	**13.66**	**18.33**	**5.00**	**3.33**

Figure 15. Field infection of *Mikania* with rust fungus (upper leaf surface, so no pustule visible). Infection of Kerala plant showing fully-compatible (score 4) pustule type (left). Infection of Assam plant showing semi-resistant (score 3) pustule type, note petiole infection (right).

Work is continuing at AAU to establish new release sites, in areas where resistant *Mikania* populations are not present, in time to start the more intensive release programme, when the main rains start in March/April 2006.

An isolate of *Puccinia spegazzinii* collected in Peru was recently found to have a better compatibility with the semi-resistant genotypes in Assam. The screening of a few selected plant species closely related to *M. micrantha* at CABI, has suggested that the selectivity of this pathotype, out side of its host species, is identical to the Trinidad strain. A licence to import this strain into quarantine in India has already been obtained by PDBC, and CABI is preparing the shipment to NBPGR (planned for February 2006). Additional confirmatory host specificity screening will at undertaken at NBPGR, New Delhi, before being released in the field in Assam.

Due to the delays in the implementation of this project and the problems with establishing the rust at AAU, the situation had not been reached where the intensive inundated release strategy could be implemented within the funding time frame.

The rust is being maintained in the rust propagation unit at AAU throughout the dry season, and distribution will be initiated once the main rains start. Large quantities of inoculum will be continuously produced in the rust propagation unit and distributed to tea gardens (in area where it will have been established that fully susceptible populations of the weed are present), using previously established network of extension works and farmer cooperatives. This will continue as long as necessary within the scope of the support pledged by ICAR and AAU. To this means the project has initially been given a 6-month extension to June 2006 by the director of AAU, Dr. A.K. Phatak.

3.5 Inoculative strategy implemented in Assam. On-going, see 3.4.

4. Farmer and forest department information campaign implemented:

4.1 CABI consultancy to KFRI and Assam to discuss farmer campaign. A progress report was prepared of the discussions held at the November 2004 project progress meeting, which focussed technical issues concerning the slow progress of the project, the putting into place of protocols and procedures to maximise the outputs from the host range screening, and the future farmer, forester and general public information campaign (see 4.3 below).

4.2 Workshops undertaken in Assam and Kerala.

Assam: A summary document of the workshop was produced; 'Proceedings of the workshop on ICAR-CABI-AAU collaborative project on "Classical biological control of *Mikania micrantha* with *Puccinia spegazzinii*: Implementation phase" held on 28-29 November 2005 at AAU, Jorhat, Assam (Appendix 5). At the workshop, the progress of the project, problems encountered and the optimum post project activities were discussed, with the limited funding available from AAU and ICAR. The possibility of additional international funding to continue the *Mikania* project and expand into tacking other weeds was deliberated, particularly through the National Agricultural Innovation Programme (NAIP) (see 6.3 below), and the replacement for the DfID-RNRRS programme.

Kerala: At the stakeholders meeting at KFRI in Kerala, in depth and open discussions were conducted concerning the rust and its perceived risks. Although the representatives from KFD were reticent to begin with, it became clear by the end of the meeting that they were, themselves, very pro the rust and would be reporting back positively to their seniors. Following this meeting, more discussions and deliberations were conducted between senior personnel at KFD and KFRI, and the approach of the forest officials has become positive. An official letter of permission to release the pathogen is expected very soon (see 2.2 above). Although no official releases of the rust were made in Kerala by the end date of this project, the rust is not under quarantine in the KFRI glasshouses, so in effect the rust has been 'released' in Kerala.

4.3 Educational material produced & campaign implemented (linked to 6.1):

Once the rust validation trials have been completed, the local information campaign will be undertaken, targeting: farmers, foresters and the general public. A number of pathways will be utilized:

- *Publicity through the mass media*. This will include local press releases, writing popular articles for the local press and bulletins, radio interviews and television programmes (linked to Short Project R8502).
- *News releases*. CABI will provide a news release both in the UK and India (as undertaken at the beginning of Phase II).
- *Peripatetic demonstrations*. A display will be taken to farmer mela (fairs) and local meetings consisting of leaflets, posters and the video produced under Short Project R8502.
- *Long-term demonstration plots*. At AAU and KFRI field plots will be established on site, composed of variety of crop plants grown in the regions, with *Mikania* plants growing over them covered in rust. Open days will be held for farmers and extension workers and other interested stakeholders. The plots will provide clear evidence of the specificity of the rust and the fact that it is not a threat to crop plants.
- *Field demonstrations*. A series of field demonstrations will be undertaken, starting at the main rust release sites and targeting farmers and extension workers. These demonstrations could continue through Kerala and Assam as the rust spreads.

Important information about the nature of control that the rust provides will be explained at these demonstrations and will include opportunities for questions.

5. Impact studies:

5.1 Selected sites regularly monitored; impact studies initiated in both Kerala and Assam. Although there are no results to date on the rust impact studies, the following protocols were discussed between collaborators concerning the monitoring of the rust after release:

- When collecting data to measure the potential efficacy of a classical biological control agent, the approach that is considered to be of most value is to record the impact of the weed 'before and after' release of the agent, i.e. over time. Working on the basis that *P. spegazzinii* is a good agent then it will be impossible to keep the rust from infecting the 'the uninfected plots', (unless you spray the plots with fungicide, which is impractical and unlikely to be effective, and could prove to be a health hazard). Thus the data gathered on the level of infestation in the permanent sample plots in tea will be compared with the level over time, post release of the rust.
- The need to establish the rust in other ecosystems in addition to the tea plantations; e.g. native forest and plantations.
- In each of the ecosystems, more than one (4 preferably) sampling plots should be recorded of 1 hectare each. Within each plot multiple e.g. 20 sub-sample quadrates should be recorded of the level of rust infection and plant biomass.
- The natural spread of the rust from the initial release sites should also be measured. Sampling needs to be radial: transects should be established (e.g. north, south, east and west from the sample plots) and sampling should be carried out, perhaps every 25 metres on a weekly basis. However, the sampling will need to be modified according to the rate of spread of the rust. At each sample time, plants should be observed at each selected site (e.g. every 25 metres) getting further way from the rust source, until no further rust is found.

Permanent *Mikania* sample plot monitoring in Assam

The trends in densities over time of the *Mikania* infestations in Cinnamora Tea Estate (CTE) and the Experimental Garden for Plantation Crops (EGPC) during 2004 are presented in Figure 16. The 'out break' of *Mikania* was recorded in middle of April as 500 plants/ha and 100 stalks/ha in CTE and EGPC respectively. A temperature range of 19.7-26.10 ^{0}C, RH of 76-92 % and RF of 336.6 mm was recorded during the month. After this a gradual increase of *Mikania* density was recorded in the following months at both sites and reached a maximum populations in the period late August - mid September. A temperature range of 24.5 - 30.4^{0}C and RF of 378.8 mm in the month of September and 25.5 - 32.5^{0}C and RF of 194.6 mm in the month of August was recorded (Figure 17). After this period the *Mikania* densities were found to decrease and reaching minimum populations during the last part of December. Temperature ranges of 11.4 – 24.0 ^{0}C, RH of 72-95 % and RF of 2.5 mm was recorded during the month of December.

From the multiple regression analysis, it was found that in CTE, maximum temperature and evening RH have a significant positive impact on number of stalks of *Mikania*. However, the minimum temperature showed a negative impact on the density of plants. In EGPC, the maximum temperature and evening RH showed a significant positive impact on the density of *Mikania*. Thus, from the analysis the weather parameters, maximum temperature and evening RH seem to have high impact on *Mikania* growth.

It is clear from the data at both sites that *Mikania* plant densities increase rapidly during the main monsoon period. The reasons for the plant mortality during the following period (August/September until December) are unclear but mortality factors may include intra-specific competition and detrimental levels of RH and temperature.

Impact of Mikania from the fixed plot sampling

This experimental study allowed a calculation of actual costs of the increased labour needs and yield losses. Table 11 shows that there is significant difference in the cost of maintenance of tea plantations with and without *Mikania*; the latter being weeded blocks. The cost incurred by the estate in the plot without *Mikania* was Rs.2304.00 per ha in CTE and Rs. 4200.00 per ha in EGPC. With *Mikania* it was Rs. 1872.00 per ha in CTE and Rs. 3500.00 per ha in EGPC. Thus around 23.07% and 20% of additional cost due to labour is incurred to make these tea gardens free from *Mikania* in CTE and EGPC respectively.

The study also revealed conversely that if *Mikania* is not weeded, costs of yield loss of 41.8 % in CTE and 18.90 % in EGPC result because of the *Mikania* problem.

Table 11. Cost of cultivation in two different fixed plot surveyed area

Particulars	CTE (Divn. Hatigarh)			EGPC, AAU (TPU)		
	Without *Mikania* (weeded)	*With* Mikania	**Loss due to Mikania (%)**	**Without *Mikania* (weeded)**	**With *Mikania***	**Loss due to *Mikania* (%)**
Labour (unit) used / ha	32	26		60	50	
Cost involved (Rs / ha)	2304.00 (@ 72 per unit)	1872.00 (@ 72 per unit)		4200.00 (@ 70 per unit)	3500.00 (@ 70 per unit)	
% increase of cost	23.07			20		
Yield of made tea (kg / ha)	1725.90	1003.90		1686.30	1367.00	
Amount (Rs)	146701.50 @Rs. 85 per kg	85331.50 @ Rs85 per kg	41.8	168630.00 @Rs. 100 per kg	136700.00 @Rs. 100 per kg)	18.90

CTE : Cinnamora Tea Estate
EGPC : Experimental Garden for plantation Crops

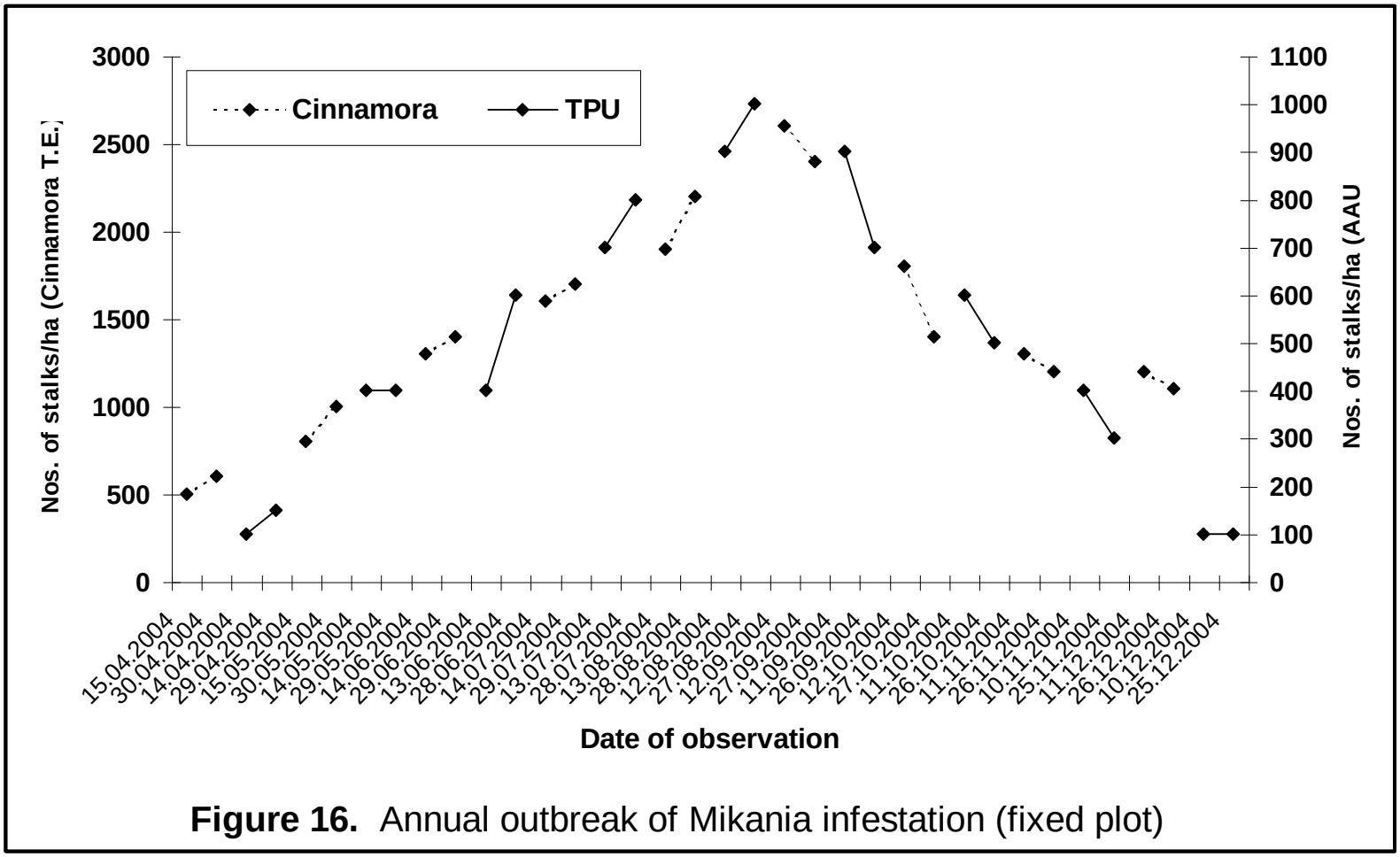


Figure 16. Annual outbreak of Mikania infestation (fixed plot)

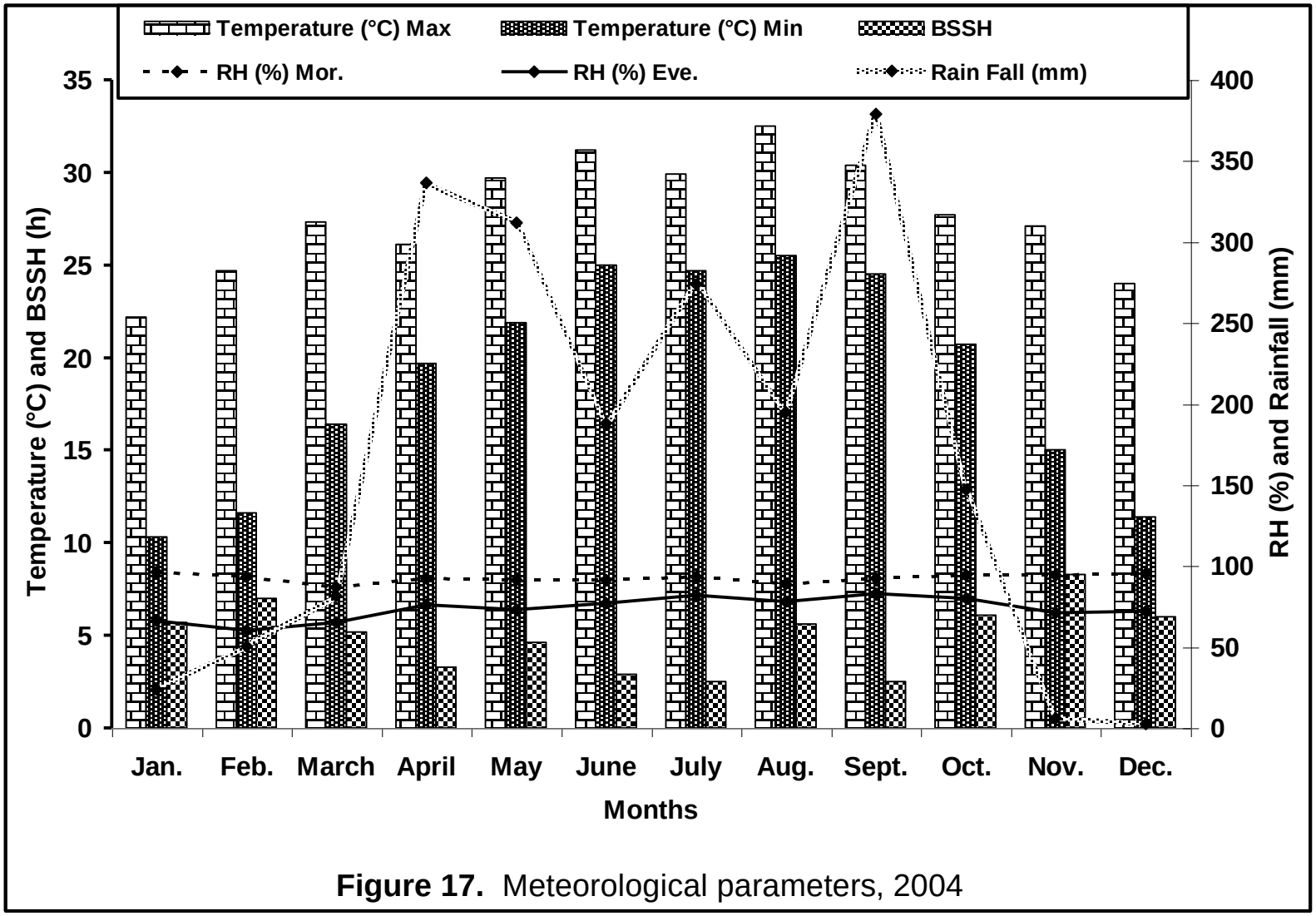


Figure 17. Meteorological parameters, 2004

5.2 Inundative release monitored through farmer surveys. Not within the scope of this phase of the project. It is planned that the same farmers surveyed during the *Mikania* impact studies (Kerala – Phase I; Assam Phase II) will be revisited as appropriate to the spread of the rust.

5.3 Economic assessment initiated

Distribution, agricultural importance and impact of *Mikania* in the tea gardens of Assam

Distribution and agricultural importance
All the gardens surveyed were observed to be infested by *Mikania*. In North Bank Plain Zone (NBPZ) 94.44% gardens have *Mikania* densities of up to 200 plants/ha; for the Upper Brahmaputra valley zone (UBVZ) and Barak valley zone (BVZ) the figures are 82.86% and 42.86% respectively. The highest densities - more than 1000 plants/ ha - were found in the UBVZ and BVZ zones (Table 12)

Table 12. Zone wise distribution of *Mikania* in Assam (%)

Zone	Grade of infestation (based on abundance)							Total Gardens Surveyed	% of localities infested
	Absent (0)	0 (0-200)	1 (201-400)	2 (401-600)	3 (601-800)	4 (801-1000)	5 (>1000)		
UBVZ	-	82.86	8.57	-	-	-	8.57	35	100
NBPZ	-	94.44	2.77	2.77	-	-	-	36	100
BVZ	-	42.86	14.28	21.43	7.14	7.14	7.14	14	100

Impact studies from the geographical survey

Mikania as a constraint and associated labour costs: Table 13 shows the different constrains faced by farmers out of which 40.00% gardens reported *Mikania* is the main constrain for tea cultivation (Rank I); this was followed by pest and disease problems (12.94%) and poor infrastructure facilities (11.76%).

Table 13. Constraints faced by surveyed tea growers

Constraints	No. of tea gardens (%)	Rank
Labour shortage	8 (9.41)	IV
Cost of production	6 (7.05)	V
Poor infrastructure facilities	10 (11.76)	III
Weed (Mikania)	35 (41.17)	I
Pest and disease Problem	11 (12.94)	II
Insurgency problem	6 (7.05)	V
Market price of I) Green Leaf	2 (2.35)	VIII
II) Made tea	4 (4.70)	VII
Government Policy	5 (5.88)	VI
Total	**85 (100)**	

Figures in the parenthesis are percentage value

The *Mikania* infestations result in an additional involvement of labour for garden maintenance, which is observed to be >50% in 13.32% gardens of UBVZ (Table 14). Most of the gardens of UBVZ (46.66%) and NBPZ (50%) have reported to use an additional labour of up to 10% to manage *Mikania*. 35.71% of gardens of BVZ have reported to use 11-20% increase in labour use due to *Mikania*

Table 14. Effect of *Mikania* on percent increase of labour used in different surveyed gardens.

Zone	Garden surveyed	Percent increase of labour use due to *Mikania*					
		0-10%	11-20%	21-30%	31-40%	41-50%	>50%
		Number of gardens					
UBVZ	15	46.66	20.00	6.66	6.66	-	13.32
NBPZ	20	50.00	15.00	20.00	15.00	-	-
BVZ	14	4.29	35.71	21.57	7.14	14.28	-

Yield loss: Results from the two methods were similar. Table 15 revealed that 75% and 66.66% gardens of NBPZ and UBVZ respectively have faced a yield loss up to 10% in terms of made tea (kg/ha); 7.14 % gardens of BVZ have recorded a yield loss of more than 50% (>1000kg made tea /ha).

Table 15. Effect of *Mikania* on yield of made tea in different surveyed gardens.

Zone	Gardens (Numbers)	Percent decrease of yield (Kg made tea /ha)					
		0-10% (0-200)	11-20% (200-400)	21-30% (400-600)	31-40% (600-800)	41-50% (800-1000)	>50% (>1000)
UBVZ	15	66.66	13.33	-	-	20.0	-
NBPZ	20	75.00	15.00	13.33	-	-	-
BVZ	14	28.57	21.43	35.71	7.14	-	7.14

- Average yield of made tea =2000 kg/ha.
- Average yield of green leaf=9540 kg/ha.
- Recovery % = 21%
- Conversion factor = 4.77
- Average price of made tea = Rs. 80 per kg.

5.4 CABI Consultancies in years 3 and 4. See 4.1 and 4.2

6. Results publicised with and adopted by stakeholders

6.1 Public awareness campaign implemented (see 4.3)

The outputs from the international media campaign are listed below:

- 'British Scientists to save tea plantations from invasion'. News release by CABI Bioscience Marketing and Communications Group, 29th February 2003.
- 'Anglo-Indian team to save tea plantations from invasion'. News release by CABI Bioscience Marketing and Communications Group, 13th March 2003
- S.T. Murphy, phone interviewed by the Canadian National Radio and local New Delhi radio station (both while attending Inception Workshop in New Delhi), June 2003
- Sankaran K.V. and Sheji, R. (2003) *Weeds: the Biological Invaders*. Produced by the Audiovisual Research Centre (AVCRC), University of Calicut, in collaboration with Kerala Forest Research Institute (KFRI), India. K.V. Sankaran (KFRI): scripting, consultant, supervision and presenting. R. Sheji (AVCRC): Director. AVCRC: editing, sound, mixing, production. The film was telecast all over India through the National Television Network. The Government of India adjudged it as the best documentary film on humanity, environment and human rights, in 2004.
- ANON (2003) Fungus in your tea, sir? *New Scientist*, 28 June 2003, p.10.
- Ellison, C.A. and Murphy, S.T. (2003) Invasive alien species (including the *Mikania* story). Film for news item. British Satellite News. 3 July, 5-10 minutes. [International].
- Biological control 'silver bullet'. New Agriculturist, *on-line,* February 2004 http://www.new-agri.co.uk/04-2/focuson/focuson4.html
- Ellison C.A. and Rabindra R.J. (2005) Invasive alien species in India (including Mikania). Taped interview for radio (press release) at DfID-Pathways out of Poverty conference. Wrenmedia. 23 September 5-10 minutes, [International].

6.2 Preparation of scientific reports and papers

The main reports and scientific papers completed under this project are listed below. This list excludes those prepared for: the normal project reporting procedures, CABI internal back-to-office reports and short project meetings where only minutes were recorded:

Reports

- Poudel, A., Sagar Baral, H., Ellison, C.A., Subedi, K., Thomas, S., and Murphy, S.T. (2005) *Mikania micrantha* weed invasion in Nepal. A summary report of the first national workshop for stakeholders, held on 25th November, Kathmandu, Nepal. Himalayan Nature and IUCN, Nepal; CAB International, UK. (See Additional Activities and Outputs above) ('Published' and distributed report on a workshop – 50 copies)
- Anon (2003) Inception Workshop on the collaborative project; 'Classical Biological Control of *Mikania micrantha* with *Puccinia spegazzinii*—implementation phase'. 27-28 June, National Bureau of Plant Genetic Resources, New Delhi, India. (See 1.1 above). (Informal report on workshop)
- Kumar, S. P., Rabindra R.J., Dev, Usha, Sankaran K.V., Puzari, K.C., Ellison, C.A. and Murphy, S.T. (2005) Supplementary dossier on: *Puccinia spegazzinii* de Toni (Basidiomycetes: Uredinales) a Potential Biological Control Agent for *Mikania micrantha* Kunth. ex H.B.K. (Asteraceae) in India. (Supporting documentation to the application to the MoA for a rust release permit; includes additional host specificity testing carried-out in India). (See 1.3)
- Puzari, K.C. and Ellison, C.A. (2005) Proceedings of the workshop on ICAR-CABI-AAU collaborative project; 'Classical biological control of *Mikania micrantha* with *Puccinia spegazzinii*: Implementation phase'. 28-29 November, Assam Agricultural University, Jorhat, Assam. (Informal report on workshop) (See Appendix 5 and 4.2 above).

Scientific papers and bulletins

- ANON (2003) Can the 'Green Tidal Wave' of Asia Be Curtailed? *Biocontrol News and information* **23(3):** 50N-52N. (News article in Journal)
- Ellison C.A. (2004) Biological Control of Weeds Using Fungal Natural Enemies: a New Technology for Weed Management in Tea? *International Journal of Tea Science* **3:** 4-20. (Peer reviewed paper)
- Ellison, C.A. and Evans, H.C. (2004) Case studies: classical biological control of *Mikania micrantha* and *Cryptostegia grandiflora* using rust pathogens, with special reference to quarantine issues. In: *Quarantine Procedures and Facilities for Biological Control Agents.* Technical Document No. 54, Project Directorate of Biological Control, Bangalore, 560 024, India, pp.79-84. (Presentation at workshop and book chapter)
- Ellison, C.A., Evans, H.C. and Ineson, J. (2004) The significance of intraspecies pathogenicity in the selection of a rust pathotype for the classical biological control of *Mikania micrantha* (mile-a-minute weed) in Southeast Asia. In: *Proceedings of the XI International Symposium on Biological Control of Weeds* (eds Cullen, J.M., Briese, D.T., Kriticos, D.J., Lonsdale, W.M., Morin, L. and Scott, J.K.). CSIRO Entomology, Canberrra, Australia, pp. 102-107. (Peer reviewed paper in proceedings)
- Ellison, C.A., Murphy, S.T. and Rabindra, R. J. (2005) Facilitating access for developing countries to invasive alien plant classical biocontrol technologies: the Indian experience. In: *Aspects of Applied Biology 75, Pathways out of Poverty* (eds Harris, D., Richards, J.I., Silverside, P., Ward, A.F. and Witcombe, J.R.). Association

of Applied Biologists, Horticultural Research International, Wellesbourne, Warwick, U.K., pp 71-80. (Edited book chapter)

- Evans H.C. and C.A. Ellison (2005) The biology and taxonomy of rust fungi associated with the neotropical vine *Mikania micrantha*, a major invasive weed in Asia. *Mycologia* **97:** 935-947. (Peer reviewed paper)
- Kumar, S.P., Ellison, C.A., Rabindra, R.J. and Murphy S.T. (2003) *Puccinia spegazzinii* for the classical biological control of *Mikania micrantha* in India. *ICAR News*. (News bulletin)
- Kumar, S. P, Rabindra R.J., Dev, Usha, Puzari, K.C., Sankaran K.V., Khetarpal R.K., Ellison C.A. and Murphy S.T. (2005) India to release the first fungal pathogen for the classical biological control of a weed. *Biological Control and Information* **26:** 71N-72N. (News article in Journal)
- Ryan, M.J. and Ellison, C. A. (2002) Development of a cryopreservation protocol for the microcyclic rust-fungus *Puccinia spegazzinii*. *CryoLetters* **24**: 43-48. (Peer reviewed paper)

6.3 Long-term dissemination:

a) **Development of government policy**: In order to secure the rust release permit, a significant amount of interaction was required between project personnel (particularly Prof. Rabindra, PDBC) and senior government official policy makers in ICAR and the MoA during the course of this project. Official communications, meeting and presentations to officials were necessary to achieve this end. This has enabled India to develop protocols for the introduction of fungal biological control agents.

b) **Interactions with ICAR**: CABI has a history of collaboration with ICAR, not only through the DfID-*Mikania* project, but also through the ICAR-CABI Workplan, funded through the National Agricultural Technical Programme (NATP) (Donor: World Bank). Under the NATP, CABI undertook a workshop, organised by PDBC: 'Quarantine Procedures and Facilities for Biological Control Agents' in May 2002 (Ramani *et al.*2004). This workshop was supported in order to strengthen capacity in India in the procedure for the import, quarantine, safety testing and evaluation of biological control agents, and was stimulated by the imminent DfID Phase II *Mikania* project. The NATP has finished, and a new programme; the National Agricultural Innovative Programme (NAIP) has taken–over. Under the ICAR-CABI Workplan, discussions are underway to continue with the invasive alien species theme and develop further initiatives under this broader programme, following-on from and complementary to the DfID-*Mikania* project.

c) **Short project R8502**. In order to develop the theme of long-term dissemination further, NRI funded the Short Project: Promotion of Weed Biocontrol in Asia: the *Mikania micrantha* Experience (2005-2006). The aim of this project is to enhance and increase the dissemination outputs from the Phase II *Mikania*, by the production of user-friendly publications and an awareness raising media production. There are three outputs from this project:

- *Policy Level Support*: Production and publication of a 'popular-style' book aimed at policy makers; 'Invasive Alien Plants as a Developmental Constraint'.
- *Guidance for Researchers and Extension Services*. Production of a Training Manual; 'Best management practices for *Mikania micrantha*'.
- *Awareness Raising*. Video/DVD programme on invasive alien weeds and their control for National TV broadcast and for use by extension services.

Additional Outputs

First National Stakeholders' Workshop, Kathmandu, Nepal. The meeting focused on *Mikania* but other invasive alien weed problems were also highlighted. A recent survey (2004), commission by IUCN, placed *M. micrantha* as the worse invasive weed in Nepal. The agricultural sector has still not fully recognised the agricultural importance of the weed, but there were growing reports of its impact in tree fruit production. The forestry section, have particularly noted that the weed is becoming problematic in Community Forests. *Mikania* is a relatively new invasion in Nepal, and was noticed and reported to officials by Harry Evans of CABI in 1997 during a survey in the Terai. *M. micrantha* has been recorded in other countries (e.g. China) as an environmental weed before it becomes significant in agricultural habitats.

Control options were discussed in detail from grazing cattle in the nature reserves to manual removal. None were seen by the participants to be worthwhile control measures. Despite care by CABI not to over promote the biocontrol option, it was concluded that there was no other possible approach to control *Mikania*. This will be a completely new weed control approach for Nepal, but there was much enthusiasm (by Nepalese nationals) to proceed with approaching donor organisations to import and release the rust for the control of this weed.

The workshop was a great success and a bound booklet of the proceedings was produced: '*Mikania micrantha* weed invasion in Nepal. A summary report of the first national workshop for stakeholders'. The original concept had been to use this meeting and the recommendations to support applications to donors to fund a biological control implementation project in Nepal. A successful application was made to NRI'nt for a short project to start this work, but unfortunately, the coup by King Gyanendra in February 2005, meant that it was not possible to implement the project. Currently, CABI is included in a Darwin Initiative (DEFRA) application by Seb Buckton of the Wildfowl and Wetlands Trust, UK. CABI will look at the impact of invasive alien weeds in his target sites (pond fisheries), where *Mikania* is a major problem. Unfortunately, again, recent civil unrest may curtail this project as well.

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Contribution of Outputs to developmental impact

Classical biological control (through the introduction of exotic fungal pathogens) is a self-perpetuating and thus long-term and sustainable management option for the *Mikania* problem in India (and all affected region in Asia). This technology is appropriate for resource poor farmers, since it require no financial or time inputs from them in order to be implemented.

Once the rust is at a sufficient concentration to be impacting on the density of *Mikania* (which is anticipated to take from 3-10 years to achieve), the main anticipated benefits for the poor will be:

- A decrease in cultivation costs; this will be largely through less need to employ additional labour for weeding or by freeing up family time that is currently being occupied by weeding. In northeastern India some smallholders will also use less or no herbicide as a result of the project. It should be noted that the casual labourers do have other forms of income in the Western Ghats such as coffee picking.
- An increase in crop yields (e.g. pineapple, banana, tea) and/or opportunities to grow some crops that have had to be abandoned because of *Mikania* infestations.
- An increase in crop quality (e.g. tea) because of a reduction/cessation in herbicide use.

The project has been conducted in the States of Kerala and Assam, where many of the holdings in these areas are marginal or smallholder and there is significant investment in cash and/or tree crops. The rust, over time, will potentially aid all farmers in these states affected by the *Mikania,* and the surrounding states where the weed is now encroaching. The collaborating institutions of KFRI, AAU, PDBC and NBPGR have benefited from an increased capacity to undertake research and implementation of biological control utilizing fungal pathogens, for the management of important alien weeds.

Within India, The directors of AAU (Dr. Phatak) and KFRI (Dr. Sharma) have committed to extend the project by six months to allow for additional field releases to be made during the 2006 rainy season (May/June). This will enable the project to achieve the developmental benefits outlined above. To aid the continuation of this work and potentially extend the time frame, ICAR have committed financial support, particularly for field monitoring of the rust and impact assessment. AAU and KFRI will further develop the farmer awareness campaign during 2006, once the rust has been evaluated in the field.

There is great potential for wider scale impact, and this has already been realised in China, where a similar project has been implemented, funded by the UK-Department for Environment Food and Rural Affaires (DEFRA), Darwin Initiative. Other countries/regions are also implementing the results from this project: an Australian Centre for International Agricultural Research (ACIAR) – funded project has just started in the South Pacific, focussing on releasing the rust in Fiji and Papua New Guinea. Other countries have requested implementing a rust release project, e.g. Taiwan, Nepal, Indonesia and Malaysia, pending funding, although seed funding has already been secured for Taiwan and Nepal.

In order for this technology to have a wider impact, addition weed problems need to be addressed, which obviously requires long-term donor support. The pathways that have been developed that have allowed this technology to be implemented (technical and political) will greatly benefit future programmes.

Biometricians Signature

The projects named biometrician must sign off the Final Technical Report before it is submitted to CPP. This can either be done by the projects named biometrician signing in the space provided below, or by a letter or email from the named biometrician accompanying the Final Technical Report submitted to CPP. (Please note that NR International reserves the right to retain the final quarter's payment pending NR International's receipt and approval of the Final Technical Report, duly signed by the project's biometrician)

I confirm that the biometric issues have been adequately addressed in the Final Technical Report:

Signature:

Name (typed):	DR S T MURPHY
Position:	Research Group Leader, Invasive Species Management, CABI Bioscience, UK Centre
Date:	30th January 2006

Appendix 1. The release permit issued by the Indian MoA in June 2005

Fax - 080 - 2 3 41 1941

egram -PROTECTION

Tel: 0129 -2418506
Fax: 0129- 2412125

F. No.99-3/2004-PQD(pt)
Government of India
Ministry of Agriculture
(Department of Agriculture & Cooperation)
DIRECTORATE OF PLANT PROTECTION, QUARANTINE & STORAGE
NH IV, FARIDABAD – 121 001 (Haryana)

Dated: 27.6.2005

To

Dr. R.J.Rabindra,
Project Director ,
Project Directorate of Biological Control (ICAR),
P.B.No. 2491, H.A.Farm Post, Bellary Road, Hebbal,
Bangalore –560 024 (Karnataka).

Subject: **Limited field release permission for Puccinia spegazzinii for Biological Control of Mikania micrantha – regarding.**

Sir,

Please refer to your letter .No. PDBC/12-4(a)/2005-829, dated 26th April,2005 on the subject cited above. As per detailed deliberation of the experts, permisson for limited field release of Puccinia spegazzinii has been given in Kerala and Assam state subject to the terms and conditions specified in the proceedings of the meeting (copy enclosed).

Yours faithfully,

(O.R.Reddy)
Joint Director(PQ)
for Plant Protection Adviser
to the Government of India

Copy for information along with proceeding of the meeting forwarded to:

1. Dr.P.S.Chandurkar, PPA.
2. Dr. O.P.Dubey, ADG(P&O), Krishi Bhavan, New Delhi.
3. Dr.Ravi Khetarpal, NBPGR, Pusa Campus, New Delhi.
4. Dr.Usha Dev. Principal Scientist, NBPGR, Pusa Campus, New Delhi.
5. Dr.Sreerama Kumar, Senior Scientist, PDBC, P.B..No. 2491, HA, Farm Post, Bellary Road, Hebbal, Bangalore – 560 024.

(O.R.Reddy)
Joint Director(PQ)
for Plant Protection Adviser
to the Government of India

Proceedings of the meeting to review the dossier on "*Puccinia spegazzinii* de Toni (Basidiomycetes: Uredinales) a potential biological control agent for *Mikania micrantha* Kunth. ex H.B.K. (Asteraceae) in India" for the issue of permission for limited field release of the rust pathogen *P. spegazzinii*, held at 10:30 AM on 15 June 2005 in the chamber of the Plant Protection Advisor (PPA) to the Government of India.

Present: Dr P. S. Chandurkar (PPA)

The following scientists participated:

Dr O. P. Dubey, ADG (P & O)
Dr O. R. Reddy, JD (PQ)
Dr R. J. Rabindra, Project Director, PDBC
Dr Ravi Khetarpal (Representative of Dr B. S. Dhillon, Director, NBPGR)
Dr Usha Dev, Principal Scientist, NBPGR
Dr P. Sreerama Kumar, Senior Scientist, PDBC

The Chairman, Dr P. S. Chandurkar, welcomed the participants and requested the Project Director, PDBC to give an account of the project. Dr Rabindra gave a brief background of the project on the "Classical biological control of *Mikania micrantha* with *Puccinia spegazzinii*: Implementation Phase" and gave details of the host-specificity testing carried out at CABI Bioscience, UK under Phase I as well as the extensive species testing under phase II at the NBPGR's National Containment-cum-Quarantine Facility by the Indian collaborators of the project. In total, 74 plant species were tested and none of the plants were found to be susceptible to infection by the rust.

The next phase of the project will include limited filed release in Kerala and Assam for which permit was sought from the PPA. Following this, there was a detailed deliberation on the proposal covering very critical aspects of the safety of the exercise and various procedures to be followed from the time of release of the rust pathogen to the establishment and assessment of biocontrol potential of the pathogen in the targeted areas as well as the impact in terms of suppression of *M. micrantha*. After detailed deliberation the following points were identified for further action:

1. A voucher specimen should be deposited with Herbarium Cryptogammae Orientalis in the Division of Plant Pathology, IARI. *Puccinia. spegazzinii* that is to be released should be subjected to molecular characterization (DNA fingerprinting) and deposited in the Division of Plant Pathology, IARI, NBPGR and PDBC. The live *P. spegazzinii* should be maintained by PDBC until 6 months beyond the end of the project period (Action: PDBC).
2. A team of experts must be formed to closely monitor the establishment and biocontrol potential of the rust in the targeted areas of Kerala and Assam (Action: PPA in consultation with PDBC).
3. It was suggested that the actual field workers who will be involved in the production and release of the rust in both Kerala and Assam should be trained thoroughly in the processes and a standard protocol should be followed. Apart from trained personnel no one else should be involved (Action: PDBC, KFRI and AAU).

4. It was decided that the Departments of Agriculture/ Horticulture/ Forestry in Kerala and Assam should be informed about the on-going research programme. (Action: PPA).
5. The concerned universities will also be informed about the programme. (Action: PDBC).
6. It was emphasized that guidelines adopted in the ISPM-3 should be followed to satisfy international standards. (Action: PDBC, KFRI and AAU).
7. It was suggested that a workshop involving all the collaborating scientists, CABI partners, DPPQ&S and the Plant Protection wing of the ICAR should be conducted in December 2005 to assess the field performance of the rust. (Action: PDBC).
8. The Project Director, PDBC was asked to ensure that rust propagation facilities are available at both KFRI and AAU to enable large-scale production of rust for limited field release. (Action: PDBC).
9. It was suggested that a clear-cut work plan for the above actions should be drawn out and the PPA should be kept informed. (Action: PDBC).
10. It was also recommended that a further follow-up proposal for a supplementary project of at least 3 years' duration should be proposed by PDBC for funding through appropriate agencies so that the biocontrol potential can be thoroughly studied as per the project norms beyond December 2005 when the current project comes to an end. (Action: PDBC).

After a very careful consideration it was decided that permission may be accorded for the limited field release of the rust fungus *P. spegazzinii* for the classical biological control of *M. micrantha* in the following target areas:

S. No.	Location	Area (Ha)	Time of release
Kerala			
1	Vazhachal, Thrissur Forest Division	1.0	June 2005
2	Kottapara, Malayattoor Forest Division	1.0	June 2005
Assam			
1	Cinamara Tea Estate, Hatigarh Division, Jorhat	0.5	July 2005
2	AAU Tea garden, Jorhat	0.2	July 2005

Appendix 2. Farmer questionnaire

SURVEY SCHEDULE

Project : "Classical biological control of *Mikania micrantha* with *Puccinia spegazinnii*"

Mycology Research Section
Department of Plant Pathology
Assam Agricultural University
Jorhat –785013, Assam.

Objective: To study the distribution, abundance and economic significance of *Mikania* on Tea

Name of the Investigators:

Dr. K. C. Puzari
Principal Scientist
Dept. of Plant Pathology
AAU, Jorhat

Mr. R. P. Bhuyan
Associate Professor
Dept. of Tea Husbandry & Technology
AAU, Jorhat

A. General Information:

1. Name the Garden :
2. Whether Estate/STG/ATC:
3. Name of respondents :
4. Location and District :

B. Specific information:

1. Area under Tea:

Type of Pruning	Total area under Tea cultivation	Area infested by Mikania	No. Stalks of Mikania per ha.
Light Pruning			
Skiff			
Unpruned			

2. Cost of cultivation:

Items	Variety Grown	Labour used (total)	Working capital (Rs)	Total cost of cultivation	Average production per ha
Mikania infested					
Mikania free					

3. Management practices:

i. Type of weeding followed: Manual/ Mechanical/ Chemical:

Why ?

ii. Total cost of weeding:

Labour charge :Rs.

Chemicals :Rs.

Others :Rs.

Total :Rs.

iii. Is there any chances of recurrence?

iv. What is the approximate loss of production due to Mikania?

4. Cost of weeding

Types of weed	Area affected	Method of weeding	Labour employed for weeding	Wage rate (Rs)	Chemical/ Machinery cost (Rs)	Total cost of weeding

C. Impact analysis:

Type	Area under Tea	Production of Tea	Productivity of Tea	Market price of Tea
Mikania infested				
Mikania free				

D. Constraints in Tea production:

i.	Price of Green Tea leaf.	Yes/No.
ii.	Price of made Tea.	Yes/No
iii.	Reduced export.	Yes/No
iv.	Quality control measures.	Yes/No
v.	Labour problem.	Yes/No
vi.	Govt. policy.	Yes/No
vii.	Socio-economic consideration.	Yes/No
viii.	Weed problem (Including Mikania).	Yes/No
ix.	Costs and Price.	Yes/No
x.	Pests Control.	Yes/No
xi.	Labour availability.	Yes/No
xii.	Any other as reported by the Grower.	

Signature of the Respondent with seal Signature of the Surveyor Date:…….

Appendix 3. The results of the host specificity testing undertaken at NBPGR, New Delhi

Ref. No.	Scientific name	Common name	Family	Cultivar	Source/place of collection	Rust symptoms
A. Plant species closely related to *Mikania micrantha*						
1	Ageratum houstonianum *Mill.*	Floss flower, mist flower	Compositae (Tribe: Eupatorieae)	-	Sunder Nursery, N. Delhi	X
2	*Artemisia annua* L.	Sweet sagewort, Wormwood	Compositae (Tribe: Anthemideae)	EC 202429	NBPGR Regional Station, Bhowali	X
3	Aster chinensis *L.*	China aster	Compositae (Tribe: Astereae)	-	Sunder Nursery, N. Delhi	X
4	*Bellis perennis* L.	Daisy	Compositae (Tribe: Astereae)	-		X
5	*Brachycome iberidifolia* Benth.	Swan river daisy	Compositae (Tribe: Astereae)	-		X
6*	*Calendula officinalis* L.	Calendula	Compositae (Tribe: Calenduleae)	-	NBPGR, New Delhi	X
7*	*Carthamus tinctorius* L.	Safflower	Compositae	-	Bangalore, Karnataka	X
8	*Centaurea cyanus* L.	Cornflower, Bachelor's-button	Compositae (Tribe: Cynareae)	Frosty mix	Namdhari Seeds Pvt. Ltd., Bidadi, Karnataka	X
9*	*Chromolaena odorata* (L.) R.M. King & H. Robinson	Siam weed, Chromolaena	Compositae (Tribe: Eupatorieae)	-	Hebbal, Bangalore, Karnataka	X
10*	*Chrysanthemum carinatum* Schousb.	Tricolor chrysanthemum	Compositae (Tribe: Anthemideae)	-	NBPGR, N. Delhi	X
11	*Cosmos bipinnatus* Cav.	Cosmos	Compositae (Tribe: Heliantheae)	-	PDBC, Bangalore	X
12	*Dimorphotheca sinuate* DC.	Cape-marigold	Compositae (Tribe: Calenduleae)	-	Sunder Nursery, N. Delhi	X
13	*Eupatorium adenophorum* Spreng. (Banmara)	Crofton weed	Compositae (Tribe: Eupatorieae)	-	Ootacamund, Tamil Nadu	X
14	*Gazania rigens* R. Br.	-	Compositae (Tribe: Arctotideae)	-	Sunder Nursery, N. Delhi	X
15*	*Gerbera jamesonii* Bolus ex Hook. f.	Transvaal daisy, Barberton daisy (Gerbera)	Compositae (Tribe: Mutisieae)	-		X
16	*Guizotia abyssinica* Cass.	Niger-seed, Rantil	Compositae (Tribe: Heliantheae)	-	Bangalore, Karnataka	X

Ref. No.	Scientific name	Common name	Family	Cultivar	Source/place of collection	Rust symptoms
17*	*Helianthus annuus* L.	Sunflower	Compositae (Tribe: Heliantheae)	AHT-16	AAU, Jorhat, Assam	Mild chlorosis
				AHT-17		X
				IH-673		Mild chlorosis
				IH-662		X
				CO-2	Tamil Nadu	X
				Morden		Mild chlorosis
				Swarna Hybrid		X
				CO-4 (TNAUSUF-7)		X
				TCSH-1 (TNAU)		Mild chlorosis
18	*Matricaria aurea* Boiss.	-	Compositae (Tribe: Anthemideae)	-	Sunder Nursery, N. Delhi	X
19*	*Parthenium hysterophorus* L.	Congress weed	Compositae (Tribe: Heliantheae)	-	NBPGR, N. Delhi	X
20	*Solidago canadensis* L.	Golden rod	Compositae (Tribe: Astereae)	-	PDBC, Bangalore	X
21	*Sonchus arvensis* L.	Field sowthistle	Compositae (Tribe: Lactuceae)	-	NBPGR, N. Delhi	X
22	*Tagetes erecta* L.	Big marigold, Aztec marigold	Compositae (Tribe: Helenieae)	African marigold (Tall)		X
23	*Tagetes tenuifolia* Cav.	Striped marigold	Compositae (Tribe: Helenieae)	Single signet		X
24	*Tithonia diversifolia* (Hemsl.) Gray	Mexican sunflower, tree marigold	Compositae (Tribe: Heliantheae)	-	Ganganagar, Bangalore, Karnataka	X
25	*Vernonia anthelmintica* (L.) Willd.	-	Compositae (Tribe: Vernonieae)	-	NBPGR, N. Delhi	X
B. Other economically important plant species						
26	*Lobelia erinus* L.	-	Campanulaceae	Crystal palace	Sunder Nursery, N. Delhi	X
27	*Ochlandra travancorica* (Bedd.) Benth. ex Gamble.	Elephant grass, reed	Gramineae	-	AAU, Jorhat, Assam	X
28*	*Oryza sativa* L.	Paddy, rice	Gramineae	-	National Seeds Corporation (NSC) Ltd., N. Delhi	X
				NDRK 5026-R	Genetics Division, IARI, N. Delhi	X

Ref. No.	Scientific name	Common name	Family	Cultivar	Source/place of collection	Rust symptoms
29	*Pennisetum typhoides* (Burm. f.) Stapf. & C. E. Hubb.	Pearl millet	Gramineae	HHB-117	NBPGR, N. Delhi	X
30	*Triticum aestivum* L. emend.Thell	Wheat	Gramineae	PDW-343	Genetics Division, IARI	X
31	Sorghum vulgare *Pers.*	Sorghum	Gramineae	GV UP CHARI-2	NBPGR, N. Delhi	X
32*	Zea mays *L.*	Maize	Gramineae	Composit 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 Agricultural University (KAU), Thrissur, Kerala	X
37	*Cinnamomum zeylanicum* Blume.	Cinnamon	Lauraceae	IISR Navasree		X
38	*Syzygium aromaticum* (L.) Merr. & Perry	Clove	Myrtaceae	-		X
39	*Piper betle* L.	Betel-pepper, Betel vine	Piperaceae	-	KAU, Thrissur, Kerala	X
40*	*Theobroma cacao* L.	Cocoa, 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.f.	Teak	Verbenaceae	-	Kerala	X
46	*Bambusa arundinacea* (Retz.) Willd.	Thorny or Spiny bamboo	Bambusaceae	-		X
47	*Mangifera indica* L.	Mango	Anacardiaceae	Mallika	N. 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	Zingiberaceae	-	N. Delhi	X
51	*Elettaria cardamomum* Maton	Cardamom	Zingiberaceae	CCS-1	Indian Inst. of Spices Res. (IISR) Cardamom Res. Centre, Appangala, Karnataka	X
52	*Anacardium occidentale* L.	Cashew	Anacardiaceae	Vengurla	Bangalore, Karnataka	X

Ref. No.	Scientific name	Common name	Family	Cultivar	Source/place of collection	Rust symptoms
53*	*Arachis hypogaea* L.	Peanut, Groundnut	Papilionaceae	TG-45	NBPGR, N. 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. (MAHYCO)	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.) Sm.	Lesser swinecress	Cruciferae	-		X
62	*Matthiola incana* (L.) Ait. f.	Tenweeks stock, gilli flower	Cruciferae	-	Sunder Nursery, New Delhi	X
63*	*Raphanus sativus* L.	Radish	Cruciferae	Pusa Desi	NSC Ltd., N. Delhi	X
64	*Capsicum annuum* L.	Chilli, red pepper	Solanaceae	Pusa Hyper-2		X
65	*Nicotiana tabacum* L.	Tobacco	Solanaceae	-	NBPGR, N. Delhi	X
66*	*Solanum melongena* L.	Brinjal	Solanaceae	PK	NSC Ltd., N. Delhi	X
67	*Ricinus communis* L.	Castor	Euphorbiaceae	DCH 519	NBPGR, N. Delhi	X
68	*Viola tricolor* L.	Pansy, heart's ease	Violaceae		Sunder Nursery, N. Delhi	X
69	*Tropaeolum majus* L.	Garden nasturtium	Tropaeolaceae			X
70	*Antirrhinum majus* L.	Snapdragon	Scrophulariaceae	-		X
71	*Linaria bipartite* Willd.	Toad flax	Scrophulariaceae	-		X
72	*Phlox drummondii* Hook.	Drummond phlox, annual phlox	Polemoniaceae	-		X
73	*Dianthus* sp.	Dianthus	Caryophyllaceae	-		X
74*	*Linum usitatissimum* L.	Linseed, flax	Linaceae	RLC 81	NBPGR, N. Delhi	X

Species also tested at CABI UK

Appendix 4. Note from smallholder tea farmer in Assam (transcribed from handwritten)

MIKANIA – Farmer's Burden

In Assam during rainy season different types of weeds disturb the farmer. Among them a creeper like weed called 'Mikania' local name 'BURBOK LATA' (maniac creeper). The name indicates its character. Mikania is one of the major enemies of the farmer, because is covers it ties up the plant, thereby resist photosynthesis as a result of which some economically important plant badly affected. Production of the plant decreases even the plants die sometimes. Continuous quilt like layers of Mikania is visible in Assam especially on tea bushes where maintenance is poor.

Due to small area, generally the small grower (tea) up root it – when come to notice and destroy it – away from the garden by weedicides or by burning after two or three days. Manual control is better than mechanical control. In case of mechanical control 2-4-D powder at recommended dose mix with water can be sprayed. It has been observed that when 2-4-D issued the leaf of the plant becomes curly for which host plant also becomes weak for some days, and reduces it's productivity. This is the drawback, use of their herbicides cannot be imagined on tea bushes, some times the plucker pluck it at the same level of the tea bud, then its limb multiplies so, it is not a good practice.

Mikania as a secondary host: Mikania harbours Hellopeltis (Tea Mosquito bug) one of the dangerous insects which attack the soft tissue of tea buds. To control Hellopelties when we spray insecticides they fly to the nearest Mikania and take shelter there for a few days and return to the original plant and starts to attack again.

To get rid of Hellopeltis spray the same solution on Mikania also, which indicates more expenditure.

Effect of Mikania on fences: To protect any crop especially in Assam a boundary fence is necessary. Generally is Assam berdidaire goat proof fence even wood and Bamboo fences are used. These items are costly here, but the life of the fence is very short only because of Mikania. Because Mikania covers the entire fence or tie up (if measures are not taken immediately) rainwater absorbed by it and finally destroys the boundary fence. Then wild or domestic animals destroy the fenceless crop.

To protect the environment, to keep the globe free from pollution, ecologically balance world this type of weed must be controlled 'Biologically' enviroment friendly substance to make pollution free world for our future generation.

Note prepared by:-

Rajen Ch. Kolilu
Hill-View Tea Plantation
JEC Road
Jorhat, Assam
India

June 2003
(transcribed from handwritten)

Appendix 5. Proceedings of the workshop on ICAR-CABI-AAU collaborative project on "Classical biological control of *Mikania micrantha* with *Puccinia spegazzinii*: Implementation phase" held on 28-29 November 2005 at AAU, Jorhat, Assam.

Members Present

1. Dr. S. Murphy, Ecologist, CABI Bioscience, UK
2. Dr. C. A. Ellison, Plant Pathologist, CABI Bioscience, UK
3. Dr. R. J. Rabindra, Project Director, PDBC, Bangalore
4. Dr. M. N. Borgohain, Director of Extension Education, FA, AAU, Jorhat
5. Dr. R. C. Bora, Dean, FA, AAU, Jorhat
6. Dr. A.K. Roy, Rtd. Director of Research, AAU,Jorhat, Assam
7. Dr. G.N. Hazarika, ADR, AAU, Jorhat, Assam
8. Dr. U.N. Saikia, Director of Post Graduate Studies, AAU,Jorhat, Assam.
9. Mr. Deepak Baruah, Manager, Assam Tea Corporation, Cinnamora,Jorhat, Assam
10. Dr. A. K. Phookan, Prof. and Head Department of Plant Pathology, AAU,Jorhat.
11. Dr. L.K. Hazarika, Prof. and Head Department of Entomology, AAU,Jorhat
12. Dr. A.K. Sinha, Prof. and Head Department of Nematology, AAU,Jorhat
13. Dr. Paran Boruah, Scientist E-II, Plant Pathology, RRL, Jorhat, Assam
14. Dr. B. K. Saikia, Rtd Associate Prof. Department of Plant Pathology, AAU, Jorhat
15. Dr. A. Basit, Principal Scientist, Bio-control, Dept. of Entomology, AAU, Jorhat
16. Dr. Y. Rathiah, Prof. Department of Plant Pathology, AAU, Jorhat, Assam
17. Dr. D.J. Rajkhoa, Principal Investigator, AICRP Weed science, AAU, Jorhat
18. Dr. N. C. Deka, Senior Scientist, AICRP Weed science, AAU, Jorhat
19. Dr. P. Devnath, Associate Prof, Department of Plant Pathology, AAU, Jorhat
20. Dr. R.P. Bhuyan, Associate Prof. Tea Husbandry and Technology, AAU, Jorhat
21. Dr. Madhumita Barua, associate Prof. Agril. Biotechnology, AAU, Jorhat
22. Dr. A.K. Saikia, Prof. Department of Plant Pathology, AAU, Jorhat
23. Dr. (Mrs.) Daisy Senapaty, Sr. Scientist, Department of Plant Pathology, AAU,Jorhat
24. Dr. M.K. Saikia, Assoc. Prof. Department of Plant Pathology, AAU,Jorhat
25. Dr. Robin Gogoi, Sr. Scientist, Department of Plant Pathology, AAU,Jorhat
26. Dr. B. C. Das, Assoc. Prof. Department of Plant Pathology, AAU, Jorhat
27. Dr. J.J Pathak, Assoc. Prof. Department of Plant Pathology, AAU, Jorhat
28. Dr. S. Ali, Principal Scientist, RARS, Titabor,
29. Dr. Prabhat Das, Sr. Extn Specialist, AAU, Jorhat, Assam
30. Dr. D. K. Saikia, Sr. Scientist, Bio-control, Department of Entomology, AAU,Jorhat
31. Dr. D. K. Sarmah, Sr. Scientist, Department of Plant Pathology, AAU, Jorhat.
32. Dr. K.C. Puzari, Principal Scientist, Department of Plant Pathology, AAU, Jorhat,
33. Mr. Pranab Dutta, Research Assoc., Dept. of Plant Pathology, AAU, Jorhat
34. Mr. Hiranya Kr. Devanath, Research Assoc., Dept. of Plant Pathology, AAU, Jorhat
35. Mrs Purnima Das, SRF, Department of Entomology, AAU, Jorhat, Assam
36. Mr. Runita Sinha, PG Student, Department of Plant Pathology, AAU, Jorhat
37. Mrs Tasvina Rahman Borah, SRF, Department of Plant Pathology, AAU, Jorhat
38. Mr. Gaurav Aditya Saikia, Department of Plant Pathology, AAU, Jorhat
39. Mr. Donbor Singh R Sohlia, Department of Plant Pathology, AAU, Jorhat.

Introduction and Inaugural Session

A workshop on "Classical biological control of *Mikania micrantha* with *Puccinia spegazzinii*: Implementation phase" was held on Nov, 28-29, 2005 at the conference hall, Directorate of Research, Assam Agricultural University, Jorhat, Assam. The inaugural session was attended by 35 scientists from: CAB International, Bioscience Division, U.K. (CABI); Project Directorate of Biological Control (PDBC), Bangalore; Regional Research Laboratory (RRL), Jorhat; and Assam Agricultural University (AAU), Jorhat. The session was initiated with a welcome address by Dr. A.K. Phookan, Head, Department of Plant Pathology, AAU, in which he expressed gratitude to CABI and PDBC for support and encouragement.

Dr. M. N. Borgohain, Director, Extension Education, FA, AAU delivered his inaugural address and narrated the importance of *Mikania micrantha* in the northeastern region of India. Dr. R.C. Borah, Dean, Faculty of agriculture, AAU, also addressed the gathering and express satisfaction for taking up biocontrol approaches to manage this weed.

Dr. R. J. Rabindra, Project Director, PDBC, Bangalore, emphasized the need for biocontrol of *Mikania* because of its threat to the biodiversity of the forest ecosystem and tribal welfare. In controlling Mikania, weedicides are extensively used which may lead undesirable residues in tea. Dr. Rabindra commented on the need to generate data on residues of weedicides in the tea product.

Dr. S. Murphy, Scientist from CABI Bioscience, UK emphasized the importance of classical biological control in managing weeds like *Mikania*. He delivered an overview of the importance of invasive alien species, government policy and collaboration between organisations.

The inaugural session ended with a vote of thanks from Dr. R. P. Bhuyan, Associate Professor, Dept. of Tea Husbandry and Co-PI of the project.

Technical Session

Dr. Carol A. Ellison

In the technical session Dr. C. A. Ellison, Plant Pathologist, CABI Bioscience, UK delivered a talk on classical biological control (CBC). She mentioned in her deliberation that invasive alien species are of global concern, and cause enormous costs to society and the environment. The work on CBC of *Mikania* has been on-going for last 10 years, and that this approach is inherently safe, cost effective, target specific, environmentally benign and sustainable method of controlling invasive alien species. She mentioned that India has many invasive alien plants species, including the following 8 major ones: *Ageratum conyzoides, Chromolaena odorata, Ageratina adenophora, Mikania micrantha,*, *Parthenium hysterophorus, Lantana camara, Mimosa invisa* and *Eichhornia crassipes*. In India biocontrol work has already been successfully implemented for a number of these species using insect natural enemies, viz., *Eichhornia crassipes* (water hyacinth), *Salvinia molesta* (water fern), *Parthenium hysterophorus* (parthenium weed), *Chromolaena odorata,* (Siam weed). The following biocontrol agents were released against these weeds: *Neochetina* spp. and *Orthogalumna terebrantis, Cyrtobagous salviniae, Zygogramma bicolorata, Cecidochares connexa,* respectively.

Use of fungal pathogens in CBC of weeds was first initiated in 1971 in Australia. Since then, a total of 26 fungal pathogens have been released up to 2004, in seven countries, with a number of success stories to date. Out of all the different pathogen biocontrol agent's, fungal pathogens are mostly exploited.

Dr. Carol mentioned that, *Mikania* is a problem over vast areas of the moist tropical zones of Asia, and that many areas still under threat. *Mikania* invades natural and

agricultural ecosystems, and that conventional control methods are ineffective, expensive and environmentally damaging. She also illustrated the impact of *Mikania* infestation in various parts of India, in tea, sugarcane and teak plantation with photographs.

Mikania biocontrol programme was started in 1978, investigating arthropods as natural enemies, and culminated in release of a host specific species of thrips, in Malaysia and the Solomon Islands. Unfortunately, probably due to predation the conspicuous nymphal stage (bright red), the agent failed to establish in the field. In 1996 new initiative with India using fungal pathogens was started, funded by the UK Department for International Development. The programme has involved collaborators from: CABI, UK; Indian Council of Agricultural Research, New Delhi; PDBC, Bangalore, India; AAU, Jorhat, Assam; Kerala Forest Research Institute, Peechi, Kerala, India and National Bureau of Plant Genetic Resources, New Delhi, India. During Phase I, potential fungal pathogens were evaluated, and the impact and spread of weed in the Western Ghats evaluated. At the end of this phase a host specific and damaging co-evolved rust fungus, *Puccinia spegazzinii,* was selected as the best candidate for introduction into India.

Under quarantine conditions at CABI-UK, seven distinct pathotypes of the rust were tested against populations of *Mikania* from Kerala and Assam. For all of the populations of the weed tested from Kerala and most of those tested from Assam an isolate of the rust from Trinidad was considered to be the best pathotype. Her research work revealed that genetic types of weed in Assam are different to those in Kerala by the molecular analysis. Pathogenicity testing confirmed that, although the Trinidad isolate does not fully infect all genetic types of *Mikania* in Assam, the pathotype from Peru fully infects all semi-resistant genetic types tested from Assam.

In 2002 during the second phase of the project the Trinidad isolate of rust fungus was imported from UK to quarantine in India, for additional host specificity testing. In the UK, more than 55 test plant species had been tested with the rust (all were resistant); the rust was found only to infect *Mikania micrantha*. Similarly in China, an Argentina isolate was imported in 2003.

In her concluding remark she mentioned that *Puccinia spegazzinii* is the first pathogen intentionally released on continental Asia for weed CBC. Following the Indian lead, other countries are 'fast tracking' similar programmes. For example, China imported an isolate of the rust from Argentina in 2003. The Indian experience is a 'pilot project' for this technology; this should aid future exploitation of pathogens for weed suppression in Asia.A question was raised about the possibility of *Mikania* developing resistance against the rust pathogen. Dr. Ellison explained that *Mikania* and the pathogen are co-evolved and that both have the ability to continue their evolution. Therefore the rust should be able to keep-up with any changes in the weed, to maintain its pathogenicity.

Dr. A. C. Puzari

Dr. K. C. Puzari presented the results from India of the experiments conducted under the Phase II of the project; "Classical biological control of *Mikania micrantha* with *Puccinia spegazzinii*". The objectives of the project were:

1. Studies on distribution and abundance of *Mikania micrantha* under tea ecosystem.
2. Additional host specificity test.
3. Multiplication and establishment of *Puccinia spegazzinii* in Assam condition.

1. Studies on distribution and abundance of *Mikania micrantha* under tea ecosystem

He has completed survey work in Assam by following two different methods viz., roving survey and fixed plot survey. Fixed plot survey was made at Cinnamora tea estate and Assam Agricultural University, Tea garden, Jorhat. In both the cases quantification of the level of infestation with Mikania in tea ecosystem was made by the modified level (Grade) for numbers of stalks of plants/ha.

Roving surveys were made in 85 tea gardens situated in different agro-climatic zones of Assam. Out of these, 33 gardens were small tea growers (STG), three were Corporate sector (ATC) and the other 49 gardens were company or Estate sector. The survey was made during the peak growing season of *Mikania*. Data were collected on data sheet by interviewing the farmers, and analyzed the results. Dr. Puzari presented that all the surveyed gardens were found to have *Mikania* infestation with a maximum of more than 1000 stalks /ha. He reported that out of all the different constraints faced by the farmers, infestation of *Mikania* is of major importance (ranked I). His study on loss caused by *Mikania* revealed that most of the surveyed gardens have to incur a yield loss of 1-5% (20-100 kg made tea/ha) annually. Yield loss of 41-50% was reported from 20 % of garden from Upper Brahmaputra Valley Zone (UBVZ) and more than 50% from 7.14% of Barak Valley Zone (BVZ).

In the fixed plot survey he reported that the annual out-break of *Mikania* varied in the different months of the year and found maximum infestation at the later part of August to September in both the surveyed sites. He also reported that there is a 20% increase in labour requirements annually, in the gardens, to keep them free from *Mikania*.

2. Additional host specificity test.

Additional host specificity testing was carried out at NBPGR on 74 plant species, which were selected using the centrifugal phylogenetic testing protocol (based on the relatedness of the test plants to *Mikania*) and also a wide selection of crop plants grown in the Western Ghats and Assam. Out of the 74 plants species tested, none were found infected with the rust *Puccinia spegazzinii* (W1761-Trinidad isolate), whereas all *M. micrantha* control plants were severely infected. A resistant reaction was observed on sunflower (cv. AHT-16, IH-673, TCSH-1 and Morden), of mild chlorosis on a few top leaves, which however, recovered during further observation.

3. Multiplication and establishment of *Puccinia spegazzinii* in Assam condition.

For multiplication and establishment in Assam, three rust consignments were brought from NBPGR. Thirty plants of *Mikania* were inoculated with the first consignment and out of which only 18 plants were showed chlorotic spots. Plants were observed for 30 days after inoculation, but the infection did not progress any further.

With the second consignment 30 plants were inoculated (6 originating from Kerala and 24 from Assam) in 3 sets (2 Kerala plants were inoculated with 8 Assam plants in each set). Inoculated plants were kept at temperature 26 ± 2^0 C, RH 65-70% with 12 h light (25,000 lux) and dark period. 100% of Kerala plants were found infected by the fungus and attained the maturity stage with score 4, whereas only 12.5% for Assam plants became infected. This suggested that the genotype of the plants from Assam that were being infected were not fully susceptible to the Trinidad isolate of the rust.

Limited field release of rust fungus was made on two localities. The first release was made at Assam Agricultural University tea garden in the 1st week of October 2005. Unfortunately, after the onset of rains a sudden attack of the pathogen *Cercospora mikanicola* Stevens occurred which spread quickly and dried the leaves, which were already infected by *P. spegazzinii,* inhibiting further development of the rust pustules. However, the infection of the rust is still progressing in the field with the inoculum released from the infected stem. Another field released was made in Cinnamora Tea Estate, Hatigarh Division. The inoculation was done on 1st week of November 2005. The infection in the field was found to be quite satisfactory.

Recommendations

1) House expressed their full satisfaction over the progress of work made on the project.
2) Both the CABI scientists and Project Director, PDBC, Bangalore has agreed to extend the project for another 6 months.
3) Considering the slow progress of infection by the Trinidad isolate of *Puccinia spegazzinii* (W1761) on some *Mikania* biotypes from Assam, the Peruvian isolate (and possibly a new rust Dietelia mesoamericana) should be included in the studies at AAU. It was initially decided that the Peru isolate would be sent to NBPGR early in 2006, for limited screening, before being sent to AAU for release in the field.
4) House also suggested collecting the base-line data, before the impact of the rust is apparent, on various aspects such as: soil residue of weedicide, weedicide application status, weedicide consumption, and other socio-economic parameters. House suggested consulting with socio-economist at AAU on these aspects.
5) Dr. Rabindra, Project Director, PDBC, Bangalore suggested investigating the possibility of this work being (4 above) being funded by a local funding agency in Assam.
6) Dr. Murphy emphasized that the current investigation at AAU should concentrate on the establishment and distribution of the rust fungus under field condition and natural spread from the released sites.
7) Dr. Carol suggested that radial sampling of the rust from the initial release should be undertaken in this investigation, possibly on a weekly basis, but that sampling will need to be modified according to the rate of spread. The house agreed with the suggestion and emphasized for this investigation.

Field visit

The team comprising of scientists from CABI, Project Director, PDBC, Bangalore, scientists from AAU, and tea garden managers visited the rust released site at Cinnamora tea estate, on 28th November 2005. All the scientists were happy to see the establishment of rust fungus *Puccinia spegazzinii,* first CBC agent in India, on *Mikania micrantha*. The methodology (air-hanging of pots containing rust infected *Mikania* plants, within the tea canopy) adopted for field inoculation, was expressed as an innovative approach by the visitors. However Dr. C.A. Ellison, Plant pathologist, CABI, U.K expressed her concern with the reduced size of the rust pustules that had developed on the *Mikania* in the field. It was considered that this was probably due to the genotype in the field at Cinnamora being a semi-resistant type to the Trinidad pathotype of the rust, and thus justifying the introduction of alternate CBC isolate/agent.

Dr. Murphy and Dr. Ellison also visited the other site of inoculation at AAU, TPU garden (on 29th November), where infection of *Cercospora* masked the progress of *Puccinia* infection. They were of the opinion that the timing of field release in *Cercospora* prone areas should be adjusted in order to avoid its damage on the progress of the rust infection until the rust was fully established, and hence at a level able to coexist with the *Cercospora*. However they expressed satisfaction with the persistent infection of *Puccinia* from infected pustules on the stem.

Laboratory Visit

Scientist from CABI, Dr. S.T. Murphy, Dr. C.A. Ellison and Dr. R.J. Rabindra, Director, PDBC, Bangalore, also visited the Rust Propagation Unit (RPU) of Mycology Research section on 29th Nov 2005. They expressed their full satisfaction with the facilities developed (RPU) for propagation of rust fungus *P. spegazzinii*. However, to improve it further, enabling a better performance of inoculation and rust development, the following modifications were suggested:

1. Provision of a post-inoculation chamber of size 3m X 4m within the RPU for housing the inoculated plants, during rust symptom development.
2. Providing 50 % agro shade net on the net house, that can be easily retracted when not required.
3. Providing a rotating stand fan in the net house.
4. Provision of a small dew chamber for maintaining the rust within the RPU

Dr. Ellison also visited the facilities developed for post-inoculated plants under AC status. She was very much impressed with the efforts made to make the project successful.

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