RNRRS PROJECT FINAL TECHNICAL REPORT

The development of a biocontrol strategy for the management of the alien perennial weed, *Mikania micrantha* H.B.K (Asteraceae) in tree crop based farming systems in India

DFID PROJECT REFERENCE NUMBER:
R 6735

RNRRS PROGRAMME:
Crop Protection

PROGRAMME MANAGER (INSTITUTION):
Dr S. Eden-Green, NR International Ltd

RNRRS PRODUCTION SYSTEM:
Forest / Agriculture Interface, Purpose 2

COMMODITY BASE:
Tree crops / agroforestry trees and associated crops in tropical moist forest zones of India

RNRRS PROGRAMME PURPOSE:
Sustainability and yield from tree crop based systems at the forest / agriculture interface improved through the removal of pest constraints

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EXECUTIVE SUMMARY

Mikania micrantha H.B.K. (Asteraceae) is a Neotropical invasive weed that smothers tree and other crops in homegarden production systems in the tropical moist forest regions of India. The project purpose was to develop a management strategy based on biological control utilizing fungal pathogens. The main project collaborators include the Kerala Forest Research Institute (KFRI), the Project Directorate of Biological Control (PDBC–ICAR), Assam Agricultural University (AAU) and CABI Bioscience, UK.

The range of *M. micrantha* is now mapped in the Western Ghats; 28 permanent sample plots have been established in Kerala. The weed is present in most of Kerala and is now moving northwards into Karnataka. Significant impacts of weed in tree crop/agroforestry systems have been confirmed. Crops particularly affected include banana, coconut, coffee, cocoa, cassava, pineapple, ginger and teak. Intensive weeding of *M. micrantha* has now become necessary to reduce its effect on productivity; this has resulted in the escalation of production costs. In moist deciduous forests, the infestation by *M. micrantha* is on the increase, which affects harvesting by tribals of reeds, bamboo and other non-wood forest products difficult. Farmers support implementation of biological control

The status of *M. micrantha* in northeastern India has also been assessed. The main impact is on tea production. Herbicide residues on tea have resulted in some exports being rejected by Western Europe. AFLP analyses indicates that the weed population probably originates from Central America rather than elsewhere in the indigenous range from Mexico to Paraguay.

Surveys for potential exotic fungal pathogens have been made in Brazil, Mexico, Trinidad and Costa Rica; twenty nine suspected fungal pathogens have recorded of which four are considered to have most potential as classical biological control agents. Surveys in India revealed only nine minor fungal pathogens.

The exotic rust pathogen, *P. spegazzinii* was selected for detailed assessment in view of the damaging nature in the field; eleven isolates have been collect and the high biocontrol potential of *Puccinia spegazzinii* (ex Trinidad) confirmed in the quarantine labs of CABI Bioscience. Also the life cycle of the rust has been elucidated. None of the Indian fungal pathogens were found to be damaging and thus inappropriate for mycoherbicide development.

A protocol for the introduction of exotic fungal pathogens was discussed and agreed with relevant institutions. For this protocol, a high specificity/biocontrol potential of *P. spegazzinii* has been demonstrated *and* support for introduction from *all* collaborators confirmed (recorded in meeting memoranda and workshop recommendations). A dossier has been prepared for GoI.

KFRI staff have been trained in ecological and socio–economic survey techniques for alien invasive species. Also pathologists from KFRI/ICAR were trained in classical biological control and mycoherbicide development and application, through training attachments at CABI Bioscience, UK Centre (Ascot).

An alert sheet was distributed in 1997 (particularly in Karnataka) and a national workshop held at KFRI in November 1999.

A meeting was held at ICAR's PDBC in June 1999 and they are now included in the project workplan. Recommendations were made by the national workshop particularly for a follow-on phase; support was also expressed for the introduction of the exotic rust fungus, *P. spegazzinii*.

CONTENTS

ACRONYMS

KFRI	Kerala Forest Research Institute, Peechi, Kerala
ICAR	Indian Council of Agricultural Research
AAU	Assam Agricultural University, Jorhat, Assam
TTES	Tocklai (Tea) Experimental Station, Jorhat, Assam
DPP Q&S	Directorate of Plant Protection, Quarantine and storage (Min. ag. Ag.), New Delhi

BACKGROUND

Introduction

In the moist tropical forest region of the Western Ghats in southwest India, agroforestry is the principal form of agriculture of the local people. The type of system depends on the type of community and can range from semi-permanent holdings of hill tribes to the more common permanent holdings, or 'homegardens' characteristic of the region. The term 'homegarden' refers to the intimate association of tree crops, multipurpose trees and shrubs with annual and perennial crops and, invariably livestock within the compounds of individual houses (Nair, 1993). In the state of Kerala, for example, agriculture is the major land use, accounting for 58% of the total geographic area. Here most homegardens (approx. 76%) belong to marginal farmers with less than 1.5 ha. of land holding; the second largest category are small-holders with 1.5 – 5 ha. (approximately 35%).

Both food crops and commercial crops are grown throughout the Western Ghats but the cropping pattern differs broadly in relation to physiographic parameters. For example, in the highlands (above 75m) coffee, tea and cardamom are predominantly cultivated; bamboo and reed are also harvested from the natural forests by local tribes. The midlands (8 – 75 m) are an area of intensive cultivation, where crops such as banana, plantain, cashew, coconut, arecanut, cocoa, cassava, yam, ginger, pepper and vegetables are grown. These homegardens are interdispersed with multipurpose trees such as teak, casuarina and *Ailanthus*. Coconut is the major crop in the lowland areas (< 8m).

Cultivation costs has always been a primary factor in the economics of homegardens, particularly for marginal and small-holder farmers. Weeds, in particularly, form a major proportion of cultivation costs. Over the last few decades, the Neotropical invasive weed, *Mikania micrantha* H.B.K. (Asteraceae) has recently spread throughout the southern part of the Western Ghats causing severe damage to several tree crops, multipurpose trees and seasonal crops (e.g. cassava/ yam). The weed has a vigorous vegetative and sexual reproductive capacity (Swarmy and Ramakrishnan, 1987), but cannot tolerate dense shade (Holm *et al*., 1977). Growth of young plants is extremely fast (8 –9 cm in 24 hours; Choudhury, 1972) and will use trees and crops to support its growth; the weed rapidly forms a dense cover of entangled stems bearing many leaves (Holm *et al*., 1977). Damage is caused to crops because the weed can smother, penetrate crowns, choke, and even full over plants. There is also evidence from studies on rubber in Malaysia that the weed can retard plant growth probably through the production of allelopathic substances (Holm *et al*., 1977; Cronk and Fuller, 1995). Initial reports of tree and other crops severely affected in the Western Ghats include: plantains, bananas, bamboo and reeds (Nair, 1988; KFRI, unpublished) although other agricultural crops were undoubtedly being affected.

Besides the problems caused in homefarms and other agroforestry systems, there were also many reports of severe damage being caused in other land-use systems in the Western Ghats and from the tropical moist forest zone of northeastern India. Examples include:

Western Ghats – forest plantations (teak and eucalyptus) and disturbed natural forest (Muniapan and Viraktamath, 1993; Nair, 1988; KFRI, unpublished).

Northeastern India – tea (Parker, 1972; Holm *et al*., 1977; Sen Sarma and Mishra, 1986; Choudhury, 1972) subsistence crops grown in short rotation shifting agricultural systems

(Swarmy and Ramakrishnan, 1987) and forest plantation and disturbed natural forest (Parker, 1972; Palit, 1981; Choudhury, 1972).

The genus *Mikania* consists of about 250 species of herbaceous or slightly woody creeping or twining plants (Holm *et al*., 1977). *M. micrantha* is native in South and Central America and the Caribbean. Some authors support the view that the weed was introduced into northeastern India as a non-leguminous ground cover for crops (Parker, 1972; Borthakur, 1977; Palit, 1981). It is also reported that it was introduced into the same region during the Second World War for camouflaging purposes (Choudhury, 1972). *Mikania micrantha* was first observed in the Western Ghats region in the 1980s (KFRI, pers. comm.).

Demand

The Kerala Forest Research Institute (KFRI, pers. comm.) emphasized the increasing problems that *M. micrantha* causes (economic and social) and needs for action in different tree crop based systems, forest plantations and natural forest areas in southwest India. Their concerns were based on their own work (local surveys, research) and interaction with farmers and plantation owners but they also drew attention to the fact that the problems caused by *M. micrantha* has been documented in the Indian scientific literature. The demand from farmers has since been confirmed during a socio–economic study (see later).

The problems caused by *M. micrantha* in tea and other crops in north eastern India were brought to attention during the second year of the project by the Indian Council of Agricultural Research (ICAR). These problems were confirmed during a project survey and discussions with tea growers in Assam and Meghalaya in January/February 1998 (see later).

Previous research on weed management, particularly biological control

Current control options centre around slashing (Sen Sarma and Mishra 1986, Muniappan and Viraktamath 1993), and the use of herbicides (Choudhury, 1972; Parker, 1972; Palit, 1981; Muniapppan and Viraktamath, 1993;). Both forms of control are prohibitively expensive, ineffective, not sustainable, and particularly in the case of herbicides, environmentally damaging and can cause tainting problems when used in high value crops such as tea. Another approach to the control of invasive alien weeds is classical biological control (cbc). This method involves the importation and release of coevolved natural enemies from the native range of the target weed, with the aim of reducing its competitive ability and hence of achieving control. Classical biological control offers an environmentally benign, cost effective, sustainable and safe method of weed control (McFadyen, 1998)

Research into cbc of *M. micrantha* was started in 1978, and concentrated on insect natural enemies (Cock, 1982a). Extensive surveys were undertaken in Latin America and *Liothrips mikaniae* (Priesner) (Thysanoptera, Phlaeothripidae) from Trinidad, was selected. Host-range screening was carried out using thirty-seven test plant species: three *Mikania* spp., thirteen other Asteraceae, and twentyone further species from fifteen other plant families. Although there was slight feeding on a few other species, *L. mikaniae* was shown to be host specific to *Mikania*, and would only feed on species closely related to *M. micrantha* (within the genus *Mikania*) (Cock 1982b). Releases of the trips were made in the Solomon Islands in 1988, and in west Malaysia in 1990, but neither led to establishment (Cock *et al*., 1999). A number of possible reasons for this failure have been proposed, and are given below:

- *L. mikaniae* is a conspicuous insect (red coloured nymphs) and attract opportunistic indigenous natural enemies, e.g. ants;
- Disruption of dispersal, mate location or host finding behaviour, because of the continuous distribution of *M. micrantha* compared to its distribution in small patches in the area of origin;
- Unidentified pupal mortality;
- Host plant incompatibility, whereby the thrips may not have been able to attack all the biotypes of *M. micrantha* present in the areas of release;
- Sub-optimal release strategy; since the optimal approach had not been fully investigated prior to release.

However, from the available information, it would appear that the impact of indigenous natural enemies was likely to be the most significant factor (Cock *et al*., 1999).

The failure of *L. mikaniae* at this time led to a loss in interest in the potential for classical biological control of *M. micrantha.* No further investment was made in this approach to the control of the weed, until this new initiative with India, part of which is to look at an alternative group of natural enemies with great potential: co-evolved fungal pathogens (Evans, 1995). The evaluation of the diversity of pathogens recorded from *M. micrantha* was given a head start by the previous work of Evans (1987) and Barreto and Evans (1995), who highlighted a number pathogens with potential as classical biological control agents.

PROJECT PURPOSE

The project purpose is – 'Biological control by use of fungal pathogens, of the perennial weed, *M. micrantha* in tree crop and agroforestry systems in the moist tropical region of southwest India developed'.

At the end of 1997, the purpose was expanded to include the moist tropical zone of northeast India (through add-on funding). Here, *M. micrantha* has a particularly serious impact on tea growing. The area was included as a direct request of ICAR, Assam Agricultural University (AAU) and Tocklai (Tea) Experimental Station (TTES).*
The project sought to assess and develop the potential for management through biological control utilizing fungal pathogens; *in particular* exotic pathogens as classical biological control agents but also native pathogens for the development of a mycoherbicide for localized use in some tree – crop plantations. Exotic pathogens, once they have become widespread, have the potential to reduce the abundance of the weed. Such an approach is self – perpetuating and this provides a long-term *sustainable* management option for the *M. micrantha* problem. Furthermore, exotic biological control agents require little or no input from resource – poor farmers and are environmentally benign. Local marginal and small – holder farmers will benefit from increased yields and decreased labour costs to control the weeds by other means.

There are good precedents for this approach, e.g. the introduction of a white smut from Central America into Hawaii against mistflower (*Ageratina riparia*) (Trujillo, 1985) and the rust fungus, *Puccinia chondrillinae* from Europe into Australia against skeleton weed (Cullen and Hasan, 1998).

* At the start of the project, KFRI also decided to include some herbicide trials. This was done to increase the chance of developing a short-term management option for localized use in some tree–crop plantation areas. However, herbicides are not appropriate for wide–spread or a long–term management strategy.

The project purpose was addressed through six outputs as follows:

1. Distribution agricultural importance and the economic and social impact of *M. micrantha* in selected areas of the Western Ghats determined.
2. Mycobiota characterized and potential pathogenic fungi biocontrol agents identified from the weed in Brazil and India.
3. The biological control potential of fungal pathogens from Brazil and India on the weed biotypes present in India determined and compared.
4. Host specificity listing requirements determined, host specificity lists completed and import/release protocol developed.
5. Indian scientists trained in tree-crop and forestry weed management research and implementation.
6. Project research outputs publicised and promoted.

Some outputs were broadened through add-on funding as described in the following sections. However, one new output (no. 7 below) was added in 1999.

7. Implementation phase for the biological control of *M. micrantha* in India formulated and agreement reached on the role/activities of new/current collaborators.

RESEARCH ACTIVITIES

1. Distribution, agricultural importance and the economic and social impact of *Mikania micrantha* in selected areas of the Western Ghats determined.

(Note, all training activities are described under activity number 5)

a. Distribution and agricultural importance.

Surveys to assess the distribution and abundance of *M. micrantha* were conducted by the KFRI from July 1997 to February 1999. This was to assess the regional significance of the weed, whether or not it is still in the invasive phase, and to provide data for monitoring and the design of biological control agent release strategy. All districts of Kerala State were covered together with the western districts of Karnataka and Goa (covering the windward side of the Western Ghats). In each district, efforts were made to visit several sites representing home garden systems, tree crop and forest plantations, and natural forest areas; natural forests reported to be affected by *M. micrantha* include evergreen, semi-evergreen, moist deciduous and dry deciduous. In Kerala, agriculture is the major land use accounting for 58% of the total geographic area followed by forests constituting 28%; the land put to non-agricultural use accounts for only 8% (KFRI, pers. comm.).

Within each district, sites were chosen at random where possible but this was constrained by the extent of accessibility of the homegardens, plantations and natural forests. At each site, the abundance of *M. micrantha* was assessed by selecting, at random, five 10 x 10 m quadrats within a 0.5 ha. plot. The number of *M. micrantha* stems (individual plants) in the quadrats were counted, the mean number calculated, and the total number per 0.5 ha estimated; these were assigned a grade number (table 1). These grades were arbitrary but were assigned to enable some measure of abundance to be applied. For some of the analysis of the data, the grades were grouped into: not present; low (1-50 stems); medium (51-100 stems) and high (>101 stems). This grouping was also used for some of the socio–economic survey.

Table 1:
Scale for quantification of the ecological distribution of *Mikania micrantha*

GRADE	NO. OF STEMS OF PLANT / 0.5 HA.
-	Not present
0	1 – 10 (Isolated)
1	10 – 25 (Scattered)
2	25 – 50 (Low)
3	50 – 75 (Moderate)
4	75 – 100 (Medium)
5	Above 100 (High)

In natural forest areas, the degree of disturbance to the forest was also graded at each site according to whether the canopy was either open or closed.
In Kerala, a total of 163 sites were surveyed; 39 were homegardens, 46 plantations and 78 natural forest (close to the gardens). Fifty-seven sites were surveyed in Karnataka and 29 in Goa. The altitude, latitude/longitude, details of the locality, water availability, degree of disturbance (in the case of natural forests) were recorded in each case. Phenological characteristics of the plant including flowering, seed-set and seed dispersal were recorded. Samples of *M. micrantha* were collected from several sites along a north – south transit in Kerala; these were sent to the herbarium of the Royal Botanic Garden Kew, for identification.

Variations in the distribution of *M. micrantha* in different geographical zones in Kerala were analysed using the chi-square test.

b. Socio–economic impact in Kerala.

The socio–economic aspects of the impact of *M. micrantha* were examined separately in three production systems: homegardens, tree forest plantations and natural forests in Kerala. Data was gathered through an interview schedule and one or more of the following: observation, participatory experiment, and participatory rural appraisal (PRA). This was undertaken by the socio–economic section of KFRI.

Before initiating the data collection, a pilot survey was carried out in the three production systems in different districts in the State. It was found that the *M. micrantha* infestation in the home garden and the other production systems was highest in central and southern Kerala than that in northern Kerala. Of the fourteen districts, the infestation of *M. micrantha* was found to be high in seven districts: Thrissur, Ernakulam, Alappuzha, Idukki, Kottayam, Kollam and Pathanamthitta. Data relating to the homegarden system as gathered from a sample of farmers from the above seven districts. With the help of Range Officers in the Forest Department and the District Agricultural officers, *M. micrantha* infested areas were located for the survey. A list of farmers with their size of holding in the *M. micrantha* infested areas was collected from the respective village offices. The farmers in the agroforestry system were then selected randomly. A total of 100 households were selected for detailed study; these also included the sites selected for the ecological survey in these districts. Based on size of holding, the selected farmers were classified into marginal (<1.5 ha), small (1.5-2.5 ha), medium (2.5-5.0 ha) and large (>5.0 ha). Since there was discrepancy in the size of holdings in the list collected from the village office and the actual size of holding reported by the farmers at the time of interview, the classification of size of holding was done after the data collection. About thirty households having the same cropping pattern but with no infestation of *M. micrantha* were also selected and their cost of cultivation and income from farming estimated. Those who actually cultivated the land and agricultural workers

were interviewed for gathering data using an interview schedule. Their opinions on the use of biological control were also discussed.

Mikania micrantha affects the production systems in a number of ways. In homegarden systems, the most obvious of these is the negative effects on crop production, i.e., either increased cost of production due to weeding or reduced yield (income) or both. One common method to study the effect of pests on the profitability of perennial crop production is to use a financial cost-benefit analysis, using a discounted cash flow technique. However, farmers do not maintain reliable long-term data on many aspects of raising crops. Further, *M. micrantha* commonly affects younger plants, which are yet to yield. Again, in most farms a variety of crops which, fall under different age groups (with and without yield) are grown. This makes a financial cost-benefit analysis, difficult. Thus, instead of undertaking financial cost-benefit analysis, we compared the annual cost of cultivation and the income received by the farmers with and without *M. micrantha*, assuming that the difference would indicate the impact of this weed.

In Kerala, there are both agricultural and forest plantations. A total of thirty-nine plantations were visited for the socio–economic survey. The effect of *M. micrantha* on profitability in the plantation sector was estimated only for teak plantations, partly because *M. micrantha* infestation is more severe in teak plantations and partly due to lack of reliable cost and income data for other types of plantations. The infestation was found to be more in younger teak plantations and older plantations are usually not severely affected. Considering this, the cost and benefit of teak plantations with and without (any infestation) *M. micrantha* up to a period of second mechanical thinning was calculated using the discounted cash flow technique.

In the natural forest areas, the impact of *M. micrantha* was worked out in term of cost of weeding and reduced income of the tribals. Fifteen sites were selected at random. The cost of weeding was calculated by selecting a few of these sites at random and employing laborers to clean and weed the sites using sickles.

c. Monitoring studies.

For the purposes of monitoring changes in the abundance of *M. micrantha* over time, 28 permanent sample plots were established in Kerala covering all districts. Seven were in agroforestry systems, eight in crop plantations, and thirteen in natural forests (mostly moist deciduous and evergreen). More were chosen in natural forest areas because of the intensity of weeding in the homegardens. The plots were re-visited six monthly – one year after the first assessment. This pattern will be continued by KFRI for the foreseeable future.

d. Assessment of *Mikania micrantha* in northeastern India (from add-on funding, January/February 1998)

The impacts of *M. micrantha* on tree crops, tea growing, forest plantations and natural forests in northeastern India were stressed by the Indian Council of Agricultural Research (Natural Resources Division), New Delhi in 1997; ICAR requested that the northern States be included in the project. Although an assessment of the weed, similar to the one undertaken in the Western Ghats was not possible, a two week survey was undertaken by staff of CABI Bioscience in January / February 1998. The main objective was to establish the status of the weed in different production systems and natural habitats.

This was done by:

- Direct survey of selected sites in different production systems.
- Discussions with farmers, tea growers, extension staff, ICAR, AAU, Forest Departments and TTES.

The survey was restricted to Assam and Meghalaya because of time restrictions and the difficulties of gaining access to other States in the region.

e. Characterisation of the *Mikania micrantha* populations (from add-on funding, October–December 1998).

During the course of the distributional surveys in India (see a.) the morphological variation of the weed was found to be greater than originally supposed. Within the native range of the weed (tropical Latin America and the Caribbean) considerable morphological variation was also observed. Thus, in order to facilitate the matching of isolates of exotic fungal agents with populations of *M. micrantha* in India, a study was undertaken to understand the degree of *genetic* variation within and between populations within India and to match these with populations from the Neotropical region (also see output 3).

The genetic variability of weed samples was assessed by amplified fragment length polymorphism (AFLP). Fifty *M. micrantha* samples were included in the analysis (table 2); these were from northeastern and southwestern India and from various parts of the Neotropics.

DNA was extracted from fresh leaf material using a Phytopure DNA extraction kit from Nucleon Bioscience. The AFLP protocol used was adapted from Mueller *et al*. (1996). The only variation from the published protocol was the introduction of a pre-amplification step to increase help increase the yield and uniformity of the selective AFLP profiles. A total of six selective primers were used with the following selective nucleotides; AC, AG, CG, CT, GC and CT. The AFLP profiles were separated by electrophoresis through 1.5% (w/v) agarose gels, which were run at 100V for 6 hours, stained with ethidium bromide and photographed with a Polaroid camera. Gel photos were scanned and imported into GelCompar (Applied Maths) and similarity values calculated for each primer using the Dice coefficient. The data matrices were exported to Excel and an overall data matrix calculated which summed the data from each primer. The resulting matrix was analyzed either by using the Neighbor package in PHYLIP (Felsestein, 1993), to produce a dendogram showing the relationships between the isolates, or by Mvsp 3.0 to produce the principal co-ordinate plots. These data were analyzed to show the relationships between both the *M. micrantha* isolates and for all of the *M. micrantha* species examined in this study.

Table 2.
***Mikania micrantha* populations investigated and their location. Details about these samples are given in appendix 1**

Number	Location	Number	Location
1	India 3-1 Vettilappara	26	India Kaziranga West W1842
2	India 3-2 Athirampilly	27	Brazil Mariana W1692
3	India 3-3 Vazhachal	28	Brazil Abre Campo W1696
4	India 3-4 Porimgal (Teak)	29	Brazil Prados W1690
5	India 3-5 Porimgal (Natural)	30	Brazil Vicosa W1693
6	*M.*sp Peru 5-1	31	Brazil Caparao W1695
7	*M.*sp Peru 28-1	32	Brazil Maripo W1694
8	Peru San Martin W1707	33	India 4-1 Kuddaram
9	*M.* sp Hairy ex Costa Rica 16-1 Siquirres	34	India Peechi
10	*M. micrantha vitifolia* ex Columbia	35	India 12-1 Varandhara
11	*M. micrantha guaco* ex Columbia	36	Nepal Chitwan
12	*M.* sp Medicinal Brazil	37	Sri Lanka 13-3 Monaragala
13	*M. micrantha* sp ex Columbia	38	Malaysia Type 2 Hairy
14	Costa Rica Turrialba 15-1	39	Malaysia Type 1 Hairless
15	Costa Rica Moravia 15-3	40	Philippines Lagawe 20-1
16	Costa Rica 15-4 W1869	41	Australia Queensland W1921
17	Costa Rica Siquirres 16-1	42	Panama Barro Colorado 29-1
18	Costa Rica 16-2 Limon	43	Panama Bocas del Toro 31-1
19	Costa Rica 17-1 Siquirres W1868	44	Trinidad Non-hairy
20	India Shillong W1848	45	Trinidad Hairy
21	India Jarhat W1844	46	Mexico Vera Cruz W1904 Laguna
22	India Geleki W1846	47	Mexico Vera Cruz 15-6-98 Angel2
23	India Garampani W1845	48	Mexico Tapachula 7-4-98
24	India Nameri W1847	49	Mexico Vera Cruz 28-4-98 Angel1
25	India Kaziranga East W1843	50	Mexico Vera Cruz 15-6-98 Angel1a

2. Mycobiota characterized and potential pathogenic fungal biocontrol agents identified from the weed in its native range and India.

a. Surveys in the native range of *Mikania micrantha*

Previous work of Evans (1987) and Barreto and Evans (1995) had established that there was a rich mycobiota on *M. micrantha*. They listed 43 suspected fungal pathogens on the weed, and of these, 8 were found only in the exotic range, 6 occurred in both native and exotic ranges and 29 were found exclusively in the native range. From this information, previous field observations, and evidence from other successful cbc programmes, four of the most promising agents from the native range of the weed were initially selected: the rusts *Puccinia spegazzinii* de Toni and *Dietelia portoricensis* (Whetzel & Olive) Buriticá & J.F. Hennen (Uredinales), a downy mildew *Basidiophora montana* R.W. Barreto (Chromista), and *Mycosphaerella mikania-mikaniae* R.W. Barreto (Ascomycotina). None of these agents have been recorded from the exotic range of the weed, and all were found to cause significant damage to their host plant under field conditions.

Under the DFID programme, field surveys were undertaken in a number of countries within the native range of the weed, including Brazil, Ecuador, Trinidad and Tobago, Costa Rica and Mexico. Surveys conducted in the latter three countries were included as part of the add-on funding (Oct.-Dec. 1996). Infected plant material of the four selected fungi was collected, dried

in a plant press, and then taken to the laboratory in the UK for study. Observations were also made in the field concerning the abundance of the pathogens and damage to the target plant. In addition, the surrounding vegetation, particularly other *Mikania* species, was observed for infection with the selected pathogens. Living plants, infected with the two rust species were collected and sealed in inflated plastic bags for transport into CABI Bioscience quarantine in the UK. This was necessary, since these rusts do not survive on dried specimens, which has been the traditional method of collection.

b. Surveys in India

During the ecological surveys in the Western Ghats, diseased parts of the *M. micrantha* plants (leaf, stem, root) were collected from the localities surveyed, stored in fresh polythene bags and transported to the laboratory for further study. The symptoms of the disease were recorded in the field.

3. The biological control potential of fungal pathogens from the native range and India on the weed biotypes present in India determined and compared

a. Fungal pathogens from the native range of *Mikania micrantha*

From observations during the field surveys, *P. spegazzinii* was selected for glass house based investigations in to its classical biological control potential. The following culturing methodology was used in all the experimental work undertaken with the rust:

Propagation and maintenance of plant material

All plant material used in the inoculation experiments was young and healthy, with maximum quantities of meristematic tissue (developing shoots and expanding stems). *Mikania* species are propagated from cuttings (seeds are not easy to germinate); roots are readily produced at the leaf nodes, once pegged into moist soil. Host range test plants were grown from seed where possible, or else obtained as rooted plants. All plants were grown in a 50:50 mixture of general-purpose peat-based potting compost and JI number 2 soil-based compost. Test plants were grown in a propagation glasshouse on a 12-hour -light/dark cycle, with a minimum temperature of 20^{o}C (fluctuating to 30^{o}C) until use. After inoculation, all plants were maintained in a quarantine glasshouse chamber with an air-conditioning unit set at 23 +/-5^{o}C, also with a 12-hour -light/-dark cycle.

Inoculation procedure

Puccinia spegazzinii produces basidiospores under high humidity conditions, from a cushion 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 variable quantities infected plant material (leaf, petiole and stem). Test plants were sprayed with a fine layer of distilled water, and the inoculum suspended 2-6 cm above the test plants using plastic coated wire ties attached to the top of plastic plant supports. The plants were then placed in a dew chamber (Mercia Scientific, UK) at 20^{o}C for 24 hours (unless otherwise stated). The inoculum was removed after 12 hours to help reduce the potential risk of hyperparasite transmission to the new plants. 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.

Due to the nature of the pathogen and the growth form of *Mikania* species, it is difficult to accurately quantify the amount of inoculum, or the potentially susceptible plant material that is used in each experiment. However, since at least four replicate plants are used in each inoculation, and each test run is repeated, with susceptible controls are used in each inoculation, the results are considered to be scientifically robust.

(i) Life cycle and description of *Puccinia spegazzinii*

During the survey in Brazil, extended studies were undertaken on the life cycle of the rust *P. spegazzinii* at Viçosa University, Minas Gerais, under the guidance of Dr. Robert Barreto. *P. spegazzinii* is recorded in the literature as a microcyclic (reduced number of spores stages in the life cycle), autoecious (completes life cycle on one host species) rust, with only teliospores and basidiospores having been recorded from the field.

(ii) Intraspecies pathogenicty of *Puccinia spegazzinii* isolates

The 11 isolates of *P. spegazzinii* were assessed for their pathogenicity towards the range of populations of *M. micrantha,* from its native and exotic ranges (see appendix 1). This screening focused on identifying an isolate of the rust that was aggressive and attacked all the populations of the weed present in the Western Ghats. A scoring system was developed, and is given below:

Pathogenicity score for the evaluation of *P. spegazzinii:*

0 No macroscopic symptoms
1 Necrotic 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 with low teliospore production in relation to compatible host-pathogen interaction.
4 Normal pustule formation, in relation to compatible host-pathogen interaction.

(iii) Environmental requirements for selected *Puccinia spegazzinii* pathotype (W1761)

For a pathogen to be successful as a cbc agent it is necessary for it to be capable of infecting the host plant under the environmental conditions that prevail in the exotic range. Glasshouse-based studies are useful in helping to predict the likely environmental tolerance of a pathogen. The majority of pathogens require free water on the plant surface in order to infect, and the length of time this film of water is needed is referred to as a 'dew period'. The minimum and optimum dew period requirements, together with temperature tolerance, are fundamental to this prediction. These two significant parameters for infection were investigated for *P. spegazzinii.*

Dew Period: the effect of six dew periods; 5, 8, 11, 14, 17 and 20 hours, on the level of infection of *M. micrantha* by *P. spegazzinii* was investigated at 20^{o}C. Four replicate plants, two apices per plant, were used for each dew period. The basic inoculation procedure was used (see above), but care was taken to give each apex the same quantity of inoculum, so that comparisons could be drawn between each dew period. Infected petioles containing a 1cm long pustule of teliospores covering half the circumference were suspended 4cm above each apex. Each apex was tagged

above the last pair of open leaves (held at right angles to the ground), and the number of sori on all the leaves above the tag were counted four weeks after inoculation. A mean of the two apices for each plant was taken. *Temperature*: the same procedure was used as described for the dew period experiment, but 5 temperatures; 12, 15, 20, 25 and 28^{o}C were investigated using a forty-eight hour dew period. The data were analysed using a one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) test. The analysis was carried-out using the statistic software package SPSS for MS Windows, release 6.1.4.

(iv) Potential impact of *Puccinia spegazzinii*

It is difficult to predict the impact that the rust will have on populations of *M. micrantha* in its exotic range. Glasshouse-based studies can only provide information on what some major limiting factors may have on the establishment, range and spread of the agent. Field observations in the native range of the weed, likewise, cannot give the complete picture, since freed of their coevolved natural enemies (e.g. hyperparasites), and in a different environment, the rust should have an even greater impact than it had in its native range. However, it is considered relevant to look at examples of where other, related rusts have been used as cbc agents, and have had a significant impact on a weed population, and extrapolate this to the rust *P. spegazzinii*.

b. Fungal pathogens from India

Isolation of pathogens: Plant samples were first surface sterilized 0.1% mercuric chloride solution, washed in several changes of sterlized water and plate on Potato Dextrose Agar medium (PDA). The petriplates were incubated at 25±2°C. The morphology of the isolates was studied and identify attempted. The colour chart used in describing the symptoms of the disease and cultural characters of the pathogens was that of Methuen (Kornerup and Wanscher, 1967).

Pathogencity: Pathogencity of different fungi isolated from *M. micrantha* was tested on healthy leaves/collar region of the plants grown in the glass house. For inoculating leaves, small droplets of conidial and mycelial suspension prepared by flooding a 10-day old culture of individual pathogens were placed at three marked regions on abaxial and adaxial surfaces of six leaves of *M. micrantha* on individual plants. Separate plants (three each) were used for inoculating with each fungus. The inoculum was applied either after wounding the leaves by pin pricking or leaving them unwounded. Controls were healthy leaves inoculated (wounded and unwounded) with 0.5 cm diameter PDA discs (with no fungal growth) at three marked regions on abaxial and adaxial surfaces as described earlier. The inoculated and control plants were transferred to a humidity chamber where r.h. was >95% and temperature 24± 1°C.

Pathogencity of all isolates was also attempted on the following economically important plants: pepper. (Piper nigram L.), eucalyptus (*Eucalyptus tereticornis* Sm.), teak (*Tectona grandis* L), bamboo (*Bambusa arundinacea* (Retz.) Roxb) and reed (*Ochlandra travancorica* (Bedd.) Benth. Ex Gamle). Leaves of healthy young saplings of each of the species were inoculated as described earlier. The collar region of the saplings was inoculated with Fusarium solani. The inoculated plants were transferred to a humidity chamber (r.h.>95 and temperature 24±1°C) for development of symptoms. Re-isolation of the pathogens was attempted in all cases.

c. Characterisation of neotropical rusts on *Mikania micrantha*

(i) Morphological characterisation

Using data from the literature, survey observations and microscopic analysis of rust-infected *M. micrantha* samples, an analysis of the neotropical rusts on *M. micrantha* was undertaken.

(ii) Molecular characterisation

DNA purification was attempted from various stages of leaf and stem infections using a variety of extraction methods. A PhytoPure DNA extraction kit (Nucleon Bioscience) was used with direct digestion of the samples with Proteinase K, disruption of the samples with an Ultra Turrax homogeniser or by grinding in liquid nitrogen with and without the addition of carborundum powder. Similar disruption methods were also tested using the CTAB extraction method of Cubero *et al*. (1999). DNA was obtained from most of these methods and was subjected to PCR amplification. Initial amplifications were tried using primers specific for the ITS region of the ribosomal DNA repeat unit in fungi.

4. Import/release protocol developed, host specificity testing requirements determined and host specificity tests completed

a. Import/release protocol and host specificity testing requirements determined.

There is no written protocol for the introduction of biological control agents into India, particularly pathogens (although this is now being revised by ICAR and the CPP *M. micrantha* project is contributing to the process by providing an example project involving an exotic pathogen). However, India has a long history of exotic insect agent introductions. Permits for introduction are issued by the Ministry of Agriculture's Directorate of Plant Protection, Quarantine and Storage (DPPQ&S). Key issues for the Government of India (from initial discussions at ICAR's Project Directorate of Biological Control) are demonstration of host specificity, broad support from relevant stakeholder institutions, demonstration of the lack of potential local biological control agents, and the nodal agency within India to handle pathogen introductions.

During the course of the project, discussions were held with a number of state and national institutions about the protocol (including host specificity testing) for the introduction of weed fungal agents. The socio–economic department of KFRI also discussed biological control as a management tactic with farmers in Kerala (see output 1); CABI Bioscience discussed the subject with a sample of tea growers during the survey in Assam and Meghalaya. Finally, views on the introduction of an exotic fungal pathogen agents was raised during a national project workshop on alien weeds, held in November 1999.

b. Host specificity tests completed (including extension funding, April-Dec. 1997)

Prior to the introduction of any natural enemy into a country or region where it does not occur, it is essential to establish, unequivocally, the complete specificity of the candidate agent to the target weed. Host testing provides the information that is required to enable the appropriate authority to make a decision, or pest risk analysis (PRA), based on balancing the risks of releasing versus the risk of no control or the consequences of other control methods (FAO, 1996). Wapshere (1974)

proposed a centrifugal, phylogenetic testing sequence that is used in most current screening programmes, based on the taxonomic classification of the target weed (see below).

Classification of *Mikania micrantha* (Cronquist's system [Mabberley, 1987]):

Class:	Dicotyledonae
Subclass:	Asteridae
Order:	Asterales
Family:	Asteraceae (Compositae)
2 Subfamilies:	I. Lactucoideae (7 tribes) to which *Mikania* belongs
	II. Asteroideae (6 tribes)

Test plants were chosen from each of the tribes within the Lactucoideae and Asteroideae, and in addition, six other *Mikania* species were also selected. Plants of economic importance in the potential area of introduction were also included, in accordance with the FAO guidelines (FAO, 1996), so as to allay the understandable concerns of decision-makers and the general public. In total, 55 species were screened (table 3). Test plants were inoculated using the inoculation procedure given in 3(a) above, except that a 48 hour-dew period was used, and plants were monitored for twice the normal time expected for full symptom development on a susceptible host. The prolonged dew period ensured that every opportunity was given for infection of the test plants, and the extended monitoring time ensured that delayed or latent disease expression would be picked-up. Susceptible control plants were included with each run. A microscopic analysis of a selection of test plants was also carried out using a leaf clearing-staining technique (Bruzzese and Hasan, 1983).

Table 3.
Plant test list for host specificity screening of *Puccinia spegazzinii* (isolate W1761), rust pathogen of *Mikania micrantha*

Plant Species	Source
Subfamily I: Lactucoideae	
Tribe: Lactuceae	
Lactuca sativa L. (lettuce)	(Egypt*)
"All the year round"	U.K.
"Unrivalled"	U.K.
Mutisieae	
Gerbera hybrida "Jamesonii" Bolus	South Africa
Arctotideae	
Arctotis hybrida "Harlequin"	South Africa
Gazania hybride	U.K. (South Africa*)
Cynareae	
Carthamus tinctorus L. "goldtuft"	Egypt
Cynara cardunculus L.(globe artichoke)	U.K. (W. Mediterranean*)
Vernonieae	
Stokesia laevis (Hill)	U.K. (USA*)
Vernonia novaboracensis	USA
Liabeae (no representative species obtained)	
Eupatorieae (Asteroideae)	
Ageratina riparia Regel (mist flower)	New Zealand
Ageratum F1 Hybrid "Adriatic"	U.K. (C. America*)
Chromolaena odorata L.	India
(*Eupatorium canabum)*	U.K.
Liatris pycnostachya Michx.	U.K. (N. America*)
Mikania guaco Bonpl.	Colombia
Mikania vitifolia DC.	Colombia
Mikania sp. 1 (Medicinal)	Brazil
Mikania sp. 2	Peru
Mikania sp. 3	Peru
Mikania sp. 4	Costa Rica
Stevia rebaudiana Bertoni (Stepa®)	Holland (Tropical America*)
Subfamily II: Asteroideae	
Tribe: Senicioneae	
Senecio cineraria DC. "silverdust"	U.K. (Mediterranean*)
Heliantheae	
Helianthus annuus L.	India
Parthenium hysterophorus L.	Bangalore, India; Australia
Inuleae	
Inula ensifolia L.	U.K.(Old World*)
Anthemideae	
Chrysanthemum carinatum L. "court jesters"	Japan
Pyrethrum roseum Adam.	U.K. (N. Persia*)
Calenduleae	
Calendula officinalis L.	U.K. (Mediterranean*)
Asteraceae	
Aster alpinus L.	U.K.

Table 3
Continued

Plant Species	Source
Selected Crop Species	
Arachis hpogaea L. (ground nut)	Bangalore, India
Brassica juncea L. (Mustard)	Jabalpur, India
Camellia sinensis L. Kuntze (Tea)	U.K. (S.E. Asia*)
Cajanus cajan L. Millsp. (Arhar / Pigeon pea)	Kurukshetra, India
Cicer arietinum L. (Chick Pea / Gram)	Kurukshetra, India
Cocos nucifera L. (Coconut)	U.K. (South America*)
Coffea arabica L. (Coffee)	Kenya
Elaeis olifera (Kunth) (Oil Palm)	Malaysia
Eucalyptus bridgesiana R. Baker	Australia
Eucalyptus cypelocarpa	Australia
Glycine max (L.) Merr. (Soybean)	Bangalore, India
Hevea brasiliensis Willd.(Rubber)	India
Lens esculenta Moench (lentil)	India
Linum usitatissimum L. (linseed)	India
Lycopersicon esculentum Mill. (Tomato)	Bangalore, India
Manihot esculenta Crantz (Cassava)	India
Oryza sativa L. (Rice)	Jalapur, India
Phaseolus aureus Roxb.(moong bean)	India
Phaseolus vulgaris L.(Faba bean)	Jabalpur, India
Pisum sativum L. (Pea)	Kurukshetra, India
Raphanus sativus L. (Radish)	Kurukshetra, India
Solanum melongena L. (Brinjal)	Bangalore, India
Sorghum bicolor L. Moench. (Sorghum)	Yugoslavia
Tectona grandis L. f. (Teak)	India
Theobroma cacao L. (cocoa)	Peru
Zea mays L. (maize)	Kurukshetra, India

Country of origin/ native distribution

5. Indian scientists trained in tree-crop and agroforestry weed management research and implementation (including external funding, April-December 1999)

Training in homegarden, tree-crop/agroforestry weed management research and implementation has been targeted by the research staff of the collaborating institutions: KFRI Peechi, Kerala; ICAR's PDBC, Bangalore, Karnataka; and AAU, Department of Crop Physiology, Jorhat, Assam. Training has focused on the following subjects:

- Weed surveys
- Socio–economic impact assessment of weeds
- Weed biological control methods
- Weed pathology

Training has been provided in two forms:

- One-to-one training in India, delivered by CABI Bioscience and NRI (socio–economics).
- Training attachments in weed pathology at CABI Bioscience UK Centre for Indian scientists from the above institutions.

6. Project research outputs publicised and promoted (including extension funding, April-December, 1999)

During the project the emphasis has been on the synthesis, dissemination and promotion of information generated by the project within India. Activities have included the production of an alert sheet, geographical information system (GIS) mapping, promotion meetings at key institutes throughout India, and the convening of a national workshop at KFRI at the end of the project (held in November 1999). The workshop was focussed on *M. micrantha* but other alien weeds important in moist regions were also included.

7. Implementation phase for the biological control of *Mikania micrantha* on the role/activities of current/new collaborators (from extension funding, April-December, 1999)

In view of the need to broaden the project to include the northeastern states and also to include ICAR's PDBC as the representative nodal point for biological control agent introductions, two meetings were held to discuss and formulate a plan for implementation of biological control including agreement on the roles of current and new collaborators. One was ICAR's PDBC at Bangalore in June 1999 and the other at the end of the project national workshop, held at KFRI, Peechi in November 1999.

OUTPUTS

1. Distribution, agricultural importance and the economic and social impact of *Mikania micrantha* in selected areas of the Western Ghats determined.

a. Distribution and agricultural importance.

In Kerala State the agricultural systems surveyed covered homegarden systems (coconut, banana, pepper, cashew, arecanut, cassava etc.) and monoculture systems (tea, coffee, pineapple, ginger, rubber etc.) The major forest plantations surveyed were teak (52%) eucalypt (26%) and miscellaneous species (26%) (bamboo, *Acacia auriculiformis, Albizia falcataria, Casuarina equisetifolia*, etc.).

Of the one hundred and sixty-three localities surveyed in Kerala, one hundred and eleven (68%) showed some level of infestation of *M. micrantha* the rest were free from infestation. None of the sites surveyed in Kararatalea and Goa, states were infested with *M. micrantha*. The highest level of infestation in Kerala (grade 5) was recorded in 24.5% of the areas (table 4). A zone -wise analysis for the distribution of the weed in the state (table 5) indicated that the southern zone has maximum proportion of sites infested (82.5%) compared to central (75.5%) and northern zones (45.3%). The level of distribution varied significantly between southern and northern ($P>0.001$) and central and northern ($P>0.001$) zones. The level of distribution did not vary significantly between southern and central zones. The southern zone also had the highest proportion of sites with the highest level of infestation compared to other zones.

Table 4.
Number of sites surveyed for *Mikania micrantha* in Kerala State arranged according to grade of infestation.

GRADE	NO. OF SITES WITH *M. MICRANTHA*	PERCENTAGE
-	52	31.90
0	1	0.60
1	8	4.90
2	29	17.80
3	18	11.00
4	15	9.20
5	40	24.50
Total	163	100.00

Table 5.
Zone-wise distribution of *Mikania micrantha* in Kerala.

ZONE	LEVEL OF INFESTATIONS PER SITE				TOTAL	% OF AREA INFESTED
	Not present	Low (1-50 stems of plants)	Medium (51-100 stems of plants)	High (> 101 stems of plants)		
Southern Zone	10 (17.5)	14 (24.6)	13 (22.8)	20 (35.1)	57 (35)*	82.5
Central Zone	13 (24.5)	10 (18.9)	12 (22.6)	18 (34)	53 (32.5)*	75.5
Northern Zone	29 (54.7)	14 (26.4)	8 (15.1)	2 (3.8)	53 (32.5)*	45.3

Figures in the parenthesis are row wise percentage (* column wise percentage). Chi-square value =28.79, P<0.001

Analysis of the level of infestation of *M. micrantha* in different districts revealed that over 75% of the sites surveyed were infested in Kollam, Alappuzha, Idukki, Kottayam and Pathanamthitta districts in the southern zone, Trichur and Ernakulam districts in the central zone and Calicut district in the northern zone (table6). Kasargode district appeared to be free from infestation. The distribution of *M. micrantha* was summarized in map form using a geographical information system (GIS) at KFRI (appendix 2). This was mainly for information dissemination purposes (see output 6).

Table 6.
District-wise distribution of *Mikania micrantha* in Kerala State.

ZONE/DISTRICT	Grade of *Mikania micrantha* infestation (per site)							TOTAL	% OF AREA INFESTED
	-	0	1	2	3	4	5		
SOUTHERN ZONE	10 (17.5)	-	2 (3.5)	12 (21.1)	9 (15.8)	4 (7)	20 (35.1)	57 (34.96)	82.5
TRIVANDRUM	5 (50)	-	1 (10)	3 (30)	-	-	1 ((10)	10 (6.1)	50
KOLLAM	2 (15.4)	-	1 (7.7)	4 (30.8)	3 (23.1)	2 (15.4)	1 (7.7)	13 (8)	84.6
ALAPPUZHA	-	-	-	2 (40)	2 (40)	-	1 (20)	5 (3.1)	100
IDUKKI	3 (25)	-	-	1 (8.3)	2 (16.7)	2 (16.7)	4 (33.3)	12 (7.4)	75
KOTTAYAM	-	-	-	1 (20)	1 (20)	-	3 (60)	5 (3.1)	100
CENTRAL ZONE	13 (24.5)	1 (1.9)	3 (5.7)	6 (11.3)	4 (7.5)	8 (15.1)	18 (34)	53 (32.5)	75.5
TRICHUR	4 (21.1)	1 (5.3)	2 (10.5)	1 (5.3)	1 (5.3)	4 (21.1)	6 (31.6)	19 (11.7)	78.9
ERNAKULAM	-	-	1 (5.9)	2 (11.8)	1 (5.9)	4 (23.5)	9 (52.5)	17 (10.4)	100
PALAKKAD	9 (52.9)	-	-	3 (17.6)	2 (11.8)	-	3 (17.6)	17 (10.4)	47.1
NORTHERN ZONE	29 (54.7)	-	3 (5.7)	11 (20.8)	5 (9.4)	3 (5.7)	2 (3.8)	53 (32.5)	45.3
KANNUR	7 (35)	-	2 (10)	4 (20)	4 (20)	1 (5)	2 (10)	20 12.3)	65
KASARGOD	10 (100)	-	-	-	-	-	-	10 (6.1)	0
KOZHIKODE	1 (25)	-	-	1 (25)		2 (50)	-	4 (2.5)	75
WAYANAD	6 (54.5)	-	-	4 (36.4)	1 (9.1)	-	-	11 (6.7)	45.5
MALAPPURAM	5 (62.5)	-	1 (12.5)	2 (25)	-	-	-	8 (4.9)	37.5

In general, the maximum proportion of sites infested with *M. micrantha* were in the low (81.8%) and medium altitude (72.5%) areas compared to the high altitude area (43.8%). Over 56% of higher altitude sites were free from infestation (table 7). Areas with highest proportion of infestation was also in the low and medium altitude areas (over 27%). The level of infestation was significantly different between areas with different altitude ($P>0.05$).

Table 7.
Distribution of *Mikania micrantha* at different altitudes

ALTITUDE (msl)	LEVEL OF INFESTATION (stems per ha.)				TOTAL	% OF AREA INFESTED
	Not present	Low (1-50 stems of plants)	Medium (51-100 stems of plants)	High (>101 stems of plants)		
>100	4 (18.2)	5 (22.7)	7 (31.8)	6 (27.3)	22 (13.5)*	81.8
101-499	30 (27.5)	26 (23.9)	23 (21.1)	30 (27.5)	109 (66.9)*	72.5
>500	18 (56.3)	7 (21.9)	3 (9.4)	4 (12.5	32 (19.6)*	43.8

Figures in the parenthesis are row wise percentage (* column wise percentage). Chi-square value = 13.66, P>0.05 (Significant).

In agricultural plantation systems, 92% of the areas surveyed were infested with *M. micrantha*. Of these 18% of the areas were highly infested (table 8). Data on infestation in forest plantations showed that teak plantations had 75% of the surveyed plantations infested. Among eucalypt and miscellaneous plantations, 58% and 70% of the sites were infested, respectively. Areas with highest level of infestation (grade 5) were 72% for teak, 14% eucalypt and 42% for miscellaneous plantations respectively.

Among the different types of natural forests surveyed, *M. micrantha* infested areas were maximum (56.4%) in the moist deciduous forest. Evergreen forests had 50% and semi-evergreen 55% of the sites infested. The highest level of infestation (grade 5) was recorded in 45% of semi-evergreen, 41% of moist deciduous and 22% of evergreen forests.

Table 8.
Severity of *Mikania micrantha* infestation in different plantation production systems.

PRODUCTION SYSTEM	GRADE								TOTAL AREA SURVEYED	% OF AREA INFESTED
		-	0	1	2	3	4	5		
Natural Forest	Moist deciduous	17	-	-	6	4	3	9	39	56.4
	Evergreen	9	-	1	3	2	1	2	18	50
	Semi-evergreen	9	-	-	2	2	2	5	20	55
	Dry deciduous	-	-	-	-	-	1	-	1	100
Plantations	Teak	6	-	1	1	1	2	13	24	75
	Eucalyptus	5	-	-	5	1	-	1	12	58.33
	Miscellaneous	3	-	1	1	-	2	3	10	70
Agricultural system		3	1	5	12	7	4	7	39	92

Data on canopy type and infestation of *M. micrantha* in different forest systems revealed that 69.9% of open canopy sites were infested; 24% of areas had maximum infestation (grade 5). Though the level of infestation between closed and open canopies did not differ significantly (table 9), a slight trend for greater proliferation of the weed in open canopy sites was apparent.

Table 9.
Distribution of *Mikania micrantha* according to canopy type of different forest systems.

CANOPY TYPE	LEVEL OF INFESTATION (stems of plant per ha.)				TOTAL	% OF AREA INFESTED
	Not present	Low (1-50 stems of plants)	Medium (51-100 stems of plants)	High (>101 stems of plants)		
OPEN	38 (30.4)	31 (24.8)	26 (20.8)	30 (24)	125 (76.7)*	69.6
CLOSED	14 (36.8)	7 (18.4)	7 (18.4)	10 (26.3)	38 (23.3)*	63.1

Figures in the parenthesis are row wise percentage (* column wise percentage)> Chi-square value – 1.03, P>0.05 (NS).

Mikania micrantha is a perennial climber and wherever the plant dries-off during the summer season due to the scarcity of water, regeneration occurs along with the southwest monsoon (June-July) in Kerala. Flowering *M. micrantha* in Kerala starts in August and continues up to January. Fruit setting occurs between September to February. In general, fruiting setting is initiated after 17-21 days of flowering. Seed dispersal is during October-April. The average number of seeds per mg. is 108±12. *Mikania micrantha* seeds are minute and each bares a pappus.

Although great variation exists in leaf morphology among *M. micrantha* population in Kerala, all the specimens collected during this survey belong to *M. micrantha* (confirmed by taxonomists at Royal Botanical Gardens, Kew).

In Kerala, infestation by *M. micrantha* was first observed in the 1980s. The luxuriant growth of the weed along roadsides and rail lines indicates that further spread was through road and rail transport. As forestry activities were common, more in the central and southern Kerala, *M. micrantha* may have spread and established in degraded areas, and unmanaged plantations in these regions through these activities. The introduction of the weed to the north of Kerala may have occurred through the transfer of planting stock, especially of pineapple, by those who migrated to settle in the north from the south of Kerala. This view is supported by the fact that agricultural systems are the most affected in the north than natural forests or plantations. Occasionally, in certain areas, *M. micrantha* infestation was only observed in pineapple monocultures (e.g. in Koottupuzha in Kannur Districts). However, the slow spread of *M. micrantha* in the north of Kerala may be due to the limited area under natural forests and plantations and unfavourable soil (latertitic) and climatic conditions.

In the State, the spread of *M. micrantha* is now restricted to the southern banks of the Valapattanam river and its tributary, the Koottupuzha river in Kannur District. It can reasonably be assumed that the weed may spread beyond this area in the near future which will facilitate its spread to Karnataka and other States in southern India.

Though 92% of the agricultural systems surveyed in Kerala is reported to be infested by *M. micrantha*, the severity of the infestation is much lower compared to plantations and natural forests. This is because of the thorough management of the system through weeding and other operations carried out by farmers, (see next section on socio–economics). Surveys in central and southern Kerala showed that *M. micrantha* could pose a serious threat to banana, cassava, pineapple, ginger and possibly paddy cultivation in the State if these agricultural systems are not managed properly by removing the weed through mechanical or chemical means (see next

section). The proliferation of *M. micrantha* in open canopy areas is because of its heliophytic adaptation (Holm *et al*. 1977; Sen Sharma & Mishra, 1986). One observation of importance became apparent from the analysis of the homegarden sites: several of the sites in the centres of the range of the weed were uninfested. The reasons for this were unclear and thus, this needs further study.

The high infestation of *M. micrantha* in the moist deciduous forests in Kerala as observed in this study is likely to be due to the degradation of most of these forests, due to felling of trees, grazing, incidence of fire and resultant more or less open canopy. In evergreen forests, the weed is often found on the fringes of the forests where canopy is comparatively open due to human interference and degradation. As the plant appears not able to tolerate dense shade (Holm *et al.* 1977), it rarely colonizes the interior areas in evergreen forests. Water may be a limiting factor for growth of *M. micrantha* in dry deciduous forests. In most parts of the state, the weed dries-off in the summer season (March to May) wherever water availability is poor.

Forest plantations in the state face a serious threat due to the infestation of *M. micrantha*. The most affected are the young teak plantations (2-3 year old) because of the thin canopy and favourable micro and macro climatic conditions present in the plantations for the proliferation of *M. micrantha*. Plantations of *Acacia auriculiformis*, *Albizia falcataria* and *Casuarina equisetifolia* are also affected by the weed. Eucalypt plantations are comparatively less affected compared to teak. One probably reason for poor growth of the weed in eucalypts plantations may be the low moisture content of soils and allelopathic properties of the eucalypt litter.

According to Choudhury (1972), *M. micrantha* grows in the altitudinal zone of 60-1330m. Holm *et al.* (1977) reported occurrence of *M. micrantha* up to 3000m in Bolivia. However, in Kerala the infestation was low above 1000m altitude and the heavily infested areas were located mostly below 500m. Spread of *M. micrantha* in the high altitude areas has been slow in the State. Also, the higher attitudinal limit reported by Holm *et al*. (1977) may be inaccurate (H.C. Evans, pers. comm.)

Apart from causing damage to crops, *M. micrantha* is known to cause fire hazards in the production systems. It is also reported to have allelopathic properties (Muniappan and Viraktamath, 1993).

b. Socio–economic impact of *Mikania micrantha* in Kerala

Homegardens

The socio–economic survey covered 100 households in seven districts. The total population of the selected households, were estimated as 440 of which male, female and children were 161, 145 and 134 respectively. The average family size was 4.4 persons. All the members in the family were literate: 22% had primary level of education, 56% secondary level 22% college level. The selected households were closely associated with farming. In general the heads of the family, or in his absence, senior unemployed person looked after the farming operations and was assisted by other members. Farming was the main occupation of 66% of total selected households, while the rest had other sources of income such as business, wage labour, salary, etc., in addition to farming. Of the total selected households, 65% were marginal farmers, with less than 1.5 ha of land holding, 19% were small farmers (1.5 to 2.5 ha), 14% were medium farmers (2.5 to 5 ha) and 2% were large farmers (above 5 ha). Of the total 65 marginal holders, 43, accounting for 65% received income mainly from farming. About 58% small holder farmers and 72% medium

farmers received income from farming. Only 30% of the income of the large holders were from farming. About 30% of the marginal farmers and 15% of the small farmers received income from agricultural labour, in addition to farming.

Of the total selected households, eighty-two households cultivated both seasonal (including vegetables) and perennial crops (mixed cropping), one household cultivated a seasonal crop (banana) and 17 cultivated perennial crops (coconut, arecanut, rubber, coffee, cashew and black pepper) alone. The income of the farmers or size of holding does not seem to have much influence on cropping pattern since all size or income groups adopted different cropping patterns. The cropping patterns in the homegardens are derived traditionally, but changes in the prices of some of the crops, has resulted in allocation of more areas under these crops. The increase of area under black pepper, rubber and cocoa are examples for this change.

By and large, the average cost of cultivation is inversely proportional to the size of holdings. For instance, the average cost of cultivation per ha. was estimated as Rs. 15,420 in the marginal size of holding which increases to Rs. 19,207 in small size of holding. But this found to be Rs. 11,074 and Rs. 5,276 in medium, and large size groups respectively.

Mikania micrantha occurred at all the selected households, but in varying proportion. Of the total selected households, 35% had a high infestation, 19% had a medium infestation and 46% if them had a low infestation (table 10). Of the total marginal holders 38% had a high infestation of *M. micrantha*, 23% had a medium infestation and 38% has a low infestation. About 26% small, 21% medium and all the large farms had a high infestation of *M. micrantha*.

Table 10.
Size of holdings and level of infestation of *Mikania micrantha*

SIZE OF HOLDING	LEVEL OF INFESTATION						NO. OF HOUSE-HOLDS
	High		Medium		Low		
	No.	%	No.	%	No.	%	
Marginal (Below 1.5 ha)	25	38.46	15	23.07	25	38.46	65
Small (1.5 – 2.5 ha.)	5	26.31	3	15.79	11	57.89	19
Medium (2.5 – 5.0 ha.)	3	21.42	1	7.14	10	71.44	14
Large (Above 5.0 ha.)	2	100	-	-	-	-	2
Total	35	-	19	-	46		100

Data on the actual impacts of *M. micrantha* on unmanaged crops was difficult to obtain. Crops severely affected included: banana, coconut, coffee, cocoa, cassava, pineapple, ginger and teak. Examples, given by farmers, of the impact of *M. micrantha* on crops included:

- Pineapple plants which normally provided fruits weighing 3 kg or more, after infestation provided fruits weighting 1.5 – 2 kg.

- In some areas, certain crops that are susceptible to *M. micrantha* had been replaced. e.g. cassava (*Manihot utilissima*) was replaced by kurkia (*Coleus parviflorus*).

Establishing a relationship between the incidence of the weed and the extent of yield loss in gardens is difficult because of the number of crops affected and the different cropping patterns

involved. Results from the ecological survey show that *M. micrantha* is widespread in the homegardens of Kerala. Even though the general incidence of the weed is low in this production system (through intensive weeding – see last section), the results here show that a relatively high proportion of marginal farmers have a high infestation. Indeed, given the rate at which *M. micrantha* grows, even low infestations are of concern to farmers, particularly those with the smaller sized holdings. In view of this most farmers use weeding to manage *M. micrantha* and this may represent a significant impact of the weed on the farmers. In general the farmers would adopt two types of weeding: simple weeding and complete weeding. The simple weeding comprises hand weeding, slash weeding, sickle weeding and circle weeding. The farms adopt any one or more types of weeding in their farms depending upon the extent of weeds, income of the farmers and availability of family labour. In complete weeding, the weeds are completely uprooted. The weeding cost is less in the simple type but the method is less effective.

In view of the above considerations, the further analysis was focussed on weeding, the impact of *M. micrantha* on this and on total production costs and income.

Among the marginal farmers, 66% adopted simple weeding of *M. micrantha* and rest complete weeding. In the case of small, medium and large holders, the percentage of farmers who adopted simple weeding were 73, 86 and 50 respectively. About 72% of farmers, who received total garden income less than Rs. 50,000 and adopted simple weeding. Among the farmers who received total garden income between Rs. 50,000 and Rs. 1,00,000, and above 1,00,000 those who adopted simple weeding were 68% and 70% respectively (tables 11 and 12). The types of weeding adopted by the farmers according to their level of education and cropping patterns are given in tables 13 and 14.

As is evident from the tables, more households are inclined to simple weeding than complete weeding, probably due to high wage rate (Rs. 65/day for female labour and Rs. 125/day for male labour). An attempt was made to examine the possible effect of size of holding, income from farming, level of education and cropping pattern on different types of weeding undertaken by the farmers, using the chi-square test. The chi-square values were found to be non-significant in all the cases, indicating that none of the above variables influence different types of weeding of *M. micrantha* in homegarden systems.

Table 11.
Land holdings and types of weeding

NO.	LAND HOLDING (IN ha.)	TYPE OF WEEDING		NO. OF HOUSEHOLDS
		Simple	Complete	
1	Marginal (Below 1.5)	43 (66)	22 (34)	65
2	Small (1.5-2.5)	14 (73)	5 (27)	19
3	Medium (2.5 – 5.0)	12 (85)	2 (15)	14
4	Large (Above 5.0)	1 (50)	1 (50)	2
Total		70	30	100

Figures in parentheses indicate percentages to the total.

Table 12.
Income from farming and weeding

NO.	PRODUCTIVITY (in Rs.)	TYPE OF WEEDING		NO. OF HOUSEHOLDS
		Simple	Complete	
1	<50,000	21 (72.4)	8 (27.6)	29 (100)
2	50,000 – 1,00,000	23 (67.6)	11 (32.4)	34 (100)
3	>1,00,000	26 (70.3)	11 (29.7)	37 (100)
Total		70	30	100

Table 13.
Education and types of weeding

	LEVEL OF EDUCATION	TYPE OF WEEDING		NO. OF HOUSEHOLDS
		Simple	Complete	
1	Primary (1^{st}-7^{th})	18 (75)	6 (25)	24
2	Secondary (8^{th}-10^{th})	40 (73)	15 (27)	55
3	College (10^{th}-PG)	12 (57)	9 (43)	21
Total		70 (70)	30 (30)	100

Table 14.
Cropping pattern and types of weeding

	TYPE OF CROP	TYPE OF WEEDING		NO. OF HOUSEHOLDS
		Simple	Complete	
1	Seasonal	1 (100)	-	1
2	Perennial	15 (88)	2 (12)	17
3	Mixed (Simple and Perennial)	54 (65)	28 (35)	82
Total		70 (70)	30 (30)	100

Among the major constraints faced by the farmers, weeds are the most important one (table 15). A point to be noted here is that all the constraints enumerated here have an influence on cost of production.

Table 15.
Constraints faced by farmers in cultivation

CONSTRAINTS	NO. OF HOUSEHOLDS
Labour shortage	1 (1.02)
Cost of production	12 (12.25)
Poor facility	5 (5.10)
Weeds (including *M. micrantha*)	62 (63.27)
Pest problem	9 (7.14)
Any other	3 (3.06)
No problems	8 (8.16)
Total	100 (100.00)

In the selected holdings, the farmers were aware of the impact of weeds, particularly that of *M. micrantha* and as a result they undertake weeding as and when required. The cost of general weeding was estimated to be about 35% of the total cost of production in the marginal and small holdings which declined to 29% in medium size of holding and to 24% in large farms (table 16). This decline of cost as size of holding increases is because large size holders have other sources of income and are not so reliant on farming.

Table 16.
Cost of weeding in selected farms (in Rs.)

MARGINAL	SMALL	MEDIUM	LARGE
5397 (37%)*	672 2 (35%)*	365 4 (29%) *	12 66 (24%)*

* Percentage to total cost of cultivation

The cost of cultivation varies from region to region depending upon a number of factors such as weather, cropping pattern, types of agricultural practices adopted and prices of inputs. Similarly, the income from farming also varies depending upon types of crops cultivated, prices, productivity, etc. In order to indicate the impact of *M. micrantha* on cost and income of farmers, we compared these two variables in different size groups with and without *M. micrantha*, assuming that all the above factors affecting cost and income are the same.

The cost and income of different size groups with and without *M. micrantha* infestation are shown in table 17. Three broad trends are apparent: first, cost and income of different size groups decline as size of holding increases; second, better management, manifested by higher cost of cultivation, provides higher income; and third, there are significant variations in the costs and income of the farms with and without *M. micrantha*.

Mikania micrantha appears to have an impact on production costs *and* on income. This is particularly significant for marginal and smallholder farmers, who, in general, do not have other forms of income.

In discussions, the selected farmers reported that the costs of weeding *M.micrantha* accounted for 10-20% of the total weeding costs. They reported that in some cases there has been an increase in the number of laborers employed for weeding, as a result of the vigorous growth of *M. micrantha*, which requires constant weeding. A shortage of income was one of the major problems for not undertaking intensive weeding, which is essential for the management of the weed.

Given these circumstances, and the fact that *M. micrantha* is on the increase in Kerala, there is clearly an urgent need to reduce weeding costs and improve the management of the weed. Most of the farmers spoken to supported the concept of biological control.

Table 17.
Per hectare cost of cultivation and income of different size groups with and without *Mikania micrantha* (in Rs.)

LAND HOLDINGS	WITH *M. MICRANTHA* (COST)	WITHOUT *M. MICRANTHA* (COST)	DIFFERENCE	WITH *M. MICRANTHA* (INCOME)	WITHOUT *M. MICRANTHA* (INCOME)	DIFFERENCE
Marginal	15,420	14,520	900	69,915	72,815	2,900
Small	19,207	17,957	1,250	62,883	65,652	2,769
Medium	11,074	10,224	850	49,056	52,256	3,200
Large	5,276	4,826	450	24,131	27,320	3,189

Plantation production systems

In the forestry sector of Kerala, teak is the major plantation species, covering an area of 80,000 ha. The rotation age of teak plantation varies from 50 to 55 years. The major management operations of teak are planting, maintenance, weeding, thinning and final felling. Generally two types of thinning viz., mechanical and silvicultural thinning are carried out in teak plantations. The first two thinning in the teak plantations are called mechanical thinning, in which trees in the alternate diagonals are removed. The subsequent four thinnings are called silvicultural thinning, in which stunted and poorly grown trees are removed (Mammen, 1998).

The discounted cost and income per ha. of teak plantation for 8 years are given in table 18 (first part of table). After one year of planting, the forest department undertakes weeding and other maintenance operations in the field. This will continue up to the 5^{th} year and during the 6th year, the first mechanical thinning will be undertaken. The maintenance will be carried out in the 7^{th} year. The second mechanical thinning will be carried out during 8^{th} year. During first and second mechanical thinning, the Forest Department enjoys income from teak plantations. The Forest Department has scheduled rates of all operations in teak plantations. The cost of planting teak in a one ha. area is estimated to be Rs. 3,100/-. The maintenance cost for first two years are Rs. 3,900/- and 4,300/- respectively and they are normally higher than successive maintenance cost

due to intensive weeding during the first two years. In the first and second mechanical thinning, benefits are estimated to be Rs. 36,000/- and Rs. 42,000/- respectively. The discounted costs and benefits during 8 year period, at 10% rate of interest, are Rs. 18706 and Rs. 39918 respectively and the net benefit is Rs. 21,212/-.

Table 18.
Discounted cost and income per hectare of teak plantation with and without *Mikania micrantha*

Yr.	ITEM	HIGHLY INFESTED					NO INFESTATION				
		Out-flow	Discount Flow		In-flow	Dis-counted	Out-flow	Discounted		In-Flow	Dis-counted
			10%	8%				10%	8%		
0	Planting	3100	3100.0	3100.0	-	-	3100	3100.0	3100.0	-	-
1	Maintenance	3900	3545.1	3611.4	-	-	3500	3181.5	3241.0	-	-
2	Maintenance	4300	3551.8	3685.1	-	-	3900	3221.4	3342.3	-	-
3	Maintenance	2100	1577.1	1667.4	-	-	1750	1314.3	1389.5	-	-
4	Maintenance	950	648.9	698.3	-	-	750	512.3	551.3	-	-
5	Maintenance	1800	1117.8	1225.8	-	-	1700	1055.7	1157.7	-	-
6	1st Mech. Thinning	4200	2368.8	2646.0	36000	20304	3800	2143.0	2394.0	41000	23124
7	Maintenance	2000	1026.0	1166.0	-	-	1599	820.0	932.0	-	-
8	2nd Mech. Thinning	3800	1774.6	2052.0	42000	19614	3425	1774.0	1849.0	46000	21482
		26150	18710.1	19802	78000	39918	23524	17122.2	17956.8	87000	44606

In order to understand the impact of *M. micrantha* on profitability, we worked out the average discounted cost and benefits of one ha. teak plantation without *M. micrantha* and then compared with the above figures (table 18). As evident from the table, there is significant difference in the cost of maintenance of teak plantations with and without *M. micrantha* during the analysis period is estimated as Rs. 1586/- and that of income is Rs. 4688/-. The difference per ha. net present value of benefits received by the plantation with and without *M. micrantha* is Rs. 6274/-.

What are the major problems in managing *M. micrantha* in teak plantations in Kerala? Based on the discussions with the officials of Kerala State Plantation Corporation (a wing of State Forest Department exclusively set up for raising forest plantation), following problems were raised. Compared to older plantations, the infestation is severe in younger plantations where *M. micrantha* completely covers the plants. This affects its growth and productivity of teak. Efficient weeding is one of the solutions to this problem. Weeding carried out by the Forest Department is mostly inefficient, partly due of lack of adequate funds and partly due to labour shortage. As per the approved schedules, 24 unskilled workers are required for weeding one ha. of area at the wage rate of Rs. 62 per day (total cost – Rs. 1,488/-). Since the workers can earn an amount of Rs. 100 to Rs. 120.day in other sectors, they prefer to work out side. In order to complete the work using the limited amount allotted, the officials employ only 12 to 15 workers to weed a one ha. area which after leads to unsatisfactory results.

Natural Forest System

In the natural forests, especially in semi-evergreen and evergreen, high infestations of *M. micrantha* was noted along the periphery of the forests. The growth was very sparse or absent in the interior parts of the forests but only where the canopy is closed. However, infestation by the weed was extensive in certain reed growing areas in the natural forests.

A participatory rural appraisal (PRA) was carried out with the tribals to understand whether or not *M. micrantha* poses any problem to their livelihood. The tribal people are aware of the presence

and spread of this weed in the natural forests. They pointed out that collection and extraction of non-wood forest products (e.g. reed, bamboo) from the natural forests have become difficult because of creeping and twining habit of the weed. The presence of the weed makes harvesting laborious and time consuming. For example, earlier it was possible to cut and collect around six bundles of reed a day (one bundle consists of twenty reeds). But in the highly infested are they are able to collect only three to four bundles, which results in reduction of income and subsequent lack of interest in the task.

At present, weeding has not been carried out in the natural forest areas and as a result no information is available on cost of weeding. Thus, data gathered from the accounting method was used to analyse the cost of weeding in natural forests. It was found that in a highly infested area, four workers are need for sickle weeding of *M. micrantha* in one ha. of natural forest. The cost incurred for this was estimated to be Rs. 490/-. The number of workers varies according to the type of weeding undertaken; for example, complete weeding of *M. micrantha* (uprooting and removal) requires about ten workers per hectare for which an amount of Rs. 1400/- is required.

c. Monitoring studies

The 28 permanent sample plots established in the state in 1997/98 were revisited after a period of six months to one year to assess the increase/decrease in severity of infestation. (see appendix 3). Among the homegarden systems, four sites retained the original status of high infestation (grade 5), two attained a high level from an earlier low level and in the remaining one area, *M. micrantha* was cut and removed. In natural forests, three sample plots showed a high level of infestation compared to the initial low level. Ten of the remaining plots retained the high level of infestation observed initially. In the case of plantations, while seven of them retained the original high level of infestation, one attained a high level from the initial low level. Thus, *M. micrantha* infestation is clearly on the increase in all the production systems surveyed in the State.

d. Assessment of *Mikania micrantha* in north eastern India (from add-on funding)

In December 1997, the DFID CPP approved additional funding to the *M. micrantha* project (then focused on the Western Ghats region) to enable the status of the weed to be assessed in Assam and some adjoining States. Part of the rationale for this 'add-on' was a report to the CPP that *M. micrantha* in this part of India has spontaneously declined over recent years. Thus is was stated that an opportunity might exist for lessons learnt in Assam to be transferred to the Western Ghats region. It should be added that the ICAR, New Delhi, did not agree that the weed has declined in northeastern India.

The survey was confined to a visit to Assam and Meghalaya States although most time was spent in the former. Staff from KFRI were to have joined the survey but were unable to participate because of flight cancellations. To undertake the assessments, relevant information on the weed status was gathered from field visits to a selection of various types of production systems. In Assam, visits were made to: seven small holder agroforestry systems (approx. 1 ha); three large tea plantations; two natural forest areas; and three grassland sites adjoining natural forest areas. All sites lay between 40-135m in altitude. In Meghalaya, six smallholder farmers were visited, lying between 200-1000m altitude. At the tea estates, discussions about *M. micrantha* were held with the estate managers. Discussions were also held with local agricultural and forestry institutions including: AAU (including the specialist extension service for smallholder tea growers), Jorhat; TTES Jorhat; Forest Department, Assam; and the ICAR Research Complex for NEH Region, Shillong, Meghalaya.

It was clear from our surveys and discussions that *M. micrantha* is well distributed throughout Assam and Meghalaya; in the latter it occurs up to about 1100m; it is also apparent that *M. micrantha* is common in most of the other northeastern States. It appears that the weed was introduced into the region in about 1942 to provide cover for military purposes. In Assam and Meghalaya, the weed affects virtually all major land use systems apart from lowland rice and is considered a major economic and sociological problem. The weed flowers mainly in September-December and rapid growth of seedlings begins after the rains which begin in March – April. Tea plantations in Assam are particularly badly affected. Each year the large plantations have to fund considerable herbicide and hand weeding operations. Small holder tea plots, being developed in the hilly areas of the region to boost local economics, are also affected by invasion of the weed. Dr. A.C. Barbora, Agronomist, TTES, said that *M. micrantha* is also an alternative to host and provides cover for the capsid tea pest *Helopeltis theivora* (Hemiptera: Capsidae). All of the institutions visited expressed a strong interest in the initiative to use classical biological control involving exotic pathogens to manage the weed and would like to be involved in a further implementation phase of the project. In particular, the Vice-Chancellor of TTES, Professor A.N. Mukhopadhyay has expressed in a letter the problems caused by the weed in Assam and the interests in biological control. He said that recently, a tea consignment to Germany had been rejected because it was tainted as a result of herbicide residues. Tocklai confirmed this and said that buyers are now deeply concerned about residues and this is threatening India's tea industry.

e. Characterisation of the *Mikania micrantha* populations (from add-on funding)

Figure 1 shows the AFLP profiles for a selection of the *M. micrantha* isolates with selective primer GT. Lanes1 to 5 and 14 to 25 contain *M. micrantha* isolates and lanes 6 to 13 contain other *M. micrantha* species.

The dendograms drawn from the *M. micrantha* (figure 2) and all *M. micrantha* species (figure 3) both show that the isloates from India, Malaysia and Sri Lanka cluster together with a similarity value of around 87%. This group then clusters with the group from Costa Rica at about 84% similarity and that this group then links to the isolates from Mexico at 83%. Thus, according this, the Indian material appears to be relatively homogeneous, genetically, and may have originated from the Central America.

The group containing the Brazilian isolates (in itself a diverse group) clusters to the remaining isolates at 79% similarity. It is interesting that in figure 3 (the dendogram for all *M. micrantha* isolates and other *M. micrantha* species) that the Brazilian isolates cluster strongly with an unidentified species from Peru, a possible indication that the isolates from Brazil are not *M. micrantha*. Indeed when the same data were analyzed using principal co-ordinate analysis the Brazilian isolates form their own separate group in the *M. micrantha* plot (figure 4) and fall in the center of the other *M. micrantha* species in (figure 5) which contains all the isolates analyzed.

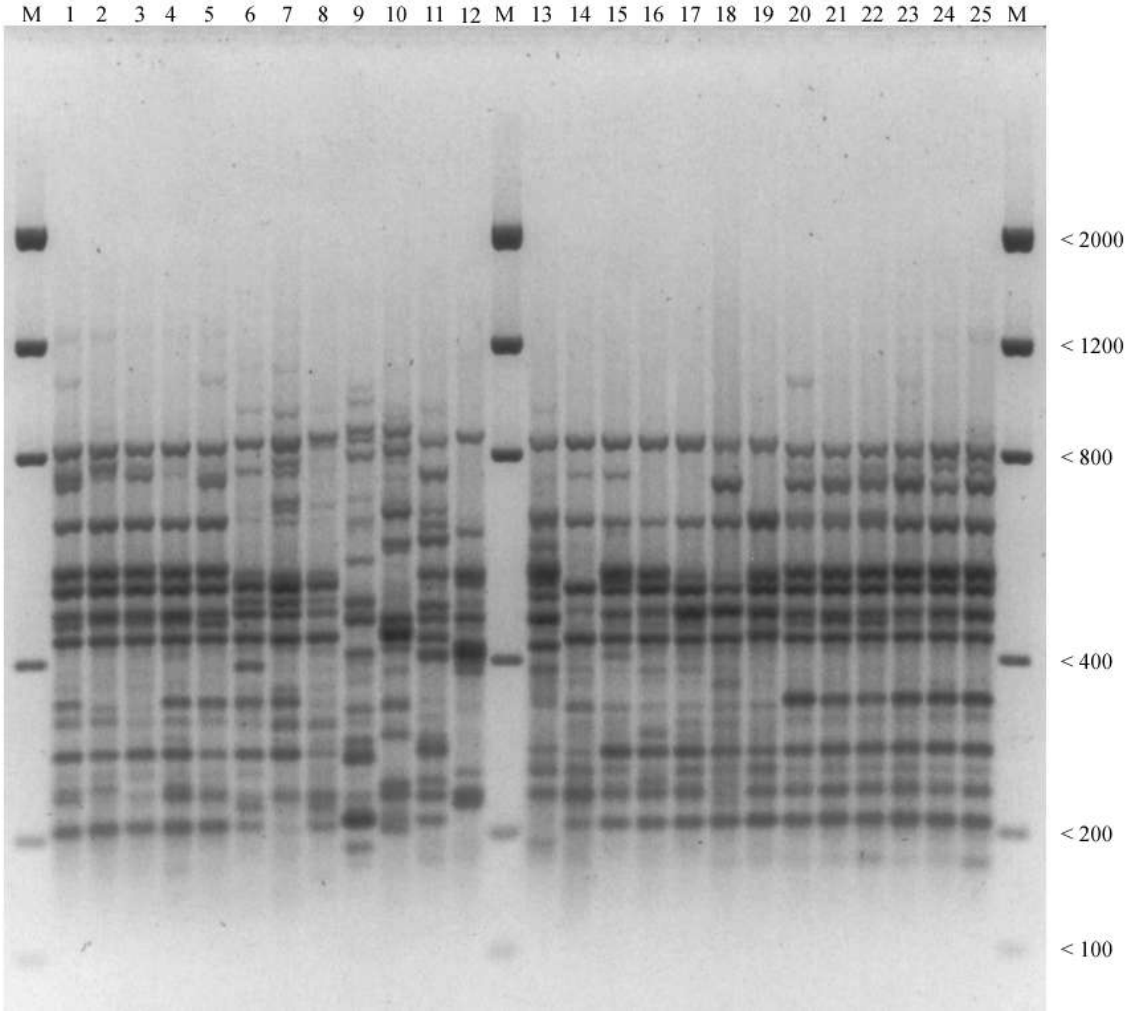


Figure 1. AFLP profiles of *Mikania micrantha* isolates (unless indicated otherwise) amplified with AFLP primer with selective nucleotides GT. **M**, molecular weight marker lanes (band sizes shown in base pairs). Lane **1**, India 3-1 Vettilappara; **2**, India 3-2 Athirampilly; **3**, India 3-3 Vazhachal; **4**, India 3-4 Porimgal (Teak); **5**, India 3-5 Porimgal (Natural); **6**, *M. micrantha* sp. Peru 5-1; **7**, *M. micrantha* sp. Peru 28-1; **8**, *M. micrantha* sp. Peru San Martin W1707; **9**, *M. micrantha* sp. Hairy ex Costa Rica 16-1 Siquirres; **10**, *M. micrantha* vitifolia ex Columbia; **11**, *M. micrantha* guaco ex Columbia; **12**, *M. micrantha* sp. Medicinal Brazil; **13**, *M. micrantha* sp. ex Columbia; **14**, Costa Rica Turrialba 15-1; **15**, Costa Rica Moravia 15-3; **16**, Costa Rica 15-4 W1869; **17**, Costa Rica Siquirres 16-1; **18**, Costa Rica 16-2 Limon; **19**, Costa Rica 17-1 Siquirres W1868; **20**, India Shillong W1848; **21**, India Jarhat W1844; **22**, India Geleki W1846; **23**, India Garampani W1845; **24**, India Nameri W1847; **25**, India Kaziranga East W1843.

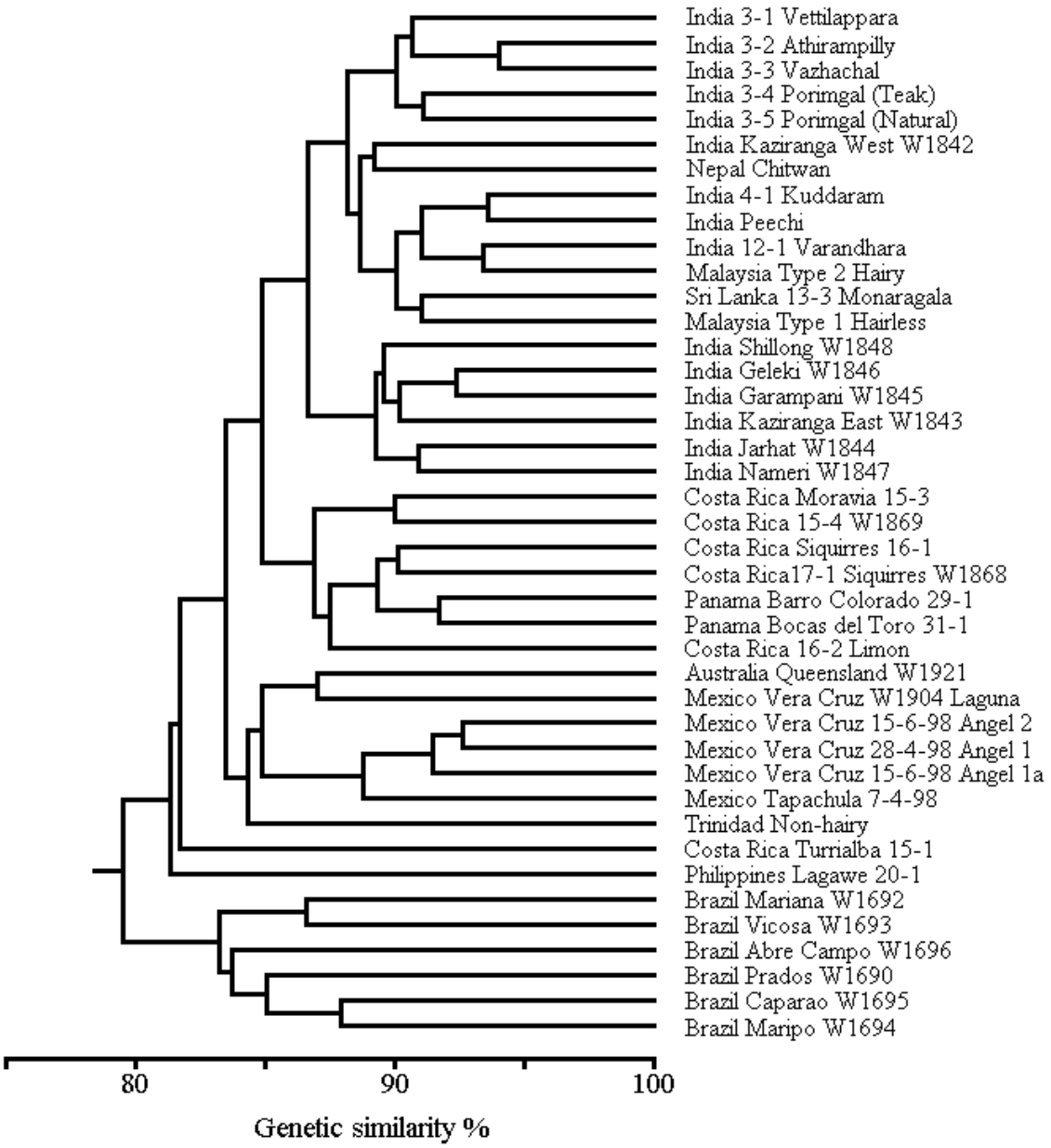


Figure 2 Dendogram of *Mikania micrantha* isolates.

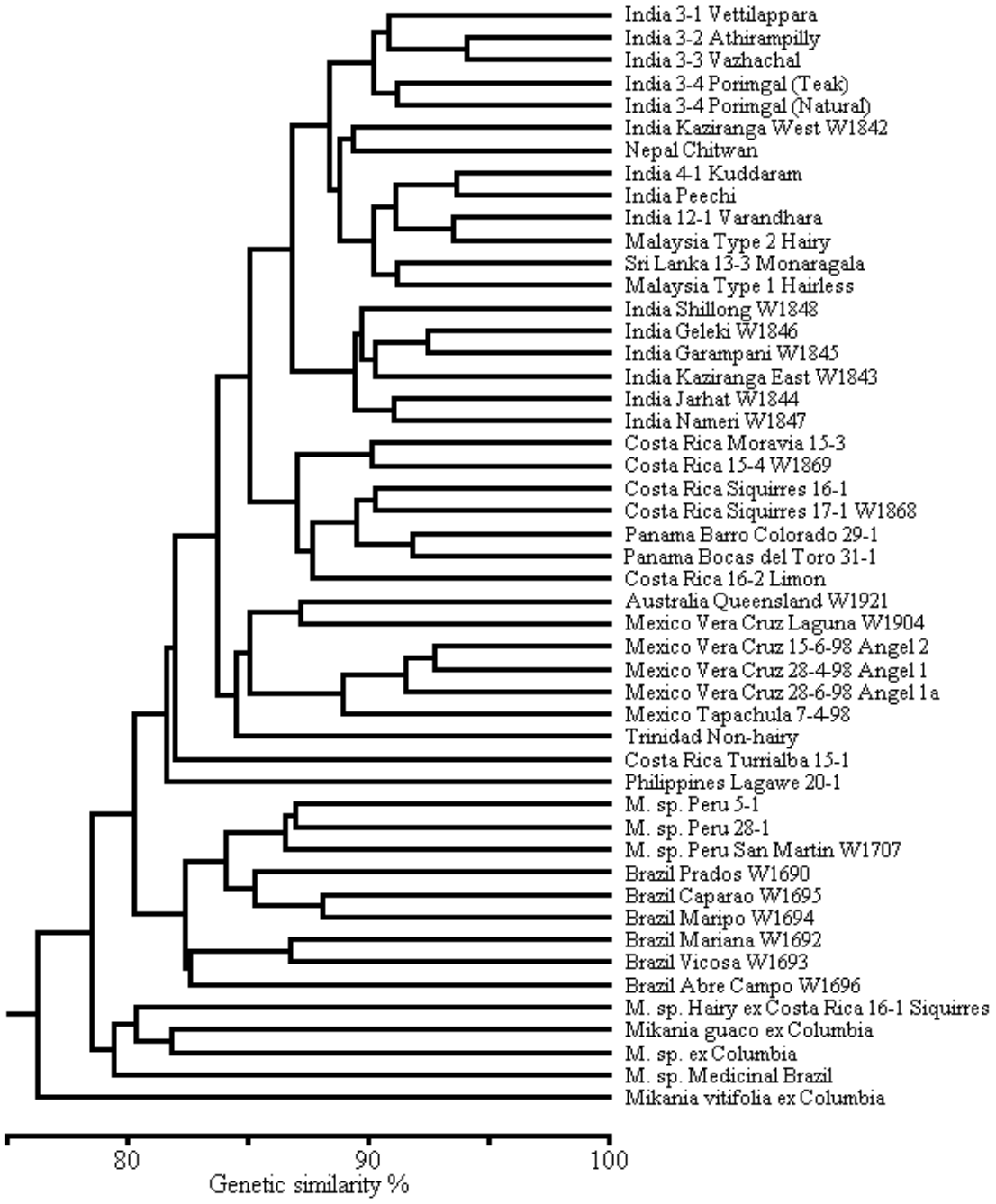


Figure 3. Dendogram of all *Mikania micrantha* isolates.

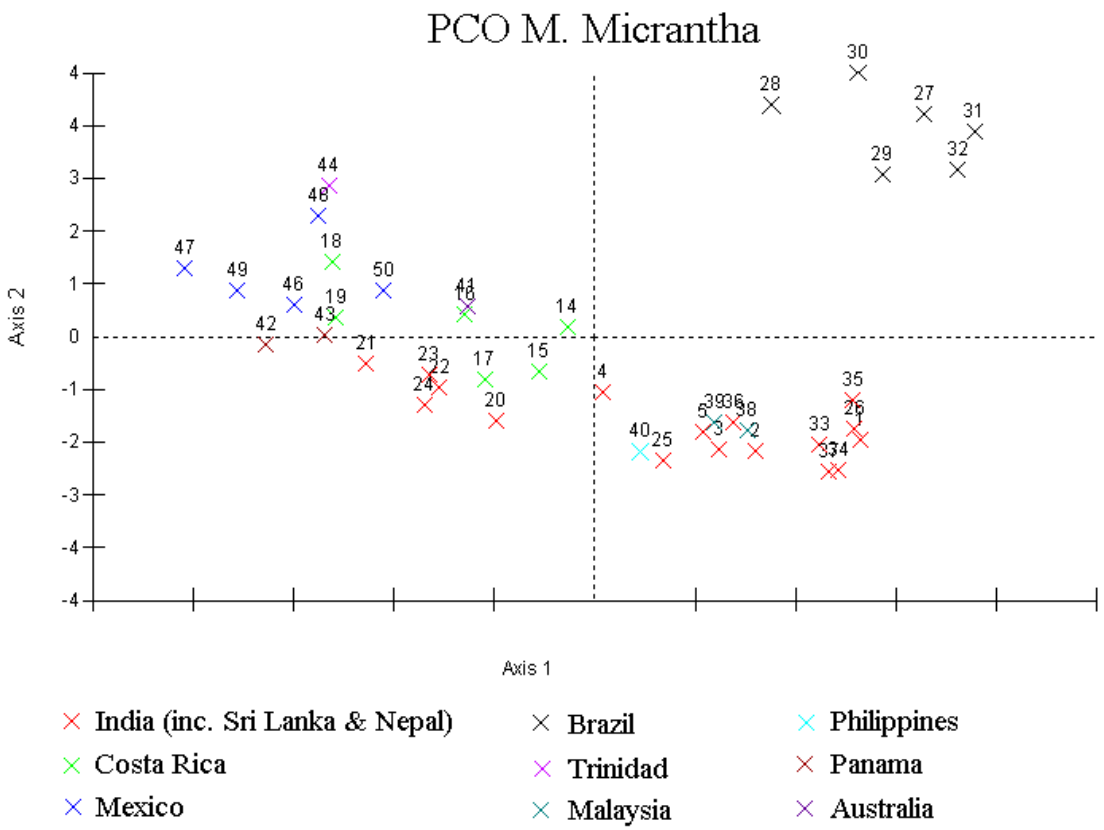


Figure 4. Principal co-ordinate analysis plot of *Mikania micrantha* isolates.

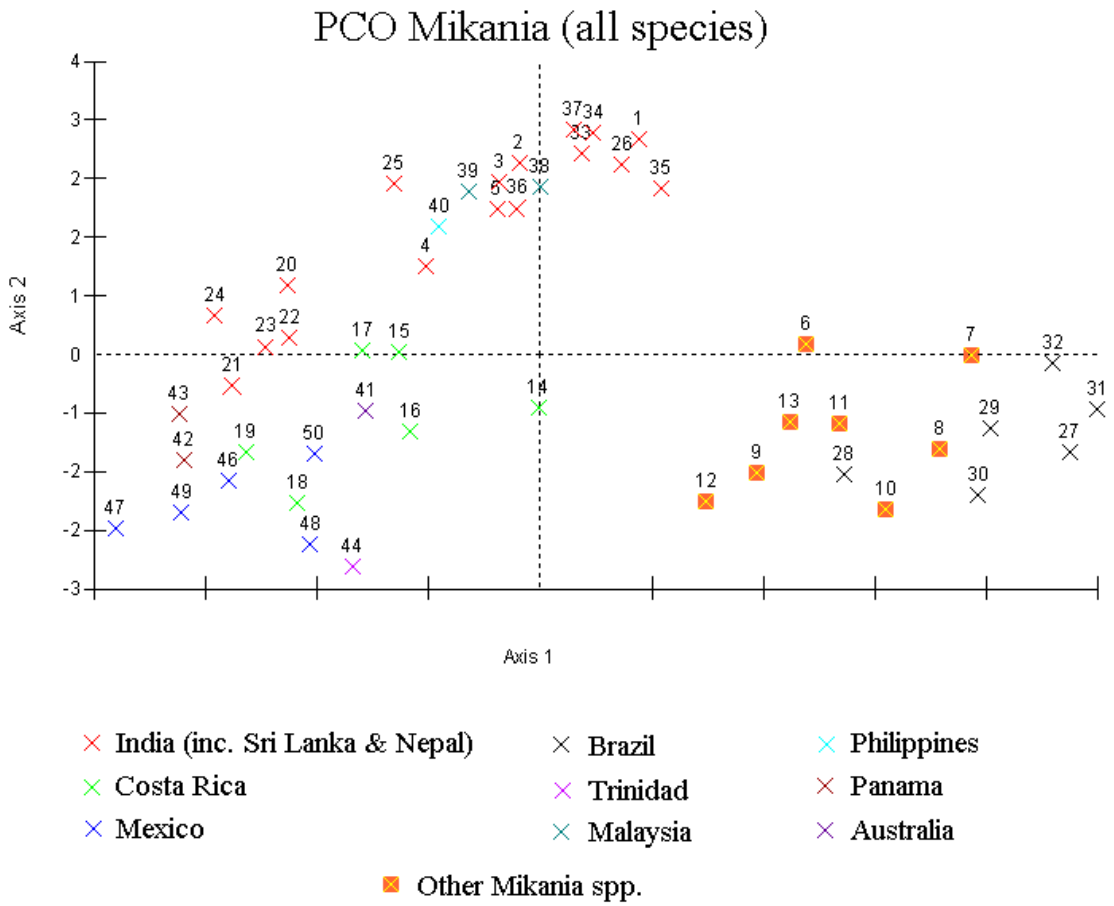


Figure 5. Principal co-ordinate analysis of all *Mikania micrantha* isolates.

2. Mycobiota characterized and potential pathogenic fungal biocontrol agents identified from the weed in its native range and India

a. Surveys in the native range of *Mikania micrantha*

The identity of the pathogens from the dried, infected plant specimens was confirmed, and specimens were then catalogued. Cultures of the rust pathogens were established on living plants in the CABI Bioscience quarantine glasshouse.

Due to limited funding, it was only possible to select one isolate of a single agent for a detailed assessment of its potential as a CBC agent. The rust *Puccinia spegazzinii* was chosen since it was found to be extremely damaging in the field, and no infection of other *Mikania* species growing in close proximity to infected *M. micrantha* plants was observed. The inherent host specificity, damage to the host, and spore dispersal qualities of rusts make them ideal candidates as CBC agents. Indeed, a significant percentage of pathogens that have been released, or are being considered for release as control agents of weeds are rusts (Julien and Griffiths, 1998). Eleven isolates of *P. spegazzinii* were collected and maintained on plants in the glasshouse in the U.K. In

addition, two cultures of the rust *Dietelia portoricensis* were also maintained, as a potential second candidate. Table 19 gives the site details of the 11 isolates of *P. spegazzinii* and the two isolates of *D. portoricensis.*

Table 19.
Details of sites where *Puccinia spegazzinii* and *Dietelia portoricensis* were collected during surveys undertaken in the native range of *Mikania micrantha*

Site Details (with identifying name)	Rust Isolate Number
Isolates of *Puccinia spegazzinii*	
'Prados' Victoriano Velosa, Prados Rd., nr. Tiradentes, Minas Gerais, Brazil.	W1690
'Mariana' Rio Guaxulo do sul. nr. Mariana, Minas Gerais, Brazil	W1692
'Viçosa' San Miguel do Anta, Viçosa, Minas Gerais, Brazil	W1693
'Maripó' Maripó, Minas Gerais, Brazil	W1694
'Trin. I' Guapo, Parrylands, south-west Trinidad	W1735
'Trin. II' Guapo, Parrylands, south-west Trinidad	W1761
'Trin. III' Valencia-Sangre Grande Road, Near WASA mains, eastern Trinidad. Sea level	W1942
'Trin. IV' Mount Catherine, Mt Pleasant range, Chaguaramas, north-west Trinidad. Alt. 100m	W1949
'Napo' Rio Pucuño, Loreto-Pangayacu Road, Napo Province, eastern Ecuador. Alt. 950m	W1960
'Imbabura' Lita, Imbabura Province, western Ecuador. Alt. 680m	W1958
'Costa R. Telia' Siquirres-Guápiles (Km 47), Limón, Costa Rica. Alt. 160m	W1868t
Isolates of *Dietelia portoricensis*	
'Costa R. Aecia' Siquirres-Guápiles (Km 47), Limón, Costa Rica. Alt. 160m	W1868a
'Mexico' Laguna Escondida, Catemaco, Vera Cruz, Mexico.	W1904

b. Surveys in India

The survey covered agroforestry systems, natural forests and plantations. A total of nine apparently pathogenic fungi were isolated from various symptoms produced on *M. micrantha*. The details on the symptoms produced by the fungi, their pathogenicity on *M. micrantha*, distribution and the economic importance of the diseases are provided in appendix 4.

3. The biological control potential of fungal pathogens from the native range and India on the weed biotypes present in India determined and compared

a. Fungal pathogens from the native range of *Mikania micrantha*

(i) Life cycle and description of *Puccinia spegazzinii*

The work undertaken at Viçosa University demonstrated that the teliospores of *P. spegazzinii*, embedded in the host tissue, liberate basidiospores under conditions of high humidity. The basidiospores are able to infect young meristematic tissue of the same host, giving rise to telia and teliospores, older plant tissue is less susceptible. This confirms the description in the literature that *P. spegazzinii* is a microcyclic, autoecious rust. *P. spegazzinii* is able to infect all vegetative parts of the plant (leaves, petioles and stems) causing necrosis, cankering and often leading to plant death. Evidence from the glasshouse suggests that the rust may survive in stem cankers for long periods, for example, during conditions unfavourable for propagation of the pathogen.

(ii) Intraspecies pathogenicty of *Puccinia spegazzinii* isolates

Appendix 5 gives the full results of this screening, and also includes the results from the screening of *D. portoricensis*. A summary of the results for *P. spegazzinii* is given in table 20. Not all possible challenges between the 11 *P. spegazzinii* isolates and all the *M. micrantha* plant collections have been undertaken, particularly with those from Ecuador, which have only recently been acquired. There was significant variation between the pathogenicity of the different rust isolates towards the different weed populations. This shows that *P. spegazzinii* exists as a number of races or pathotypes within its native range. In addition, the results confirm those from the plant DNA analyses (see section 1e), in that *M. micrantha* occurs as distinct biotypes in the Neotropics, and that there are a number of distinct populations of *M. micrantha* within India and other region in Southeast Asia. These Old World populations could be considered to be biotypes, although further studies are needed to establish this.

Table 20.
Summary of intraspecies pathogenicity of *Puccinia spegazzinii* isolates against world-wide populations of *Mikania micrantha*

	Rust Isolates (number assessed)			
***Mikania* Collections (number assessed)**	**Brazil (4)**	**Trinidad (4)**	**Costa Rica (1)**	**Ecuador (2)**
India South-west (10)	± 4 **X**	4	4	4
India North-east (7)	± 4	± 4	±	_
Nepal (1)	4	4	4	_
Sri Lanka (1)	_	4	_	_
Malaysia (2)	± **X**	4	4	_
Philippines(1)	4	4	_	_
Australia (1)	4±	4	4	_
Mexico (3)	4	**X**	4**X**	_
Costa Rica (6)	**X**	**X**	4	_
Panama (2)	±	**X(?)**	4	_
Brazil (6)	4	**X**	**X**	_
Trinidad (4)	±	4	**X**	
Ecuador (2)	_	±**X**	_	4

Pathogenicity score:
4 Fully compatible (4)
± Semi-resistance response (2/3)
X Not compatible (0/1)
- Not tested
? Unclear result, needs confirmation

An isolate of the rust from Trinidad (W1761) was found to be capable of infecting almost all the collections of the weed from the Old World against which it was challenged. For the focus area of this particular study, 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 to be semi-resistant to W1761, giving a 2 or 3 pathogenicity score. However, an isolate of the rust *Dietelia portoricensis* from Mexico infected all three collections and hence, could be considered as an alternative CBC agent in this region of India (see appendix 5).

It is interesting to note that *P. spegazzinii* was found to be more damaging on the Indian populations of the weed, with plant death frequently resulting, than on the host biotype from which it was originally isolated. This held true for inoculations carried-out in the glasshouse, and

hence, could not be attributable to an increase in susceptibility of glasshouse-grown plants (thinner cuticles etc), nor to the absence of hyperparasites (common in the field). One possible explanation could be the absence of pathogen selection pressure on the plants in the exotic range, and hence loss of resistance to the rust, since the development of resistance mechanisms carries a metabolic price.

(iii) Environmental requirements for selected *Puccinia spegazzinii* pathotype (W1761)

Figures 6 and 7 give the temperature and dew period requirements for *P. spegazzinii* isolate W1761. The ANOVA showed that there was highly significant variation in the data due to treatment for both factors (temperature: df=4, F-ratio=8.9, $p<0.001$; dew period: df=5, F-ratio=7.2, $p<0.001$). There was a considerable temperature effect, with significantly more infection achieved at 15^{o}C than at any of the other temperatures tested (LSD-test, significance level $p<0.05$). There was a trend of increasing sori number per apex with increasing dew period, although this was only significant between the longest three periods and each of the shortest three dew periods (LSD-test, significance level $p<0.05$). The results of this analysis show that the rust has a relatively broad environmental tolerance. Good infection occurred between 15^{o}C and 25^{o}C, with an optimum near 17^{o}C. It is able to infect, albeit at a very low level, at temperatures as low as 12^{o}C. The rust has a relatively low minimum dew period requirement of 8 hours, with maximum infection achieved after 14 hours. This short requirement for free water on the leaf surface is ideal for a tropical pathogen, allowing infection to occur over night, when dew forms, rather than being dependent on rainfall. These temperature and dew period requirements correlate well within those experienced in the Western Ghats region of India.

Figure 6.
Effect of temperature on the infection of *Mikania micrantha* by *Puccinia spegazzinii* isolate W1761. Bars represent standard error (SPSS for MS Windows, release 6.1.4)

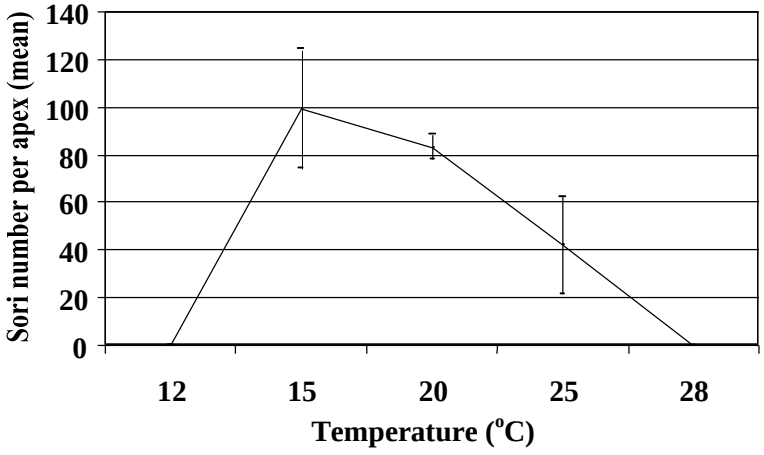
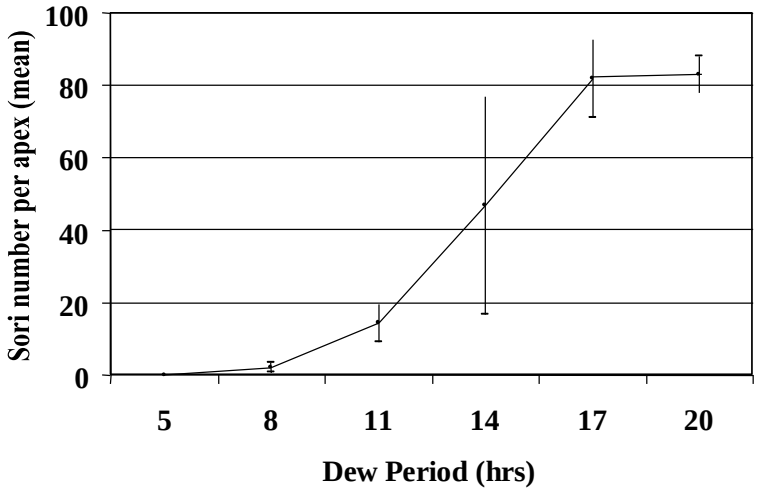


Figure 7.
Effect of dew period on infection of *Mikania micrantha* by *Puccinia spegazzinii* isolate W1761. Bars represent standard error (SPSS for MS Windows, release 6.1.4)



(iv) Potential impact of *Puccinia spegazzinii*

Although there are a number of examples of successful CBC with pathogens (Hasan and Wapshere,1992; Trujillo, 1985), one example is of particular relevance to this programme; the control of Noogoora burr (*Xanthium occidentale* L.) in Queensland, Australia with the rust *Puccina xanthii* Schw. (Uredinales) (Chippendale, 1995). This rust has the same type of life cycle as *P. spegazzinii* and has a similar impact on the weed. Noogoora burr is a rangeland weed, that is toxic to live stock, and the burrs are a serious contaminant of sheep wool. *P. xanthii* was accidentally introduced into Australia, and was first recorded in the field in 1974. By 1978, there were general observations from landholders that the rust was having a visible impact on the weed in the field. This was backed up by a significant reduction in the percentage of burrs in wool clip. An economic study on the weed concluded that the benefits from biological control in less than 10 years, was estimated at almost A$17 million. Since classical biological control is sustainable, the savings to the farmer of this programme will clearly continue in perpetuity.

b. Fungal pathogens from India

The fungal pathogens reported here represent the mycobiota of *M. micrantha* from the southern part of the Western Ghats. Of the fungal pathogens, only *Myrothecium leucotrichum, Corynespora casiicola, Ascochyta* sp. and *Phoma* sp. produced symptoms both on unwounded and wounded leaves. Others, viz., *Colletotrichum gloeosporioides, Alternaria alternata, Curvularia lunata, Fusarium solani* and *Pestalotiopsis* sp. can be considered as wound pathogens as wounding of plant parts (leaf/collar region) was necessary for initiation of infection. All the pathogens can be considered as opportunistic as none of them caused any serious damage to the host. The low virulence of these pathogens may be because of the fact that they are not co-evolved. Although, none of them caused any symptoms on economically important plants like pepper, eucalypts, teak and bamboo it is not clear whether it is due to the host specificity of the pathogens or inability of the pathogen to cause symptoms due to their opportunistic nature. Possibly, these represent weak pathogens, or secondary invaders of composite hosts, unable to

attack healthy crop plants from other families. The principal criterion on which the selection of fungal isolates for mycoherbicide development is based is not host specificity, since plurivorous pathogens have been exploited, but virulence. If isolates do not exhibit a consistently high degree of virulence to the target weed in pathogenicity tests, preferably with a high kill ratio, then they would not be considered further. None of the isolates tested during the study demonstrated this potential and, consequently the search for local pathogens which could be exploited as mycoherbicides was discontinued.

Of previously recorded fungal species on *M. micrantha* (Barreto and Evans, 1995), only *Colletotrichum gloeosporioides (Glomerella cingulata*) and *Alternaria* sp.(probably *A. alternata*) reported from India could be collected during the current survey. *Myrothecium leucotrichum, Corynesporoa cassiicola, Curvularia lunata, Fusarium solani, Pestalotiopsis* sp., *Ascochyta* sp. and *Phoma* sp. are new pathogen records on the host. *Cercospora M. micranthakola* F. Stevens reported by Barreto and Evans (1995) from India, and potentially the most damaging, could not be collected during the present study.

d. Characterisation of neotropical rusts on *Mikania micrantha*

(i) Morphological characterisation

Puccinia spegazzinii de Toni was the only rust fungus found associated with *Mikania micrantha* during surveys in southern Brazil (Barreto & Evans, 1995). However, during the present extended surveys in South, Central and North America, three taxonomic groups of rusts, based on morphological characters, can now be delimited on *M. micrantha sensu latoa. P. spegazzinii* remains by far the commonest rust with the widest distribution, and occurs on *M. micrantha* throughout its South American range and appears to be the only species associated with this host in the region, in which Trinidad can be considered to be a geographic extension. This microcyclic species, with only telia represented in the life cycle, is also present in Central America but becomes rarer farther north and was not collected during the Mexican surveys. Nevertheless, it has been recorded on an unidentified *Mikania* sp. from Mexico (Leon – Gallegos & Cummins, 1981), as well as on various *Mikania* spp. from the extremes of their range in southern USA (Arthur, 1922), and in northern Argentina (Lindquist, 1982). Germination studies have shown that the two-celled, brown teliospores produce the characteristic, "text" book basidia, each forming four basidiospores after a meiotic cycle. These haploid basidiospores function as the only disseminative as well as the only infective spore in the rust life-cycle. In evolutionary terms, this is an advanced rust species having a reduced life cycle in which the other spore stages (pycnia, aecia and uredinia) have been lost (Hennen & Buriticá, 1980).

The other two rust taxa, which have been identified on *M. micrantha* during the present study, were not found in South America during the surveys and only appeared towards the northern end of the range, in Costa Rica and Mexico. In several sites in Costa Rica, two rust species coexist on *M. micrantha*; in addition to *P. spegazzinii*, a distinctive microcyclic species with similar symptomatology is also present, which, instead of producing characteristic telia and teliospores, possesses telia that, morphologically at least, are aecioid in form. Genetically, however, they perform a sexual function since on germination, and presumably following a meiotic division, they produce basidia and basidiospores. Rusts with this reduced life cycle, and a telial stage which resembles the aecidium-like aecial stage of the parental species, are termed endophylloid (Hennen & Buriticá, 1980). In fact, a new genus was created for this taxon (*Endophylloides portoricensis* Whetzel & Olive) when it was collected on *Mikania* spp. (*M. cordifolia, M. odoratissima*) in Puerto Rico (Olive & Whetzel, 1917). The species is characterised by long,

pinkish-grey, compact chains or "horns" of unicellular teliospores, which emerge from "aecial" cups. The diagnostic feature of this genus and the new species was considered to be the presence of sterile intercalary cells between the teliospores which appear to have a structural function, binding or holding the spores together in long, persistent columns. Later, Stevenson (1975) also reported this rust from both Central and South America on several *Mikania* spp., including *M. micrantha*. More recently, the genus has been reduced to synonymy with the earlier named genus *Dietelia*, under the new combination *D. portoricensis* (Whetzel & Olive) Buriticá & Hennen, which included species with or without intercalary cells (Buriticá & Hennen, 1980). This species was also thought to extend into Mexico, where it was collected by project collaborators and considered to represent a new record for that country (A. Romero & G. Carrión, in press). However, following further study in the UK, particularly on its micro-morphology and germination behaviour, and direct comparison with the isolates of *D. portoricensis* from Costa Rica, it is now concluded that the Mexican isolates with aecioid telia represent a different, almost certainly undescribed species of *Dietelia.* This can be distinguished from *D. portoricensis* by the presence of pycnia, distinctive yellow structures produced in abundance on the upper leaf surface prior to the formation of the aecioid telia; the general absence of the intercalary cells; and the germination process.

Thus, the morphological studies show that three rust species occur on *M. micrantha* in the Neotropics. However, there are strong indications that all three species share a common genetic origin or ancestry, since the life-cycles and host symptomatology follow a similar pattern, and that Costa Rica may be the evolutionary centre of the host-rust complex association.

(ii) Molecular characterisation

No amplifiable products were obtained from any samples regardless of the extraction method used although all controls worked. Subsequent amplifications were carried out using universal ITS primers, which should amplify both the plant and rust DNA. These usually gave a product from the leaf DNA extracts from the plant host material indicating that the DNA was of sufficient purity to allow PCR amplification. No product was obtained from DNA extracted from stem material indicating the presence of inhibitors co-purifying with the DNA. The failure of amplification of a PCR product from the rust samples could be due to one of two reasons. First, the physical disruption methods used for the rust cells are so extreme that the forces required to break the cells open are so extreme that the DNA is immediately sheared into fragments to small for PCR amplification. Second, the sequence of the primers available is not compatible with sequences in the rust genome. The solution to these problems would be the development of an *in vitro* culturing technique enabling the purification of rust DNA uncontaminated with host DNA. Once DNA is obtained a genomic library would have to be constructed and screened for ribosomal sequences, which could then be sequenced, leading to the development of specific primers for *Puccinia*. Unfortunately, the length of time required for such work was outside the scope of this project.

4. Import/release protocol developed, host specificity testing requirements determined and host specificity tests completed

a. Import/release protocol and host specificity testing requirements determined.

Host specificity: Discussions about the testing procedure to be followed and the non-target plants were held with KFRI, ICAR's PDBC, AAU, TTES, Jorhat, and ICAR's National Research Centre

for Weed Science, Jabalpur, M.P. Unfortunately, as there is no written protocol for the introduction of biological control agents, there are no written guidelines for testing procedures or plants for inclusion in host specificity tests. However, the Government of India is now basing their assessment more on the centrifugal phylogenetic testing sequence (Wapshere, 1974), used in most current screening programmes (ICAR, pers. comm.) as well as the screening of relevant economically important plants. For the latter, the general advice was to screen a selection of plants from southwest and northeast India although no 'formal' was provided; the test plants recommended in the discussions are covered in in section(b) below. The information from these tests are then summarised in a dossier and submitted (by the Indian collaborating institutions) to the Directorate of Plant Protection, Quarantine and Storage (DPPQ&S). The DPPQ&S committee that reviews applications for biological control agent introductions, may then recommend direct introduction (under quarantine or) further testing, either under third country quarantine or under Indian quarantine. A dossier on *P. spegazzinii* has been prepared.

Support for the use of exotic pathogens: This was formally expressed by all the Indian collaborators at the national workshop held at KFRI, Peechi, in November 1999 (see output 6).

Lack of potential of local biological control agents: The work on this by KFRI was discussed under output 1. The work was presented at the national workshop.

Nodal agency for exotic pathogens: Recent discussions with ICAR in New Dehli suggests that exotic fungal pathogens, as well as other microbial agents will be handled by the National Bureau of Plant Genetic Resources (NBPGR), New Dehli but this still has to be confirmed.

b. Host specificity tests completed (including extension funding)

None of the 55 species that were screened became infected with *P. spegazzinii,* despite consistent infection of control plants of *M. micrantha* (Appendix 6). Chlorotic spots were observed on *Helanthus annuus* and *Eupatorium canabum*, and necrotic spots on two other *Mikania* species. A microscopic analysis was undertaken of these species to see at what stage the resistance response had been elicited in the non-host. In addition, a selection of test species, where no macroscopic symptoms were observed, were also included in the microscopic analysis (Table 21). The results revealed that, in all cases, basidiospore germination and/or penetration was inhibited by the non-host.

Table 21.
Microscopic analysis of selected test plants after inoculation with *Puccinia spegazzinii* (isolate W1761)

Plant Species	Microscopic symptoms								
	Germination					Penetration		Colonisation and Sporulation	
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Subfamily I: Lactucoideae									
Tribe: Lactuceae									
Lactuca sativa (lettuce)									
"All the year round"	+	-	-	-	-	-	-	-	-
Mutisieae									
Gerbera hybrida "Jamesonii"	-	-	-	+	-	-	-	-	-
Arctotideae									
Gazania hybride	-	+	-	-	-	-	-	-	-
Eupatorieae (Asteroideae)									
Ageratina riparia (mist flower)	-	-	-	-	-	+	-	-	-
Ageratum F1 Hybrid "Adriatic"	-	-	-	+	-	-	-	-	-
Chromolaena odorata	-	-	-	+	-	-	-	-	-
Eupatorium canabum	-	-	-	-	-	-	+	-	-
Liatris pycnostachya	-	-	-	+	-	-	-	-	-
Mikania guaco	-	-	-	+	-	-	-	-	-
Mikania sp. 2	-	-	-	+	-	-	-	-	-
Mikania sp. 3	-	-	-	+	-	-	-	-	-
Stevia rebaudiana (Stepa®)	-	-	-	-	-	+	+	-	-
Subfamily II: Asteroideae									
Heliantheae									
Helianthus annuus	-	-	-	-	+	-	-	-	-
Anthemideae									
Pyrethrum roseum	-	+	-	-	-	-	-	-	-
Calenduleae									
Calendula officinalis	-	-	-	-	-	-	+	-	-
Asteraceae									
Aster alpinus	-	-	+	-	-	-	-	-	-
Selected Crop Species									
Coffea arabica (Coffee)	-	-	+		-	-	-	-	-

Microscopic assessment categories:

0 no germination or spore lysis
1 spore germination with short germ tubes, often collapsed
2 long and short germ tubes, no apparent recognition of cell junction
3 germ tube recognition of cell junction, but no appressorial formation nor penetration
4 appressorial formation, no penetration
5 attempted penetration, defensive papillae formation around appressorium
6 normal penetration, secondary vesicles, no further development (phytoalexins, hypersensitive response)
7 internal mycelium
8 spore formation

5. Indian scientists trained in tree crop and agroforestry weed management research and implementation (including extension funding)

a. One-to-one training

Training visits to KFRI in relation to the project schedule, were made by staff of CABI Bioscience in: June/July 1997; January/February 1998; November 1998; June 1999; and October/November 1999 (see Murphy and Evans, 1997; 1998; Murphy, 1998; 1999a; 1999b). KFRI staff trained included Dr K.V. Sankaran, Mr M.A. Srinivasa, Dr P.K. Muraleedharan and Dr V. Anitha. Topics covered included: weed surveys, monitoring and mapping techniques; biological control methods and weed pathology. During the visits, pilot surveys were conducted to demonstrate various weed survey techniques.

The National Resources Institute (NRI) also made a visit to KFRI in June/July 1997 to discuss socio–economic survey techniques and analysis (Conroy, 1997).

b. Weed pathology training at CABI Bioscience, UK Centre, Ascot.

Training was provided to Dr K.V. Sankaran, KFRI, Peechi, 12-17 and 25-31 October 1998; and to Dr P Sreerama Kumar, PDBC (ICAR), Bangalore, and Dr M.C. Puzari, AAU, Jorhat (Assam), 17-28 January 2000. The main focus of the training was to learn the inoculation and assessment techniques for *P. spegazzinii*.

6. Project research outputs publicised and promoted (including extension funding)

a. Alert sheet

This was prepared by CABI Bioscience (then IIBC) in 1997 (see appendix 7). Copies were distributed to KFRI, AAU, TTES, and ICAR, New Delhi. Once KFRI had determined that the front of the weed invasion lay at the border between Kerala and Karnataka (see output 1) copies of the alert sheet were given to the Karnataka Forest Department, which is based in Bangalore. Copies were also given to the field offices of the two forest circles in the south of the state: Mysore and Madikere.

b. GIS map

See appendix 2. This was produced by the GIS unit of KFRI in 1999 in order to summarize in simple form, the current distribution and abundance of *M. micrantha* throughout Kerala. The purpose was two-fold:

- To disseminate information about the weed status in summary form to the administrators/policy makers in the Kerala Agricultural and Forestry Departments.
- To summarize changes in the status of the weed, on a regional basis, for monitoring purposes.

c. Meetings with key research and extension organisations with India.

During the course of the project meetings were arranged with a wide range of agricultural/forestry research and extension institutions for the following strategic purposes:

- To understand Indian view points on the status and damage caused by *M. micrantha*.
- To publicise and promote the main purpose of the CPP project. The institutions met are listed in appendix 8. Efforts have been made to interact with people at all levels within these organisations.

It should be stressed that these meetings were in addition to the surveys and interviews conducted by the socio–economic department of the KFRI (see output 1) and were aimed at a wider geographical area.

d. News items, publications

Several news items and 'popular publications' were produced; they are listed in appendix 9 (but also see the workshop papers in appendix 10). More traditional scientific papers, resulting from the work of the project, for referred journals, are in preparation.

e. National workshop

This was organised by KFRI and CABI Bioscience and was held at KFRI, Peechi, Thrissur, Kerala, 2-4 November, 1999. The workshop was entitled:

'Alien Weeds in Moist Tropical Zones'
Banes and Benefits

The objectives were:

- To disseminate information on the status of alien invasive weed infestation in the moist tropical forest zones of India.
- To consolidate research outputs and develop strategies for control of important weeds (particularly *M. micrantha*) in homegarden, plantation and natural forest systems..

The special focus of the workshop was *M. micrantha*. A list of speakers and papers presented are given in appendix 10. Participants included representatives of:

Kerala Forest Research Institute
Science, Technology and Environment Committee, Kerala
Assam Agricultural University, Assam
Tocklai (Tea) Experimental Station, Assam
Project Directorate of Biological Control (ICAR)
Kerala Forest Department
University of Agricultural Sciences, Dharwad, Karnataka
Karnataka Forest Department
CABI Bioscience
The outputs of the workshop and major recommendations are given in appendix 11. (Also see output 7 below).

7. **Implementation phase for the biological control of *Mikania micrantha* in India formulated and agreement reached on the role/activities of current/new collaborators (from extension funding)**

a. Meeting at Project Directorate of Biological Control, (ICAR), Bangalore, Karnataka

The initial request to broaden the focus of the project to include northeastern India came from the DDG (Natural Resources), ICAR, New Delhi. Given the nature of the project, the appropriate nodal ICAR institute is the PDBC in Bangalore; PDBC is also connected with a network of ICAR institutions and agricultural universities throughout India that work with PDBC on biological control; the nodal agency in northeastern India is Assam Agricultural University (AAU). The original plan was to have a joint meeting at PDBC, also involving AAU and KFRI to discuss the work of the first phase of the project and to design an implementation phase. This was not possible because of delays in obtaining approval for the project extension and approval from ICAR, New Delhi for inclusion of PDBC/AAU in the extension. However, a one-day meeting was held at PDBC in June 1999 to discuss the issues. PDBC had already been in discussion with AAU about working jointly in the project. PDBC showed their commitment to the project by including the project in their workplan with CABI Bioscience. A full meeting of all collaborators was delayed until the end of the national workshop (see below).

b. Meeting at KFRI, Peechi, Kerala

This was convened at the end of the workshop. Participants included representatives from: KFRI, PDBC, AAU, TTES, Karnataka Forest Department and CABI Bioscience. The recommendations for the implementation of the outputs of the project (and later endorsed by the workshop participants) are:

- 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 ICAR. In particular, an application should be made for the introduction of the *M. micrantha* – specific exotic rust fungus *P. spegazzinii*.
- Further ecological and socio–economic studies on *M. micrantha* – in particular, dispersal, prediction of spread, modeling and ecological and socio–economic impact on agroforestry farming systems.
- Adaptation of the integrated weed management technology for use and implementation through AAU, TTES and Indian Council for Forestry Research and Education (ICFRE) centres in the northeastern States.

CONTRIBUTION OF OUTPUTS

a. Contribution to project goal

The project goal (forest/agriculture 2 programme purpose) is: 'sustainability and yield from tree crop based systems at the forest/agriculture interface improved through the removal of pest constraints'.

The outputs have contributed to this goal in that the project purpose has been achieved i.e. a biological control strategy for the management of *M. micrantha* has been developed. More specifically, the outputs have made the following contributions:

	Major Results	Contribution to Project Goal
1.	a. Quantification of range in W. Ghats and establishment of permanent sample plots. b. Impact of weed on tree/agricultural crops and production costs. c. Farmer support for biological control.	• Monitoring now possible and thus information on weed status in different landscape types easily summarized for state/national government policy makers. • Major target areas in W. Ghats for management identified. • Generated co-ordinated support for action across state and national research organisations and provided important linkages. • No major conflicts of interest in major beneficiary group identified.
2.& 3.	a. High biological control potential of exotic fungal pathogen, *Puccinia spegazinii* b. Low biological control potential of native fungal pathogen.	• Classical biological control of *M. micrantha* over a wide area now a realistic option for the status in Southwest and Northeast India. Also possibility for augmenting the fungus in large tea plantations. • General acceptance in India that biological control through use of local agents is not feasible. This is an important step as government administrators need this information before backing the introduction of an exotic agent.
4.	a. Complete host specificity of *Puccinia spegazzinii*. b. Import/release protocols developed.	• A major criteria of the Ministry of Agriculture, Directorate of Plant Quarantine is that exotic agents are host specific. *Puccinia spegazzinii* meets this criteria. • There is no written protocol for the introduction of biological control agents into India, particularly pathogens (although this is now being revised and the CPP Mikania project is contributing to the process). Key issues for GoI are host specificity, broad support from relevant institutions and demonstration of lack of potential of local agents. All of these have been achieved. In particular support for the introduction of *Puccinia spegazzinii* is coming from ICAR, KFRI, AAU and Tocklai (Tea) Experimental Station and the farmers.
5.	a. KFRI staff trained in weed ecology, socio–economics, weed pathology; ICAR staff also trained in last of these (in extension).	• Human resource capability for handling exotic pathogens established.
6.	a. Factsheet, local news letter items; discussions at key research organisations; meetings with farmers and extension workers; workshop.	• Publicised the project widely in Southwest and Northeast India. • Provided broad consensus on support for biological control.
7.	a. Meeting and workshop outputs supporting immediate implementation phase for biological control of *M. micrantha*	• Commitment of India to implement outputs of first phase project. Components of implementation identified.

b. Promotion pathways to target institutions and beneficiaries

A strong connection has already been established with the target institutions in India (KFRI, PDBC, AAU and TTES as they are collaborators in the project. Once a permit has been issued for the introduction of the exotic rust fungus by the Ministry of Agriculture's DPP Q&S, the rust fungus can be imported through one of ICAR's quarantine stations (New Delhi or Bangalore). The fungus will then be available to the Indian collaborating institutions.

Once the fungus has been released from quarantine, the collaborating institutions will be able to arrange validation trials in representative homegardens, plantations and natural forest systems. Farmer support for this has been established (see output 1.)

c. Follow-up action/research

The concept of an implementation phase for the management of *M. micrantha* has been agreed by the Indian collaborators (see output 7). The major components of this implementation phase are as follows:

- As *M. micrantha* is still in the invasive phase in the Western Ghats, and now threatens Karnataka, further ecological and socio–economic studies are required to understand dispersal, and to predict spread. Further studies are also required to understand why some homestead farms in the centre of the range of the weed in Kerala, apparently have no infestation.
- The result of the application to the DPP Q&S, for the introduction of the exotic rust fungus, *Puccinia spegazzinii*, will need follow-up. This could involve further testing of additional Indian crop plants against *P. spegazzinii*.
- Validation trials for *P. spegazzinii* in homegardens, plantations and natural forests of Kerala will need to be designed. The integration of the use of the fungus with herbicides will also need to be considered for some plantation areas.
- The integrated weed management technology, developed for the Western Ghats, will need to be adapted for use and implementation in northeastern India; this will be done through AAU, TTES and ICFRE.

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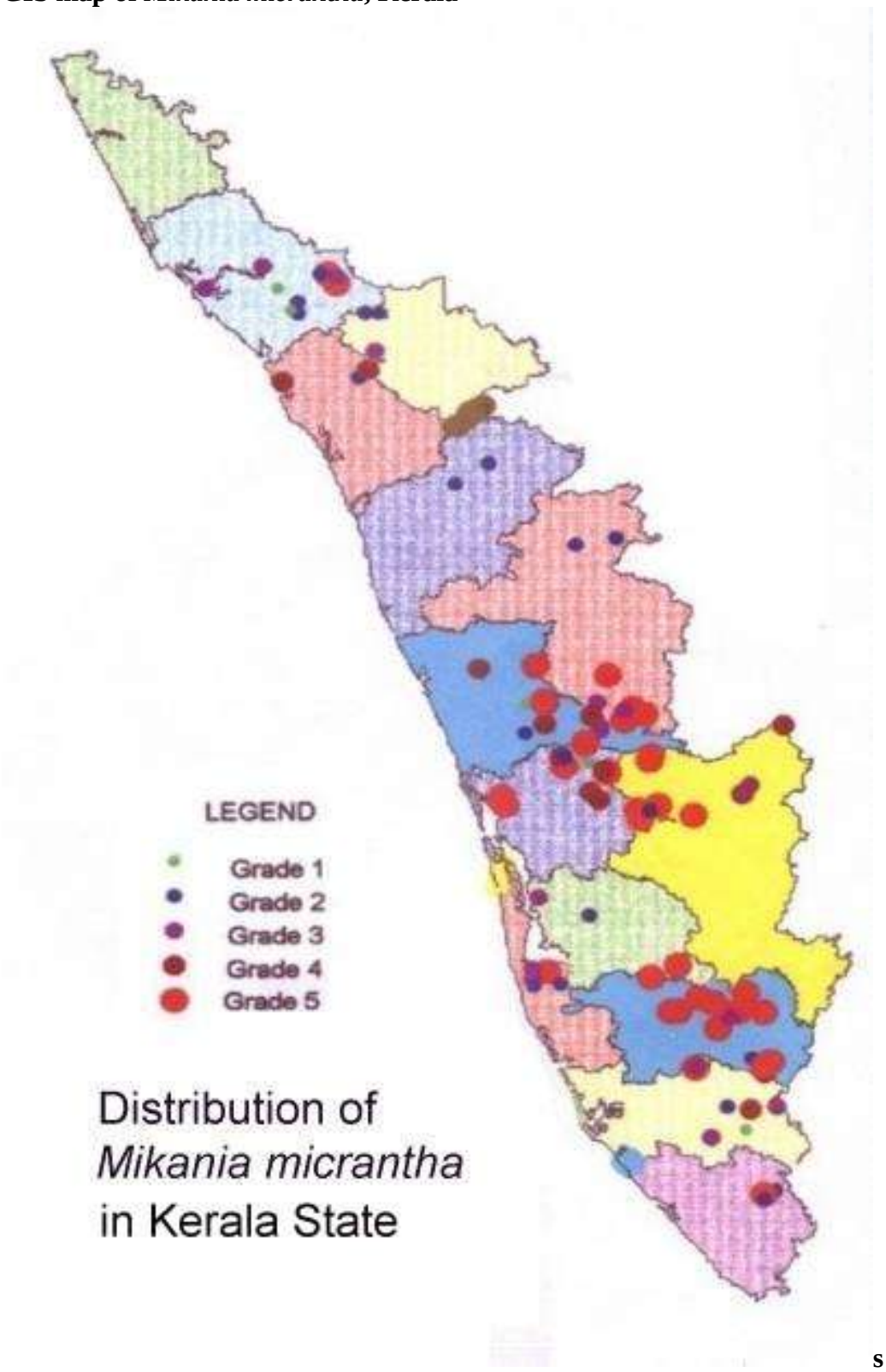
Appendix 1.
Origin of *Mikania* spp. populations in CABI Bioscience collection

Site Details (with 'identifying name')	Table 2 Ref. No.
Mikania micrantha	
BRAZIL 'Prados' Victoriano-Velosa, Prados Rd., nr. Tiradentes, Minas Gerais, Brazil. (W1690)	29
'Mariana' Rio Guaxulo do Sul. (nr. Mariana), Minas Gerais, Brazil (W1692)	27
'Viçosa' San Miguel do Anta, Viçosa, Minas Gerais, Brazil (W1693)	30
'Maripó' Maripó, Minas Gerais, Brazil (W1694)	32
'Ambré Campo' Ambré Campo, Minas Gerais, Brazil. (W1696)	28
'Caparão' Alto Caparão, Parque do Caparão, Minas Gerais, Brazil (W1695)	31
ECUADOR 'Imbrabura' Lita, Imbabura Province, west Ecuador. Alt. 680m (W1958)	
'Napo' Rio Pucuño Loreto-Pangayacu Rd., Napo, east Ecuador. (W1960)	
TRINIDAD 'Blanchisseuse' Blanchisseuse, Cookertrace loubaga, Arima, Trinidad (W1735)	44?
'Parrylands' Guapo, Parrylands airfield, SW Trinidad (W1761)	45?
'Valencia' Valencia-Sangue, Grande Rd., Trinidad (W1942)	
'Mount Catherine' Mount Catherine, (Mt. Pleasant range), Chaguaramas, Trinidad (W1949)	
COSTA RICA 'Siquirres 16-1' Siquirres, 40km Costa Rica (16-1)	17
'Siquirres 17-1' Rio de Madre de Dios, Siquirres-Guapiles, Limón, Costa Rica. (W1868)	19
'Turrialba 15-1', La Suiza-Turrialba, km6.7, Costa Rica (15-1)	14
'Moravia 15-3', nr. Moravia, km16.4, Costa Rica. (15-3)	15
'Moravia 15-4' Moravia, Turrialba, Costa Rica (W1869)	16
MEXICO 'Laguna' Laguna-Escondida, Catemaco, Vera Cruz, Mexico. (W1904)	46
'Angel 1' Vera Cruz, Mexico (28/4/98) (same site as Angel 1a and Angel 2)	47, 49, 50
'Tapachula' Tapachula, Mexico (7/4/98)	48
PANAMA 'Barro Colorado' Barro Colorado Island, Panama (29-1)	42
'Bocas del Toro' Changuinola, Bocas del Toro, Panama (31-1)	43
INDIA 'Peechi' Peechi, Thrissur Distr., Kerala Prov., India. (Sankaran)	34
'Vettilappara, 3-1' Vettilappara (Kazihehal- Sean), Thrissur District, Kerala, India. 110m, in oil palm plantation. (3-1)	1
'Athirampilly, 3-2' Athirampilly, Thrissur District, Kerala, India. 210m, near waterfall (3-2)	2
'Vazhachal, 3-3' Vazhachal, Thrissur District, Kerala, India. 275m (3-3).	3
'Porimgal (Teak), 3-4' Porimgal, Thrissur District, Kerala, India. 470m, in Teak forest (3-4)	4
'Porimgal (Natural), 3-5' Porimgal, Thrissur District, Kerala, India. 400m, in natural forest. (3-5)	5
'Kuddaram 4-1' Kuddaram, Kerala, India. (4-1)	33
'Varandhara 12-1' Varandharappally, Trichur District, India (12-1)	35
'Kalady' Kalady, Ernakulam District, Kerala India (W1925)	
'Peirumidu' Peirumidu, Idukki District. Kerala, India (W1924)	
'Geleki' Geleki, Sibsagar, Jorhat District,Upper Assam, India. 129m Tea/Rice Border. (W1846)	22
'Jorhat' Mariani-Amugri, Upper Assam, Jorhat, India. 135m. Degraded Deciduous Forest (W1844)	21

Site Details (with 'identifying name')	Table 2 Ref. No.
'Garampani' Garampani, Nambar river, Gologhat Distr., Assam, India. 101m, Degraded Evergreen Forest. (W1845)	23
'Kaziranga West' Kaziranga West, Golaghat Distr., Eastern Assam, India. 42m, Baguri Range. (W1842)	26
'Kaziranga East' Kaziranga East, Golaghat Distr., Eastern Assam, India. 65m (W1843)	25
'Nameri' Nameri, Bhalukpong, Sanitpur Distr., Central Assam, India 130m Savannah. (W1847)	24
'Shillong' Borpani, Shillong, Meghalaya, India. 976m Roadside (W1848)	20
NEPAL 'Chitwan', Chitwan National Park, Terai, Southern Nepal	36
SRI LANKA 'Monaragala' Diyaluma Waterfall, Monaragala District, Sri Lanka. (13-3).	37
'Ratnapura', Ratnapura, Halpe, Sabaragamuna District, Sri Lanka (18-2)	
MALAYSIA 'Malaysia Non-Hairy' Type 1 Malaysia (16/7/98)	39
'Malaysia Hairy' Type 2 Malaysia (16/7/98)	38
PHILIPPINES 'Philippines' Lagawe, Ifugao, Nr. Luzon, Philippines. (20-1)	40
SUMATRA Buntar, Turunan, Medan, North Sumatra	
JAVA Sajira, Rangkas Bitung, West Java.	
AUSTRALIA 'Australia, Mission Beach' Mission Beach, Innisfail, North Queensland, Australia (W1922)	41
Other *Mikania* Species	
Mikania guaco ex Colombia	11
Mikania vitifolia ex Colombia	10
Mikania sp.1 (medicinal) ex Brazil	12
Mikania sp. 2 San Martin Dept., Tabalosos, Peru (W1707)	8
Mikania sp. 3 Tingo Maria, Huánuco, Peru (W1852)	7
Mikania sp. 3 Mallaqui, Huánuco, Peru (W1851)	6
Mikania sp.4 (hairy) ex Siquirres, Costa Rica (16-1)	9

Apendix 2.
GIS map of *Mikania micrantha*, Kerala



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Appendix 3.
Permanent plots surveyed to assess the severity of *Mikania micrantha* infestation

DISTRICT	DIVISION	RANGE	LOCALITY	GRADE (Initial)	GRADE (RETAINED / CHANGED	PRODUCT-ION SYSTEM
Ernakulam	Malayattur	Kodanadu	Panali section	5	5	Teak plantation
Ernakulam	Kothamangalam	Kothamangalam	Thalikulam	5	5	Teak plantation
Ernakulam	Malayattur	Kalady	Mallana	5	5	Teak plantation
Ernakulam	Malayattur	Thundathil	Edamalayar	5	5	Evergreen forest
Idukki	Munnar	Adimali	Machipilav	5	5	Teak plantation
Kannur	Kannur	Aralam	Aralam	5	5	Agricultural system
Kannur	Kannur	Thaliparamba	Valapattanam	4	4	Agricultural system
Kottayam	Kottayam	Erumely	Karimbam	5	5	Teak plantation
Palakkad	Parambikulam	Parambikulam	Kuriyarkutty	5	5	Moist deciduous forest
Palakkad	Parambikulam	Orukombil	Orukombil	5	5	Moist deciduous forest
Palakkad	Parambikulam	Sungam	Sungam	4	5	Moist deciduous forest
Pathanamthitta	Ranni	Vadasserykkara	Adukuzhi	5	5	Semi evergreen forest
Pathanamthitta	Ranni	Vadasserykkara	Chengara	5	5	Teak plantation
Pathanamthitta	Ranni	Goodrickal	Moozhiyar	5	5	Evergreen forest
Pathanamthitta	Ranni	Goodirickal	Kakki	5	5	Evergreen forest
Trichur	Trichur	Peechi	Kuthiran	5	5	Moist deciduous forest
Trichur	Trichur	Chimminy	Chimminy	5	5	Teak plantation
Trichur	Trichur	Chimminy	Chimminy	5	5	Semi evergreen forest
Trichur	Vazhachal	Athirappally	Athirappally	2	4	Evergreen forest
Trichur	Vazhachal	Charpa	Charpa	4	5	Semi evergreen forest
Trichur	Vazhachal	Charpa	Kalady plantation	5	5	Rubber plantation
Trichur	Vazhachal	Charpa	Charpa	4	5	Oilpalm plantation
Trichur	Trivandrum	Peppara	Bonakkad	2	2	Tea plantation
Trivandrum	Trivandrum	Peppara	Peppara dam	5	5	Semi evergreen forest
Trivandrum	Trivandrum	Peppara	Kuttappara	2	3	Eucalyptus plantation
Trivandrum	Trivandrum	Peppara	Bonakkad	2	0	Tea plantation

Appendix 4.
Fungal pathogens, Kerala, India

1. ***Colletotrichum*** leaf spot

Symptoms**:** Initial symptoms were minute pale brown spots which enlarge to form irregular to circular leaf spots (up to 5mm in diam) with dull white centers and dark brown margins. Usually, there will be two margins around the inner most necrotic area, the outer margin encircling (up to 1 mm in diam) a pale brown necrotic area developed around the inner margin; reverse same colour. Shot holes are commonly produced on the lamina.

Causal organism: *Collectotrichum gloeosporioides* Penz. & Sacc.

Cultural characters: Colonies on PDA light grey (7 E 2) attaining 8.1 cm. Diam in 6 da; reverse dark grey (7 F1). Mycelium floccose; hyphae light brown, septate, branched, 2.7 – 4.5 um wide. Conidiomata acervular with conidia produced on pulvinate mass of conidiophores. Setae and selerotia absent. Conidiogenous cells determinate, integrated, cylindrical, hyaline, smooth, 6.4 – 11.7 x 2 – 3.6 um. Conidia holoblastic, hyaline, aseptate, straight, smooth, thin walled, cylindrical, rounded at the ends, 12.6 – 19.8 x 3.6 – 5.4 um.

Distribution: Widespread. Leaf spotting observed throughout the year. One of the most common leaf spots observed on *M. micrantha* in Kerala.

Pathogencity: Typical symptoms of leaf spot appeared only on wound inoculated leaves after 5 days of inoculation. This shows that C. gloeosporioides is a wound pathogen. The pathogen was reisolated from the affected leaves.

Economic importance: Nil

2. ***Corynespora*** **leaf spot**

Symptoms: Leaf spots more or less circular, up to 4 mm in diam. Necrotic areas pale brown (64D), margins think, dark brown (7F4); reverse same colour. Spots coalasce to form large dark brown necrotic areas especially near the leaf margins.

Causal organism: *Corynespora casiicola* (Berk. & M.A. Curtis) C.T. Wei

Cultural characters: Colonies on PDA light grey (21D1) attaining 4.7 cm. Diam in 6 da; reverse olive grey (3F2). Odour nil. Mycelium floccose, hyphae light brown, septate, branched, 2.7-4.5 in diam. Conidiophores light brown, smooth walled, and mononematous 3.7-5.6 um thick. Conidiogenous cells monotrectic, integrated, terminal, cylindrical, 90-108 x 3.7-4.5 um. Conidia solitary or catenate, variable in shape, obclavate to cylindrical, straight curved, pale brown, smooth walled, 4-16 pseudoseptate, 100-213 x 7-13.8 um. Wide at the truncate base.

Distribution: Widespread. Leaf spotting observed throughout the year. Most common leaf spot observed in *M. micrantha* in Kerala.

Pathogencity: Typical symptoms of leaf spot appeared on all the inoculated leaves after 5 days of inoculation. Both wounded and unwounded leaves were infected. The pathogen was reisolated from the leaf spots.

Economic importance: Causes negligible damage to the plant. No leaf shedding or leaf wilting due to Corynespora infection was noticed.

3. ***Alternaria leaf spot***

Symptoms: Leaf spots circular to irregular, up to 6mm in diam, brownish (7E5), with very narrow blackish brown margins, reverse same colour. Individual spots coalesce to form large necrotic areas. Shot holes are produced.

Causal organism*: Alternaria alternatea* (Fr.) Keissler

Cultural characters: Colonies on PDA brownish grey (8F2), attaining 6.4 cm. in diam. In 6 da., reverse dark brown (7F4). Hyphae light grey, smooth walled, septate, 1.8 – 3.6 um wide. Conidiophores, greyish, smooth, micronematous, mononematous, 21.1 – 32.8 x 3.6 um. Conidiogenous cells integrated, terminal, polytrectic, cylindrical, cicatrized 4.5-6.3 x 2.7 – 3.6 um. Conidia catenate, typically obclavate, brown, verrucose, with 3-4 transverse and 1-2 oblique septa, 24.3-42.3 x 9 – 13.5 um.

Distribution: Restricted. Collected only from localities in Kerala. (Neriamangalam; Ernakulam Dt., Peria, Wynad Dt., and Kuttiadi; Calicut Dt.)

Pathogencity: Restricted. Collected only from 3 localities in Kerala. (Neriamangalam; Ernakulam Dt., Peria, Wynad Dt., and Kuttiadi; Calicut Dt.)

Economic importance: Nil

4. ***Curvularia leaf spot***

Symptoms: Circular to irregular leaf spots 1-5 mm in diam. distributed all over the lamina. Leaf spots characteristically with brownish centres (65E) and thin dark brown (6F6) margins. Reverse same colour. Shot holes are common.

Causal organism: *Curvularia lunata* (Wakker) Boedijn

Cultural characters: Colonies on PDA brownish grey (7F2) attaining 7.8 cm in diam in 6 da; reverse bluish grey (19F2). Odour nil. Mycelium adpressed, hyphae light brown, septate, branched. Primary hyphae 2.7-4.5 um wide. Secondary hyphae 18.-2.7 um wide. Conidiophores mononematous, straight, dark brown, smooth walled, 2.7-3.6 um in diam. Conidiogenous cells integrated, mostly terminal polytrectic, cylindrical, sometimes nodose, 5.4-9 x 2.7-3.6 um. Conidia light brown, solitary, acropleurogenous, simple, straight curved, predominantly three septate, middle septum not median, smooth walled, hilum not protuberant, 13.5-24.5 x 6.3 – 9.9 um.

Distribution: Sparse. Collected only from 9 localities belonging to central and southern Kerala.

Pathogencity: Symptoms of leaf spot appeared 5 days after inoculation. Only wounded leaves were infected. C. lunata can be considered as a weak would pathogen. Re-isolation of the pathogen was done from the infected leaves.

Economic importance – Nil.

5. ***Fusarium collar rot***

Symptoms: The primary symptom was the appearance of water soaked lesions at the collar region of the plant. The colour of the lesion turned to dark brown when the infection progressed and the stem appeared slightly depressed at the infected area.

Causal organism: *Fusarium solani* (Mart.) Sacc.

Cultural characters: Colonies on PDA greyish while attaining 4.5 cm in diam in 6 da., reverse brownish (6E4). Odour nil. Mycelium affused, huphae light brown, septate, branched, 2.7 – 4.5 um wide. Microconidiophores elongate, branched, hyaline, smooth walled, 3.6 – 4.5 um diam. Conidiogenous cells phialidic, cylindrical, 7.2 – 11.4 x 2.7 um. Microconidia oval to ellipsoidal hyaline, smooth walled, 5.4 – 6.3 x 2 – 3 um. Macroconidia fusoid, curved, hyaline, smooth walled, septate, 16.7 – 23.2 x 4.5 – 6.4 um.

Distribution: Very restricted. Collected from only one locality. (Thundathil, Ernakulam Dt.)

Pathogencity: Typical symptoms of collar rot appeared in wound inoculated stem within 6 days of inoculation. The unwounded plants were not infected. Wounding appears to be a pre-disposing factor for F. solani to produce collar rot.

Economic importance: Nil

6. ***Myrothecium* blight**

Symptoms: Initial leaf spots (2-3 mm diam.) were light brown centers and dark brown margins which enlarged with age to give the leaf a blighted appearance. Necrotic areas light brown, up to 2.5 cm. in diam. Numerous greenish black pustules of Myrothecium were observed on the ventral side of the blighted area of the leaf. Shot holes were common.

Causal organism: *Myrothecium leucotrichum* (Peck) Tulloch

Cultural characters: Colonies on PDA attaining 3.5 cm. diam in 6 day., mycelium whitish grey (7B1), reverse greyish orange (6 B4). Sporulating areas coalesced, wet, olivaceous black; Hyphae light grey, smooth walled, branched, septate, mostly 2.7 um diam. Conidiophores micronematous branched, closely compacted, penicillate. Conidiogenous cells phialidic, 2-4 in a whorl, closely compacted, cylindrical, clavate or ampulliform, hyaline, 12-6-14.4 x 3.6-4.5 um. Conidia rod shaped, smooth, hyaline, both ends rounded, 6.3-8.1 x 0.9 – 1.8 um.

Distribution: Very restricted. Collected only from one locality (Eloor, Ernakulam Dt).

Pathogencity: Characteristic leaf spot symptoms appeared on all leaves after 5-6 days of inoculation. Both unwounded and wounded leaves were infected. The pathogen was re-isolated from the leaf spot.

Economic importance – Nil.

7. ***Ascochyta*** leaf blight

Symptoms: Primary symptoms were small pale brown (6d6) leaf spots (up to 5mm in diam) originated anywhere on the border of the lamina. The spots enlarged to form large necrotic areas (up to 3-6 cm. in diam) with pale brown centers and blackish brown margins giving the leaf a blighted appearance.

Causal organism*: Asochyta sp.*

Cultural characters: Colonies on PDA light grey (7E1) attaining 7.3 cm. Diam in 6 da; reverse greyish (7E3). Mycelium affused, hyaline, branched, septate, 2.7 – 3.6 µm wide. Conidomata pycnidial, solitary, amphigenous, subglobose, light brown, 89.6 – 145.6 x 83 – 140 µm. Ostiole central, circular, 26.3 – 32.1 µm diam. Conidiophores absent. Conidiogenous cells phialidic, determinate, hyaline, smooth walled, cylindrical, 16.3 – 26.1 µm x 1.8 – 2.7 µm. conidia hyaline, medianly one septate, thin walled, smooth, fusiform, constricted at the septa, 12.6 – 14.9 x 4 – 6 µm.

Distribution*:* Very restricted. Collected only from one locality in Kerala. (Eloor, Ernakulam Dt.).

Pathogencity: All the leaves inoculated with the pathogen (wound-inoculated as well as unwounded) produced typical leaf spot symptoms within 4-5 days of inoculation. The pathogen was re-isolated from the spots.
Economic importance: Nil

8. ***Pestalotiopsis*** **leaf blight**

Symptoms: Leaf spots brownish orange (6 C 6) to light brown (6D4), up to 2.5 cm. In diam, margins dark brown. The necrotic area is often characterized by the presence of 4-5 thin, light brown concentric rings; reverse of leaf spots same colour.

Causal organism: *Pestalotiopsis* sp.

Cultural characters: Colonies on PDA white, attaining 6.2 cm. in diam. In 6 da; reverse light grey (7B2). Odour nil. Mycelium affused, hyphae hyaline, septate, branched 2.7 – 3.6 µm wide. Conidiophores hyaline, branched, smooth walled, septate 7.4 – 12.6 x 2 – 2.6 µm. Conidiogenous cells holoblastic, indeterminate, integrated, cylindrical, hyaline, smooth walled, 4.7 – 7.3 x 2 – 3.6 µm. Conidia fusiform, mostly 4 euseptate, straight or slightly curved, apical cell conic, hyaline, with 2 – 3 unbranched appendages, 4.6 – 9.2 µm; basal cell hyaline, truncate, median cells brownish, smooth walled, 15.3 – 22.5 x 4.5 µm.

Distribution: Very restricted. Collected only from two localities. (Thundathil; Ernakulam Dt. And Ayyappancoil; Kottayam Dt.) in Kerala.

Pathogencity: Leaf spots were observed only on wound inoculated leaves. Symptoms appeared after 5-6 days of inoculation. *Pestalotiopsis* appears to be a wound pathogen.

Economic importance – Nil

9. ***Phoma* leaf spot**

Symptoms: Leaf spots up to 3 mm in diam with minute (up to 1mm) greyish centers and dark brown margins; margins more prominent on the dorsal side. Leaf spots distributed all over the lamina.

Causal organism – *Phoma* sp.

Cultural characters: Colonies on PDA olive grey (1F2), attaining 3.3 cm. in diam in 6 da., reverse bluish grey (21F3). Odour nil. Mycelium adpressed, hyphae ash white, septate, branched, smooth walled, 2.7-4.5 µm wide. Pycnidia dark, thick walled 64.4 – 108.1 x 52.9 – 105.8 µm; ostiole, single, central, non-papillate, 25.3 – 39.1 µm diam. Conidiogenous cells absent. Conidia hyaline, aseptate, smooth walled, cylindrical, apices obtuse, 3.6-6.3 x 1.8 µm.

Distribution – Very restricted. Collected only from one locality (Eloor, Ernakulam Dt.) in Kerala.

Pathogencity – Symptoms of leaf spots appeared on all inoculated leaves (both wounded and unwounded) after 5 days of inoculation. The pathogen was re-isolated from the leaf spots.

Economic importance: Nil

Appendix 5.
Infectivity of isolates of *Puccinia spegazzinii* and *Dietelia portoricensis* to populations of *Mikania micrantha*

	Puccinia spegazzinii											*Dietelia portoricensis*	
	Brazil				Ecuador		Trinidad				Costa Rica		Mexico
Mikania micrantha Population Origin*[1]	W1690 Prados	W1692 Mariana	W1693 Viçosa	W1694 Maripó	W1960 Napo	W1958 Imbabura	W1735 Trin. I	W1761 Trin. II	W1942 Trin. III	W1949 Trin. IV	W1868t C.R. telia	W1868a C. R. aecia	W1904 M. aecia
BRAZIL													
Prados, Brazil (W1690)	**4**	**4**											
Mariana Brazil (W1692)		**4**						**1**			**0**		
Viçosa Brazil (W1693)		**4**	**4**	**4**								**0**	
Maripó Brazil*[2] (W1694)				**4**			**0**	**2**					**0**
Abre Campo, Brazil*[2] (W1696)	**4**		**4**	**4**								**2**	
Caparao Brazil (W1695)	**4**	**3/4**	**4**				**0**	**1**			**0**	**0**	
ECUADOR													
Imbabura, W. Ecuador (W1958)						**4**							
Napo, E. Ecuador (W1960)					**4**	**4**							
TRINIDAD													
Blanchisseuse, Trinidad	**3**	**3**	**2**	**2**			**4**	**4**			**0**	**0**	**0**
Parrylands,Trinidad (W1761)							**4**	**4**					
Valencia,Trinidad (W1942)									**4**				
Mount Catherine, Trinidad (W1949)										**4**			
COSTA RICA													
Siquirres 16-1, Costa Rica		**2**										**4**	
Siquirres 17-1, Costa	**2**						**0**	**0**			**4**	**4**	**4**

	Puccinia spegazzinii											*Dietelia portoricensis*	
	Brazil				Ecuador		Trinidad				Costa Rica		Mexico
Mikania micrantha Population Origin*[1]	W1690 Prados	W1692 Mariana	W1693 Viçosa	W1694 Maripó	W1960 Napo	W1958 Imbabura	W1735 Trin. I	W1761 Trin. II	W1942 Trin. III	W1949 Trin. IV	W1868t C.R. telia	W1868a C. R. aecia	W1904 M. aecia
Rica (W1868)													
Turrialba 15-1, Costa Rica											4		
Moravia 15-3, Costa Rica												4	
Moravia 15-4, Costa Rica (W1869)											4	4	
MEXICO													
Laguna, Vera Cruz, Mexico (W1904)							0	0			0	4	4
Vera Cruz, Mexico (Angel 1 28/4/98)	4		4				2/3	0			4	0	4
Tapachula, Mexico	4							2			4	4	4
PANAMA													
Barro Colorado, Panama							1 / 2				4	4	3
Bocas del Toro, Panama (31-1)	3		2				1 / 2	2			4	2	2
INDIA													
Peechi, Kerala India	4	4	4	4		4	4	4		4	4	4	4
Vettilappara, Kerala. India. (3-1)	4	4	4	4			4	4			4	4	4
Athirampilly, Kerala, India. (3-2)	3	1	1	2			4	4		4	4	0	1
Vazhachal, Kerala, India. (3-3)	4	1	3/4	2			4	4		4			
Porimgal (Teak), Kerala, India. (3-4)	3	1	1	2			4	4			4		
Porimgal (Natural), Kerala, India. (3-5)	3	0	1	2			4	4			4		
Kuddaram, Kerala, India. (4-1)	4	4	4	4	4	4	4	4	4	4	4	1/2	4
Varandhara, Kerala,							4	4					

	Puccinia spegazzinii											*Dietelia portoricensis*	
	Brazil				Ecuador		Trinidad				Costa Rica		Mexico
Mikania micrantha Population Origin*[1]	W1690 Prados	W1692 Mariana	W1693 Viçosa	W1694 Maripó	W1960 Napo	W1958 Imbabura	W1735 Trin. I	W1761 Trin. II	W1942 Trin. III	W1949 Trin. IV	W1868t C.R. telia	W1868a C. R. aecia	W1904 M. aecia
India. (12-1)													
Kalady, Kerala, India (W1925)								4					
Peirumidu, Idukki, Kerala India (W1924)		4					4	4	4				4
Geleki, Assam, India (W1846)							4	4					
Jorhat, Assam, India (W1844)							4	4					
Garampani, Assam, India (W1845)	4		4/3				2	3/2		2	2		4
Kaziranga West, Assam India(W1842)	3	2	3				3	3/4	3	3	3	4	4
Kaziranga East, Assam India(W1843)	3	3	3				2/3	3		4/3	3	4	4
Nameri, Assam, India (W1847)								4					
Shillong, Meghalaya, India(W1848)								4					
NEPAL													
Chitwan, Nepal	4	4	4	4			4	4		4	4	4	4
SRI LANKA													
Monaragala, (13-3) Sri Lanka								4					
Ratnapura, Sri Lanka (18-2)								4					
MALAYSIA													
Type 1 hairless Malaysia	2/3	1						4		4	4		4/2 ?
Type 2 hairy Malaysia	4							4					2
PHILIPPINES													
Lagawe (20-1) Philippines	4							4					

	Puccinia spegazzinii											*Dietelia portoricensis*	
	Brazil				**Ecuador**		**Trinidad**				**Costa Rica**		**Mexico**
***Mikania micrantha* Population Origin*[1]**	**W1690 Prados**	**W1692 Mariana**	**W1693 Viçosa**	**W1694 Maripó**	**W1960 Napo**	**W1958 Imbabura**	**W1735 Trin. I**	**W1761 Trin. II**	**W1942 Trin. III**	**W1949 Trin. IV**	**W1868t C.R. telia**	**W1868a C. R. aecia**	**W1904 M. aecia**
SUMATRA													
Buntar, Sumatra								**4**					
JAVA													
Sajira, Java								**4**					
AUSTRALIA													
Mission Beech, Australia(W1922)	**3/4**	**4**	**4**	**3**			**4/3**	**4/3**			**4**	**0**	**3**

*[1] See Appendix 1 for full site details
*[2] Abre Campo and Maripó sites are very close.
? Unclear result, needs confirmation
Blank indicates not tested

Pathogenicity scores for the evaluation of *Puccinia spegazzinii* and *Dietelia portoricensis*
Teliospores of *Puccinia spegazzinii*
0 No macroscopic symptoms
1 Necrotic 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 with low teliospore production in relation to compatible-host pathogen interaction.
4 Normal pustule formation, in relation to compatible-host pathogen interaction.

Aecia of *Dietelia portoricensis*
0 No macroscopic symptoms
1 Necrotic spots on inoculated leaves - no sporulation
2 Abnormal infection site: very low aecial formation
3 Abnormal infection site: infection site reduced in size with low aecial production in relation to compatible-host pathogen interaction.
4 Normal aecial formation, in relation to compatible-host pathogen interaction.

Appendix 6.
Host specificity screening of *Puccinia spegazzinii* (isolate W1761), rust pathogen of *Mikania micrantha*

Plant Species	Macroscopic Symptoms	Microscopic Analysis of Leaf Undertaken*[1]
Subfamily I: Lactucoideae		
Tribe: Lactuceae		
***Lactuca sativa* (lettuce)**		
"All the year round"	7	4
"Unrivalled"	7	-
Mutisieae		
***Gerbera hybrida* "Jamesonii"**	7	4
Arctotideae		
***Arctotis hybrida* "Harlequin"**	7	-
Gazania hybride	7	4
Cynareae		
***Carthamus tinctorus* "goldtuft"**	7	-
***Cynara cardunculus* (globe artichoke)**	7	-
Vernonieae		
Stokesia laevis	7	-
Vernonia novaboracensis	7	-
Eupatorieae (Asteroideae)		
***Ageratina riparia* (mist flower)**	7	4
***Ageratum* F1 Hybrid "Adriatic"**	7	4
Chromolaena odorata	7	4
Eupatorium Canabum	**4 chlorotic spots**	4
Liatris pycnostachya	7	4
Mikania guaco	7	4
Mikania vitifolia	7	-
***Mikania* sp. 1 (Medicinal)**	7	-
***Mikania* sp. 2**	**4 necrotic spots**	4
***Mikania* sp. 3**	**4 necrotic spots**	4
***Mikania* sp. 4**	7	-
***Stevia rebaudiana* (Stepa®)**	7	4
Subfamily II: Asteroideae		
Tribe: Senicioneae		
Senecio cineraria *"silverdust"*	7	-
Heliantheae		
Helianthus annuus	**4Chlorotic spots**	4
Parthenium hysterophorus	7	-
Inuleae		
Inula ensifolia	7	-
Anthemideae		
***Chrysanthemum carinatum* "court jesters"**	7	-
Pyrethrum roseum	7	4
Calenduleae		
Calendula officinalis	7	4
Asteraceae		
Aster alpinus	7	4

Plant Species	Macroscopic Symptoms	Microscopic Analysis of Leaf Undertaken*[1]
Selected Crop Species		
Arachis hypogaea **(ground nut)**	7	-
Brassica juncea **(Mustard)**	7	-
Camellia sinensis **(Tea)**	7	-
Cajanus cajan **(Arhar / Pigeon pea)**	7	-
Cicer arietinum **(Chick Pea / Gram)**	7	-
Cocos nucifera **(Coconut)**	7	-
Coffea arabica **(Coffee)**	7	4
Elaeis olifera **(Oil Palm)**	7	-
Eucalyptus bridgesiana	7	-
Eucalyptus cypelocarpa	7	-
Glycine max **(soybean)**	7	-
Hevea brasiliensis **(Rubber)**	7	-
Lens esculenta **(lentil)**	7	-
Linum usitatissimum **(linseed)**	7	-
Lycopersicon esculentum **(Tomato)**	7	-
Manihot esculentus **(Cassava)**	7	-
Oryza sativa **(Rice)**	7	-
Phaseolus aureus **(moong bean)**	7	-
Phaseolus vulgaris **(Fabae bean)**	7	-
Pisum sativum **(Pea)**	7	-
Raphanus sativus **(Radish)**	7	-
Solanum melongena **(Brinjal)**	7	-
Sorghum bicolor **(Sorghum)**	7	-
Tectona grandis **(Teak)**	7	-
Theobroma cacao **(cocoa)**	7	-
Zea mays **(maize)**	7	-

*[1] See Table 21 for results

Appendix 7.
***Mikania micrantha* alert sheet.**

Appendix 8.
Agricultural/Forestry research and extension institutions visited by CABI Bioscience staff during the course of the project

Kerala Forest Research Institute
Kerala Agricultural University
Kerala Forest Department – field staff

Karnataka Forest Department, Bangalore
Karnataka Forest Department - field staff, Madikere Circle
- field staff, Mysore Circle

Project Directorate of Biological Control (ICAR), Bangalore, Karnataka

National Centre of Weed Science (ICAR), Jabalpur, M.P.

Assam Agricultural University, Jorhat, Assam
AAU - extension service
Tocklai (Tea) Experimental Station, Jorhat, Assam
Assam Forest Department, Guwahati
ICAR Northeastern Complex, Shillong, Meghalaya
ICAR, New Delhi - ADG, Plant Protection
- DDG, Natural Resources
Tea estates, Assam
Agroforestry farmers, Assam and Meghalaya

Appendix 9.
News items and other publications produced during the project (also see appendix 7).

Anon. (1998). IIBC collaborates with KFRI for *M. micrantha* research. *Evergreen No. 40 (March)*. p 13. Kerala Forest Research Institute, Peechi, Kerala, India. (Newsletter article).

Cock, M.J.W., Ellison, C.A., Evans, H.C. and Ooi, P.A.C. (in press). Can failure be turned into success for biological control of mile-a-minute weed (*M. micrantha*)? In: *Proceedings of X International Symposium on Biological control of Weeds.* Bozeman, Montana, USA. 4-14 July 1999.

International Institute of Biological Control (1997). Invasive Weed Alert! *M. micrantha* or Mile-a-minute Weed. IIBC, Ascot, Berkshire, UK (Factsheet).

Murphy, S.T. (1999). Ecology and management of the climber, *M. micrantha*. Malaysia Agricultural Research Development Institute, Kuala, Lumpur Malaysia. 8 February. (Seminar).

Murphy, S.T. and Evans, H.C. (1997). Combating the increasing menace of alien weeds in India. *Biocontrol New & Information No. 18.* Pp 67-68. CABI Bioscience UK Centre (Ascot)Berkshire, UK. (Newsletter article).

Reid, A. and Murphy, S.T. (1999). Biogeographic fine-tuning helps weed biocontrol. *Biocontrol News & Information No. 20.* Pp 51-54. CABI Bioscience UK Centre (Ascot), Berkshire, UK. (Newsletter article).

Sankaran, K.V. (1988). Control of mile-a-minute weed. *Evergreen No. 40 (March)*. p 14. Kerala Forest Research Institute, Peechi, Kerala, India. (Newsletter article).

Appendix 10.
List of speakers (first author) and papers presented at the project national workshop, held at KFRI, Peechi, Kerala, 2 – 4 November 1999

S.T. Murphy (CABI Bioscience, UK Centre (Ascot), Silwood Park, Berkshire, SL5 7TA, UK.). Alien weed ecology, population characteristics and implications for management.

H.C. Evans (CABI Bioscience, UK Centre (Ascot), Silwood Park, Berkshire, SL5 7TA, UK.). Classical biological control: A tailor-made strategy for the management of alien weeds.

M.B. Doddamani., U.V. Mummigatt., B.S. Nadagoudar and M.B. Chetti (University of Agricultural Sciences, Dharwad-580 005, Karnataka.). *Chromolaena* in Karnataka: Problems and prospects.

Maya V. Mahajan and P.A. Azeez (Environmental Impact Assessment Division, Salim Ali Centre for Ornithology and Natural History, Anaikatty P.O.; Coimbatore-641 108, Tamil Nadu.). Distribution of selected exotic weeds in Nilgiri Biosphere Reserve.

U.K. Hazarika and N.P. Singh (Division of Agronomy, ICAR Research Complex for N.E.H. Region, Umiam-793, Meghalaya.). Ecological distribution of weed flora in Meghalaya: Their density, intensity and infestation status.

U.K. Chandrashekara (Kerala Forest Research Institute, Peechi-680 653, Kerala.). *Lantana camara* in Chinnar Wildlife Sanctuary, Kerala, India

M. Muni Reddy (Karnataka Forest Department, Kodagu Circle, Madikeri-571 201, Karnataka.). *Lantana* infestation in Karnataka – An overview.

K.V. Sankaran and M.A. Sreenivasan (Kerala Forest Research Institute, Peechi-680 653, Kerala.). Status of *M. micrantha* infestation in the Western Ghats.

A.K. Gogoi (Assam Agricultual University, Jorhat-785 013, Assam.). Status of *M. micrantha* infestation in Northeast India: Management and future research thrust.

P.K. Muraleedraran and V. Anitha (Kerala Forest Research Institute, Peechi-680 653, Kerala.). The economic impact of *M. micrantha* on production systems in the Western Ghats of Kerala.

Madhu Verma and Nishita Bakshi (Forest Resource Economics & Management, Indian Institute of Forest Management, Nehru Nagar, P.B. No. 357, Bhopal-462 003, Madhya Pradesh.). Economics of alien weeds invasion: Effect on forest ecosystems.

M. Balasundaran (Kerala Forest Research Institute, Peechi-680 653, Kerala.). Alien invasive weeds: A few benefits amidst banes.

Sushilkumar (National Research Centre for Weed Science, Maharajpur, Jabalpur-482 004, Madhya Pradesh.). Biological control of *Lantana* in India: Trend, prospects and need of integrated approach.

A.C. Barbora (Tocklai Experimental Station, TRA, Jorhat-785 008, Assam.). Weed control in tea plantations: Current scenario in northeast India.

M.H. Swaminath (Forest Research and Training Institute, Malleswaram-560 003, Bangalore, Karnataka.). Management strategies of *Chromolaena odorata* in the Western Ghats forests.

A. Naseema and S. Balakrishnan (Department of Plant Pathology, College ofAgriculture, Vellayani-695 522, Kerala.) Bioherbicidal potential of fungi pathogenic to water hyacinth.

M.A. Sreenivasan and K.V. Sankaran (Kerala Forest Research Institute, Peechi-680 653, Kerala.). Management of *M. micrantha* in Kerala – The potential of biological and chemical methods.

Carol. A. Ellison ((CABI Bioscience, UK Centre (Ascot), Silwood Park, Berkshire, SL5 7TA, UK.). Classical biological control of *M. micrantha*.

M.B. Doddamani., U.V. Mummigatti,. B.S. Nadagoudar and M.B. Chetti (University of Agricultural Sciences, Dharwas-580 005, Karnataka.). Influence of chemical and conventional methods of weed control on *Chromolaena odorata*.

S.J. Hiremath,. M.B. Chetti,. H.Y. Patil,. U.V. Mummigatti and V.H. Ashwathama (University of Agricultural Sciences, Dharwad-580 005, Karnataka.). Influence of various botanical agents on physiological and biochemical parameters in *Chromolaena odorata* (L.) K & R.

M.B. Chetti,. S.M. Hiremath,. S.K. Prashanthi,. U.V. Mummigatti and Sreekant Kulkarni (Department of Crop Physiology, University of Agricultural Sciences, Dharwad-580 005, Karnataka.). Survey and screening of various pathogens inducing diseases to control *Chromolaena odorata* (L.) K. & R.

Sushilkumar and V.N. Saraswat (National Research Centre for Weed Science Maharajpur, Jabalpur-482 004, Madhya Pradesh.). Integrated management: The only solution to suppress *Parthenium hysterophorus*.

Appendix 11.
Workshop on alien weeds in moist tropical zones: banes and benefits. 2 – 4 November 1999. Kerala Forest Research Institute, Peechi-680 653, India

Workshop on 'alien Weeds' recognized the following:

1. The major alien invasive plants in the moist tropical zones are *M. micrantha*, *Chromolaena* and *Lantana*. These are characterized by having a wide distribution in several ecosystems and therefore to be classified as serious weeds and in need of management: *M. micrantha*, in particular continues to spread to new regions.
2. Overall, some impacts of these weeds on production systems, communities and biodiversity have been quantified and shown to outweigh any benefits stemming from potential uses of the weeds as these are not proven to be economically viable. However, uses of weeds could be incorporated into an integrated management plan.
3. No natural means of control of these weeds have been identified; in particular, indigenous natural enemies have not been found to have any significant impact on weed growth.
4. The regional nature of the weeds emphasize the need for integrated management strategies to be developed with appropriate action that is tailored to different landscapes and habitats; as many of these landscapes are rural, integrated weed management should be based on low cost and environmentally compatible methods of control – in particular biological control. Such methods require low inputs and would thereby improve the sustainability of the livelihoods of those dependent on production systems affected by the weeds.
5. In view of the limited, or non-availability, of local natural enemies, and the socio–economic costs of alternative control strategies, a new emphasis needs to be placed on introducing host-specific natural enemies for the management of these weeds. Such an approach needs to be implemented using Govt. of India guidelines and through project Directorate of Biological Control, Bangalore.

The following recommendations are made for activities to be pursued by the relevant Indian institutions and their collaborators:

Mikania micrantha

1. 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 ICAR. In particular, an application should be made for the introduction of the *M. micrantha* – specific exotic rust fungus *Puccinia spegazzinii*.
2. Further ecological and socio–economic studies on *M. micrantha* – in particular, dispersal, predication of spread, modeling and ecological and socio–economic impact on agroforestry, plantations and farming systems.
3. Adaptation of the integrated weed management technology for use and implementation through Assam Agricultural University, Tocklai Experimental Station and ICFRE in the northeastern States.

Chromolaena odorata and *Lantana camara*

1. Quantification of the ecological and socio–economic impact of the weeds in different land use systems and communities.
2. Characterization of weed bio-types and elucidation of weed ecology particularly dispersal, colonization and competitive ability in order to facilitate appropriate measures to restrict the expansion of the range of the weeds.
3. Identification and assessment of effective local (Indian) biological control agents for re-distribution within India.
4. A need was identified for the introduction of further classical biological control agents to complement local agents.
5. Cultural control and other proven local technologies should be implemented and integrated with biological control in order to provide a sustainable socio–economic basis for weed management.