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Exotic rust fungus to manage the invasive mile-a-minute weed in India: Pre-release evaluation and status of establishment in the field

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ABSTRACT

The mile-a-minute weed, *Mikania micrantha*, is a highly problematic and widespread invasive weed in the moist forests of the Western Ghats and in the north-eastern states in India causing significant damage to natural forests as well as to plantation crops, including tea, coffee, bamboo, coconut and teak. The microcyclic rust fungus, *Puccinia spegazzinii*, was identified as a potential classical biological control agent to replace the unsustainable or even hazardous conventional control methods. Following a successful risk analysis under quarantine at CABI (UK), a pathotype of the fungus (IMI 393067) from Trinidad and Tobago was imported into India. Prior to its release in the open field, the rust was further evaluated under strict quarantine conditions to ascertain the susceptibility of *M. micrantha* populations from three regions in India where the weed is invasive, and to confirm the safety of economically important plant species and indigenous flora. Results of host-specificity screening of 90 plant species belonging to 32 families ensured that the Trinidadian pathotype of *P. spegazzinii* was highly host-specific and could not infect any of the test plant species, though it was highly pathogenic to most of the target weed populations from Assam, Kerala and the Andaman and Nicobar Islands. The rust was released in Assam and Kerala but failed to establish at the time. However, due to the apparent success of this rust at controlling *M. micrantha* in the Pacific region, further releases in India are recommended.

Key words: Classical biological control, host-specificity, *Mikania micrantha*, *Puccinia spegazzinii*

The mile-a-minute weed, *Mikania micrantha* H.B.K. (Asteraceae), is a Neotropical invasive plant that smothers native vegetation in the tropical moist agroforests and natural forests of India, especially in Kerala and in the north-eastern states. The weed has also become a destructive factor in plantation crops causing significant damages to tea, coffee, banana, bamboo, coconut and teak in the areas with high soil moisture. It is also of major concern in the Andaman and Nicobar Islands, and in the eastern states of Odisha and West Bengal. Contemporary control methods which involve weeding and use of herbicides are laborious, expensive, unsustainable and hazardous to the environment. Also, in moist deciduous forests, the infestation by *M. micrantha* is on the increase, which makes harvesting by tribals of reeds, bamboo and other non-wood forest products difficult, affecting their livelihood (Sankaran *et al*. 2001). In north-eastern India, the main impact is on tea production, where unusually this crop is grown at low altitudes, within the invasive range of the weed.

Socio-economic studies conducted on home-garden farming systems in the Western Ghats region showed that *M. micrantha* has an impact on production costs and income of all sizes of holdings (Sankaran *et al*. 2001). In general, weeds form the greatest constraint to cultivation, and *M. micrantha* accounted for 10-20% of the total weeding costs.

M. micrantha is also a serious issue in several other countries, especially in the Asia-Pacific region (Waterhouse 1994). The first classical biological control (CBC) attempt for this weed was through the release of *Liothrips mikaniae* (Priesner) (Thysanoptera: Phlaeothripidae) in the Solomon Islands in 1988 and later in Malaysia in 1990. Unfortunately, neither release led to establishment most likely because of predation by ants of the nymphal stage of the agent (Cock *et al*. 2000).

During 1996-2000, an international collaborative project was funded by the UK Department for International Development (DFID) to investigate the

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CBC potential of fungal pathogens against *M. micrantha* in India and a microcyclic rust fungus *Puccinia spegazzinii* de Toni (Evans and Ellison 2005) was identified as a potential CBC agent (Murphy *et al.* 2000). CABI screened 11 pathotypes of *P. spegazzinii* from different origins and IMI 393067 from Trinidad was found to be promising against the Indian populations of *M. micrantha* (Ellison *et al.* 2004). After initial screening of 60 plant species, including 11 *Mikania* spp. from Brazil, Colombia, Costa Rica, Peru, Ghana, South Africa and the one Asian native species from Taiwan (not present in India), the Trinidadian pathotype was found to be specific to a very limited number of species within the genus *Mikania* (Ellison *et al.* 2008). A dossier (Ellison and Murphy 2001) on this risk assessment was submitted to the Government of India to obtain permission for introduction of the rust into India.

P. spegazzinii (pathotype IMI 393067) was imported into India in the implementation phase of the project, which was a collaborative venture between CABI (UK) and four institutes in India: Project Directorate of Biological Control (now NBAIR) and National Bureau of Plant Genetic Resources (NBPGR, New Delhi); Kerala Forest Research Institute (KFRI, Peechi); and Assam Agricultural University (AAU, Jorhat). Since *P. spegazzinii* was not known to occur in India (CABI 2006), before the rust inoculum was released in the open field, it was further evaluated under strict quarantine conditions to ensure the safety of indigenous flora against the introduced fungus (Ellison *et al.* 2008).

MATERIALS AND METHODS

Importation, establishment and maintenance of rust in quarantine

M. micrantha plants (Peechi, Kerala, population) inoculated at CABI with the Trinidadian pathotype (IMI 393067) of *P. spegazzinii* were imported into India (vide Permit No. 33/2004 in PQ Form 13; date of issue: 3 August 2004) on 2 September 2004 and established under strict quarantine conditions in the CL-4 level National Containment-cum-Quarantine Facility (NCQF) for Transgenic Planting Material of NBPGR in New Delhi. The pathogen was passed several times through *M. micrantha* (Thrissur, Kerala, population), established, and maintained without hyperparasites by re-inoculation, approximately every six weeks, onto fresh plants which were given a standard 24-hour dew period. All the used-up inoculum, as well as the inoculated test plants, was autoclaved and incinerated after the mandatory experimental period. Other used materials such as protective clothing, etc. were also destroyed in a similar manner. Used soil was autoclaved before discarding.

Target weed

Populations of *M. micrantha* in the form of young seedlings or cuttings were obtained from three different regions in India (Table 1). These comprised five populations collected from Chimmoni, Peechi, Shoranur, Thrissur and Vazhachal representing two districts of Kerala; populations collected from 15 locations of 13 districts of Assam; and 10 populations collected from Andaman and Nicobar Islands, representing North and Middle Andaman, and South Andaman districts. These planting materials were potted and regularly propagated through cuttings in NCQF. The plants were maintained at 18±1 °C for multiplying the rust inoculum. Imidacloprid (0.004%) (Confidor 200SL, Bayer) was used occasionally to eliminate sucking pests (mostly red spider mites).

Test plant species

Selection of plants for the host-specificity screening of the rust was based on the taxonomic classification of the target weed in relation to the centrifugal phylogenetic testing sequence proposed by Wapshere (1974) and also included plants of economic importance in the potential areas of release of the rust, in accordance with the International Plant Protection Convention guidelines (FAO 1996), so as to allay concerns of decision-makers and the general public.

Based on the mode of propagation, seeds, seedlings, cuttings or other vegetative propagules of 90 plant species belonging to 32 families (Tables 2, 3 and 4) were collected or procured from different parts of India and other sources like National Gene Bank, NBPGR, National Seeds Corporation Limited, and some seed companies. The list included 35 plant species representing 13 out of the 17 tribes existing in Asteraceae (Katinas 2005). Details of the 28 cultivars/ accessions of sunflower tested for host-specificity of the rust are given in Table 3. All the plants were potted in autoclavable plastic pots, containing garden soil mixed with both organic manure and inorganic fertilizers, and seedlings were maintained for rust inoculations. Some of the important plant species screened earlier at CABI (UK) were also included for screening. All the plant collections were maintained under available natural light conditions inside designated bays at 18±1 ºC till inoculations. Plants were watered at least once a day. Adequate care was taken to ensure that the plants did not harbour any insect or mite pests.

Rust inoculation and assessment of pathogenicity

The inoculation procedure prescribed by Ellison *et al.* (2004) with due modification (Sreerama Kumar and Rabindra 2005) was adopted. Young, healthy seedlings of test plants were sprayed with distilled water and kept in the dew chamber (Fig. 1). *P. spegazzinii* produces basidiospores under high humidity conditions from cushions of teliospores that are embedded in the plant tissue. The teliospores are not released, and hence, the inoculum used in the experimental work was composed of infected leaf, petiole and/or stem. The inoculum was spread over the grill-like tray just above the plants to be inoculated in such a manner that the pustules (for example, on the lower surface of the leaves) faced the healthy plants kept on the lower rack. A 48-hour dew period at 20 °C and 100% RH was used to ensure every opportunity for test plant to pick up the infection. *M. micrantha* plants from Peechi were used as control. The teliospores at high humidity produced basidiospores (Fig. 2) which were ejected from the teliospores and fell on the young seedlings below. Four seedlings/saplings of each test plant were inoculated and each experiment was repeated twice. For some plant species, populations from more than one location were tested. Symptoms were recorded as described by Ellison *et al*. (2004) on the pathogenicity score (PS) from 0-4 (0 = no macroscopic symptoms; 1 = necrotic or chlorotic spots on inoculated vegetative parts - no sporulation; 2 = abnormal infection site: chlorotic patches on vegetative parts 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). All the inoculated test plants were kept under observation for six weeks (double the time taken by the weed to express full symptoms) and thereafter, autoclaved and incinerated. The rust inoculum was maintained on *M. micrantha* plants by regular inoculations.

RESULTS AND DISCUSSION

First visible symptoms of rust appeared 5-7 days after inoculations as chlorotic spots on leaves, petiole and stem (Fig. 3). These spots further enlarged and within 12-15 days developed into cinnamon-coloured telia with teliospores embedded in sori (Figs 4a and 4b).

M. micrantha plants from Assam inoculated with the rust fungus showed a remarkable variability in the susceptibility towards the pathogen (Table 1). Weed populations from districts Barpeta, Cachar, Darrang, Kokrajhar, Lakhimpur and Nalbari were found to be highly susceptible (PS 4), while those from Sivasagar and Sonitpur exhibited medium susceptibility (PS 3-4). Weed populations from Dhemaji and Nagaon showed medium susceptibility (PS 3). *M. micrantha* populations from Karbi Anglong and Tinsukia showed resistance to the fungus with slight infection (PS 2). Three populations from Jorhat district showed mixed susceptibility with

Table 1. Susceptibility of *Mikania micrantha* populations from different regions in India to *Puccinia spegazzinii* (Trinidadian pathotype)

State/ union territory	Place of collection/ population (district)	Total no. of plants inoculated	Mean pathogenicity score
Assam	Diphu (Karbi Anglong)	9	2
	Gelapukhuri (Tinsukia)	8	2
	Jorhat (Jorhat)	20	3-4
	Kokrajhar (Kokrajhar)	17	4
	Nagajanka (Jorhat)	8	2
	North Lakhimpur (Lakhimpur)	8	4
	Orang (Darrang)	8	4
	Pathsala (Barpeta)	12	4
	Sepon (Sivasagar)	8	3-4
	Silapathar (Dhemaji)	12	3
	Silchar (Cachar)	8	4
	Silongoni (Nagaon)	12	3
	Tezpur (Sonitpur)	8	3-4
	Titabar (Jorhat)	28	4
	Tihu (Nalbari)	9	4
Kerala	Chimmoni (Thrissur)	25	4
	Peechi (Thrissur)	> 100	4
	Shoranur (Palakkad)	> 100	4
	Thrissur (Thrissur)	> 100	4
	Vazhachal (Thrissur)	25	4
Andaman and Nicobar Islands	Central Agricultural Research Institute (CARI), Garacharma, Port Blair (South Andaman)	31	4
	Garacharma village, Port Blair (South Andaman)	14	3-4
	Hut Bay, Little Andaman (South Andaman)	8	4
	Kalpong Hydroelectric Project (KHEP) Junction (North and Middle Andaman)	23	3-4
	Kalpong (North and Middle Andaman)	9	3-4
	Keralapuram (North and Middle Andaman)	8	4
	Mount Harriet National Park (South Andaman)	9	4
	Nayashahar (South Andaman)	10	4
	Radhanagar, Havelock Island (South Andaman)	24	3-4
	Not labelled by the collector	10	4

the collection from Titabar showing PS 4 and that from Nagajanka showing rust symptoms with PS 2. The third one from Jorhat had a score of 3-4. The results of screening of *M. micrantha* populations from 13 districts of Assam suggest that most of the weed populations in the North, South and West

Table 2. Asteraceae inoculated with *Puccinia spegazzinii* (Trinidadian pathotype) for host-specificity screening

Tribe	Scientific name	Common name	Cultivar	Source/ place of collection[@]
Anthemideae	*Artemisia annua* L.	Sweet sagewort/ sweet wormwort	EC-202429	NBPGR Regional Station, Bhowali, Uttarakhand
	Chrysanthemum carinatum Schousboe*	Tricolor chrysanthemum	-	NBPGR
	Matricaria aurea Boiss.	Golden cotula	-	SN
Arctotideae	*Gazania rigens* (L.) Gaertn.**	Treasure flower	-	SN
Astereae	*Solidago canadensis* L.	Canada goldenrod	-	Bengaluru, Karnataka
	Aster chinensis L.**	China aster		SN
	Bellis perennis L.	Daisy		SN
	Brachyscome iberidifolia Benth.	Swan river daisy	-	SN
Calenduleae	*Calendula officinalis* L.*	Calendula	-	NBPGR
	Dimorphotheca sinuata DC.	Cape-marigold	-	SN
Cichorieae	*Lactuca sativa* L.*	Lettuce	-	New Delhi
(= Lactuceae)	*Sonchus arvensis* L.	Field sowthistle	-	NBPGR
Cynareae	*Carthamus tinctorius* L.*	Safflower	-	Bengaluru, Karnataka
	Centaurea cyanus L.	Cornflower/ bachelor's button	Frosty Mix	Namdhari Seeds Pvt. Ltd., Bidadi, Karnataka
Eupatorieae	*Ageratum conyzoides* L.**	Goat weed	-	AAU; Andaman and Nicobar Islands
	Ageratum houstonianum Mill.	Floss flower/ mist flower	-	SN
	Chromolaena odorata (L.) R.M. King & H. Robinson*	Siam weed	-	Bengaluru, Karnataka; Nicobar Islands, Andaman and Nicobar Islands
	Eupatorium adenophorum Spreng. (Banmara)**	Crofton weed	-	Ootacamund, Tamil Nadu
	Stevia rebaudiana (Bertoni) Bertoni*	Sweet leaf	-	Assam
Helenieae	*Tagetes erecta* L.	Big marigold/ Aztec marigold	African Marigold (Tall)	NBPGR
	Tagetes tenuifolia Cav.	Striped marigold	Single Signet	NBPGR
Heliantheae	*Cosmos bipinnatus* Cav.	Cosmos	-	Bengaluru, Karnataka
	Dahlia sp.	Dahlia	Unwins Mix	Karnataka
	Eclipta alba (L.) Hassk.	False daisy	-	Nancowry, Nicobar Islands, Andaman and Nicobar Islands
	Guizotia abyssinica Cass.	Niger-seed	-	Bengaluru, Karnataka
	Helianthus annuus L.*#	Sunflower	Morden	Tamil Nadu
	Parthenium hysterophorus L.*	Congress grass	-	NBPGR
	Tithonia diversifolia (Hemsl.) Gray	Mexican sunflower/ tree marigold	-	Bengaluru, Karnataka
	Wedelia biflora (L.) DC.	Sea daisy	-	Nicobar Islands, Andaman and Nicobar Islands
	Zinnia elegans Jacq.	Elegant zinnia	Giant Dahlia Fld. Double Mixed	Karnataka
Inuleae	*Blumea junghuhniana* (Miq.) Boerl.	Blumea	-	Nicobar Islands, Andaman and Nicobar Islands
Mutisieae	*Gerbera jamesonii* Bolus ex Hook. f.*	Transvaal daisy/ Barberton daisy	-	SN
Senecioneae	*Cineraria lyrata* DC.	Wild parsley	Jubilee Mix	New Delhi
	Erechtites valerianifolia (Link ex Wolf) Less. ex DC.	Tropical burnweed	-	Assam
Vernonieae	*Vernonia anthelmintica* (L.) Willd.**	Purple fleabane	-	Andaman and Nicobar Islands; NBPGR

*Plant species (or **same genus) tested at CABI (UK) earlier and found non-susceptible to *P. spegazzinii* (Trinidadian pathotype). #Showed mild chlorotic flecks (PS 1). [@]Abbreviations: AAU: Assam Agricultural University, Jorhat, Assam; NBPGR: National Bureau of Plant Genetic Resources, New Delhi; SN: Sunder Nursery, New Delhi.

Assam are susceptible to the pathotype of *P. spegazzinii* (PS 4) while those in the East and Central Assam show some resistance (PS 2/3). On the other hand, all the five populations of *M. micrantha* from Kerala were found to be highly susceptible with PS 4 (Table 1).

All the 10 populations of *M. micrantha* from Andaman showed rust symptoms with PS not less than 3 (Table 1), thus indicating the susceptibility of the weed populations from the Islands. Two populations each from South Andaman (Garacharma village and Radhanagar) and North and Middle Andaman (KHEP Junction and Kalpong) showed medium susceptibility with PS 3-4, but the rest all were highly susceptible.

Out of the 35 plant species in Asteraceae, 34 did not show any reaction to rust inoculation (Table 2), suggesting their immunity to *P. spegazzinii*. In sunflower (cv. Morden), however, mild chlorotic flecks (PS 1) were observed on a few top leaves **(**Fig. 5**)** that were directly under the heavy inoculum inside the dew chamber, after 6-8 days of inoculation (Table 2)**.** Ellison *et al*. (2008) also observed similar chlorotic spots on sunflower in the primary host-range screening with the same pathotype in UK. They monitored the leaves showing chlorosis till senescence and found no further development of disease. In the present study, to avoid the risk of the alien rust fungus posing threat to the sunflower biodiversity in India, an additional screening of sunflower cultivars/accessions was undertaken to ascertain the results. A total of 27 more cultivars/ accessions, in addition to Morden, of sunflower collected from various sources were inoculated and the chlorotic flecks were observed in eight samples, including Morden (Table 3). During the microscopic examination no mycelial growth was observed in the leaf tissue of the plants showing chlorotic flecks. All the inoculated sunflower plants showed normal growth and flowering. Ellison *et al*. (2008) also observed such symptoms on the sunflower sample from India and noticed the germination of basidiospores and formation of appressoria on the leaves, but there was no penetration of the tissues. The plants showing chlorotic flecks were observed until flowering and the leaves had senesced. The resistance reaction of sunflower was further confirmed with microscopic and histopathological studies. The flecks did not develop further and there was no spore formation. It was concluded that sunflower is not susceptible to *P. spegazzinii* and the development of chlorotic flecks was only due to hypersensitive reaction of no economic consequence.

Table 3. Additional screening of sunflower cultivars/ accessions for reaction to *Puccinia spegazzinii* (Trinidadian pathotype)[#]

Cultivar/ accession	Source	Reaction
AHT-16	AAU, Jorhat, Assam	+
AHT-17	AAU, Jorhat, Assam	-
IH-673	AAU, Jorhat, Assam	+
IH-662	AAU, Jorhat, Assam	-
CO-2	Tamil Nadu	-
Morden	Tamil Nadu	+
Swarna Hybrid	Tamil Nadu	-
CO-4 (TNAUSUF-7)	Tamil Nadu	-
TCSH-1 (TNAU)	Tamil Nadu	+
PRO-011	Gene Bank, NBPGR, New Delhi	-
LSFH-35	Gene Bank, NBPGR, New Delhi	-
CMSH-84A	Gene Bank, NBPGR, New Delhi	-
Surya	NBPGR, New Delhi	-
MSFH-17	NBPGR, New Delhi	+
KP-AK-164 (IC-415484)	NBPGR, New Delhi	-
SM-BJ-6 (IC-411604)	NBPGR, New Delhi	-
KP-AK-76 (IC-415396)	NBPGR, New Delhi	-
SM-BJ-22 (IC-411620)	NBPGR, New Delhi	-
JBT-38/228 (IC-424494)	NBPGR, New Delhi	+
SM-BJ-50 (IC-411648)	NBPGR, New Delhi	-
SM-BJ-99 (IC-411697)	NBPGR, New Delhi	-
KP-AK-37 (IC-415357)	NBPGR, New Delhi	-
IC-328856	NBPGR, New Delhi	+
EC-512670 (France)	NBPGR, New Delhi	-
EC-512671 (France)	NBPGR, New Delhi	-
EC-512682 (France)	NBPGR, New Delhi	-
EC-512683 (France)	NBPGR, New Delhi	-
EC-68414	NBPGR, New Delhi	+

+ = chlorotic flecks observed on leaves (pathogenicity score 1); - = no chlorotic flecks observed. [#]Chlorotic spots were observed on sunflower leaves in CABI (UK), but there was no further disease development and the chlorosis faded and disappeared as the leaves aged. A microscopic analysis also proved that fungal penetration was inhibited (Ellison *et al.* 2008).

Ellison *et al*. (2008) recorded a hypersensitive response to the same rust pathotype on *Calendula officinalis*, *Eupatorium cannabinum* and *Stevia rebaudiana*; however, no such reaction was observed during the present testing, which included *C. officinalis*, *S. rebaudiana* and a different species of *Eupatorium*.

No rust symptoms were observed on any of the other 55 test plant species belonging to 31 families (Table 4) and representing various economically important crop groups, viz. cereals, vegetables, pulses, ornamentals, oilseeds, fibre crops, fruit crops, medicinal crops, spices and condiments, etc. Further, the results of 21 plant species, including nine in Asteraceae and 12 others of high economic importance, tested earlier at CABI (UK) also reconfirmed their immunity to the rust fungus.

Table 4. Other economically important plant species inoculated with *Puccinia spegazzinii* (Trinidadian pathotype) for host-specificity screening

Family	Scientific name	Common name	Cultivar	Place/ source of collection[@]
Anacardiaceae	*Anacardium occidentale* L.	Cashew	Vengurla	Bengaluru, Karnataka
	Mangifera indica L.	Mango	Mallika	New Delhi
Bromeliaceae	*Ananas comosus* (L.) Merr.	Pineapple	Mauritius	KAU
Campanulaceae	*Lobelia erinus* L.	Garden lobelia	Crystal Palace	SN
Caryophyllaceae	*Dianthus caryophyllus* L.	Carnation	-	SN
	Gypsophila muralis L.	Cushion baby's-breath	White Covent Garden	New Delhi
Cruciferae	*Brassica nigra* (L.) Koch**	Black mustard	RK-01-03	NBPGR
	Coronopus didymus (L.) Sm.	Lesser swine-cress	-	NBPGR
	Iberis sp.	Candytuft	-	New Delhi
	Matthiola incana (L.) W.T. Aiton	Hoary stock	-	SN
	Raphanus sativus L.*	Radish	Pusa Desi	NSC
Dioscoreaceae	*Dioscorea bulbifera* L.	Potato yam	Gajendra	AAU
Euphorbiaceae	*Ricinus communis* L.	Castorbean	DCH 519	NBPGR
Fabaceae	*Arachis hypogaea* L.*	Groundnut/ peanut	TG-45	NBPGR
	Vigna unguiculata (L.) Walp.	Cowpea	Pusa Phalguni	NBPGR
Lamiaceae	*Salvia splendens* Sellow ex Schult.	Scarlet sage	Bonfire salvia	New Delhi
Lauraceae	*Cinnamomum zeylanicum* Blume	Cinnamon	IISR Navasree	KAU
Linaceae	*Linum usitatissimum* L.*	Flax/ linseed	RLC-81	NBPGR
Malvaceae	*Gossypium arboreum* L.	Desi cotton	Karbi	AAU
	Gossypium hirsutum L.	Upland cotton	MECH-162 (Non- Bt); MECH-162 (Bt)	MAHYCO
Moraceae	*Artocarpus heterophyllus* Lam.	Jackfruit	-	AAU
Musaceae	*Musa paradisiaca* L.	Banana	-	AAU
Myristicaceae	*Myristica fragrans* Houtt.	Nutmeg	IISR Viswashree	KAU
Myrtaceae	*Syzygium aromaticum* (L.) Merr. & Perry	Clove	-	KAU
Palmae	*Areca catechu* L.	Arecanut/ betel-nut palm	Mangala	KAU
	Cocos nucifera L.*	Coconut	Bengal Selection	AAU
Pedaliaceae	*Sesamum indicum* L.	Gingelly/ sesame	-	AAU
Piperaceae	*Piper betle* L.	Betel vine	-	KAU
	Piper nigrum L.	Black pepper	Panniyur-1	KAU
Poaceae	*Bambusa arundinacea* (Retz.) Willd.	Thorny bamboo	-	KFRI
	Ochlandra travancorica (Bedd.) Benth. ex Gamble.	Elephant grass	-	AAU
	Oryza sativa L.*	Paddy/ rice	NDRK 5026-R	Genetics Division, IARI
			-	NSC
	Pennisetum typhoides (Burm.f.) Stapf. & C.E. Hubb.	Pearl millet	HHB-117	NBPGR
	Saccharum officinarum L.	Sugarcane	CO-1148	AAU
	Sorghum bicolor (L.) Moench*	Sorghum	UP Chari-2	NBPGR
	Triticum aestivum L.	Bread wheat	PBW-343	Genetics Division, IARI
	Zea mays L.*	Corn/ maize	Composite Lakshmi	NBPGR
Polemoniaceae	*Phlox drummondii* Hook.	Annual phlox/ Drummond's phlox	-	SN
Rubiaceae	*Coffea arabica* L.*	Arabian coffee	Kaveri	KAU
Scrophulariaceae	*Antirrhinum majus* L.	Snapdragon	-	SN
	Linaria bipartita (Vent.) Willd.	Clovenlip toadflax	-	SN
Solanaceae	*Capsicum annuum* L.	Chilli/ red pepper	Pusa Hyper-2	NSC
	Nicotiana tabacum L.	Tobacco	-	NBPGR
	Petunia sp.	Garden petunia	Multiflora Double Mix	New Delhi
	Solanum melongena L.*	Brinjal/ eggplant	PK	NSC
Sterculiaceae	*Theobroma cacao* L.*	Cocoa	CCRP-1	KAU
Theaceae	*Camellia sinensis* (L.) O. Kuntze*	Tea	TV-23	AAU
Tiliaceae	*Corchorus capsularis* L.	Jute	J-295; JRC-212; JRO-524	AAU
Tropaeolaceae	*Tropaeolum majus* L.	Garden nasturtium	-	SN
Verbenaceae	*Tectona grandis* L.f.*	Teak	-	KFRI
	Verbena officinalis L.	Common vervain/ pigeon's-grass	Time Mixed	New Delhi
Violaceae	*Viola tricolor* L.	Heart's ease/ pansy	-	SN
Zingiberaceae	*Curcuma domestica* Val.	Turmeric	Prathibha	IISR Experimental Farm, Peruvannamuzhi, Kerala
	Elettaria cardamomum (L.) Maton	Green cardamom	CCS-1	IISR Regional Station, Appangala, Karnataka; Kerala
	Zingiber officinale Rosc.	Ginger	-	New Delhi

*Plant species (or **same genus) tested at CABI (UK) earlier and found non-susceptible to *P. spegazzinii* (Trinidadian pathotype).

[@]Abbreviations: AAU: Assam Agricultural University, Jorhat, Assam; IARI: Indian Agricultural Research Institute, New Delhi; IISR: Indian Institute of Spices Research; KAU: Kerala Agricultural University, Thrissur, Kerala; KFRI: Kerala Forest Research Institute, Peechi, Kerala; MAHYCO: Maharashtra Hybrid Seeds Company; NSC: National Seeds Corporation Limited, New Delhi; NBPGR: National Bureau of Plant Genetic Resources, New Delhi; SN: Sunder Nursery, New Delhi.

Fig. 1. Inoculation in the dew chamber

Fig. 2. Basidiospore production

Fig. 3. Initial chlorotic spots on *Mikania micrantha* leaves

Fig. 4a. Mature telia on *Mikania micrantha* leaves

Fig. 4b. Mature telia on *Mikania micrantha* stem

Fig. 5. Mild chlorotic flecks on sunflower leaf

Host-specificity testing is an essential part of risk analysis and is the key tool to assess whether the biological agent is safe to release into a new environment. Based on the results from screening a wide range of plant species for host-specificity, the Plant Protection Advisor to Government of India granted permission for limited field release of the Trinidadian pathotype of *P. spegazzinii*. The rust inoculum was released at identified sites in Assam and Kerala (Sankaran *et al.* 2008). In Kerala, the rust was released in agricultural systems and forest areas in during August-October 2006. The releases were successful in the sense that the rust had spread to the native population of the weed at all release sites. The maximum distance of spread was 1.5 m away from the source plant. However, the rust persisted on the field population of the weed only till December until the temperature and humidity at the sites were suitable for survival of the pathogen and disease spread. Low inoculum load and inappropriate time of release are considered to be the main reasons for the failure in survival of the rust in the field beyond December. The ideal time for release was June-August, during the southwest monsoon in Kerala (which provided the optimum conditions for rust infection), which would have promoted wider spread of the rust and its survival during the summer. On the other hand, in Assam, the rust failed to establish probably because of plant biotypic variation in susceptibility. With this release of *P. spegazzinii*, India had become the eighth country in the world and the first on mainland Asia to deliberately and scientifically introduce an exotic fungal pathogen for CBC of a weed. Once established in the fields this CBC agent can provide a cheaper, safer and sustainable solution for the management of *M. micrantha*. The host-susceptibility results presented here indicate the potential of the rust not only on the Indian mainland but also in the Andaman archipelago, where *M. micrantha* is a perennial problem to the local flora.

In 2008, *P. spegazzinii* was imported and released (but in this case, a different isolate was used) at nearly 560 sites in 15 provinces in Papua New Guinea (PNG) and over 80 sites on four islands in Fiji (Day *et al*. 2011 and 2013). In PNG, the rust had established in 11 provinces, spreading up to 40 km from some sites, and in Fiji, it had established on two islands. Further field monitoring indicated the potential of *P. spegazzinii* to control this weed in many parts of both countries, by way of reducing the weed density (Day *et al.* 2011). Results of the release from PNG, Fiji (Day *et al.* 2011 and 2013) and also in Taiwan (S.S. Tzean, personal communication, 2013) indicate strong possibility of survival of the rust in the field in Kerala exerting an impact on the population of *M. micrantha*. It is evident from results elsewhere that once there is a critical concentration of the rust in the area, the infection will enter into an epidemic phase. It can be concluded that fresh releases of the rust, probably using the isolate released in PNG and Taiwan (Ellison *et al.* 2014), during the southwest monsoon (June/July) may help survival of the pathogen in the field in Kerala and subsequent control of the weed population.

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