The significance of intraspecies pathogenicity in the selection of a rust pathotype for the classical biological control of Mikania micrantha (mile-a-minute weed) in Southeast Asia

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Summary

Mikania micrantha, commonly known as mile-a-minute weed (Asteraceae), is a vine of Neotropical origin, which has become an important, invasive weed within the moist tropical zones of Southeast Asia. A classical biological control program focusing on the potential of fungal agents, evaluated three rust pathogens, *Puccinia spegazzinii*, *Dietelia portoricensis* and *Dietelia* sp. nov., which occur within the native range of the plant. These rusts were found to have distinct and disparate geographical distributions. *Puccinia spegazzinii* is the widest-ranging species and 16 pathotypes were collected from eight countries, together with one isolate each of the other two species. Using molecular techniques, the genetic variability of *M. micrantha* throughout its native range was analyzed (21 accessions from nine countries) and compared to that in the exotic range (29 accessions from nine countries). The results show that great genetic variation occurs within the Neotropics, whilst in the exotic or palaeotropic range the genetic base is narrow, indicating that those populations originated from only a few introductions. The molecular data were compared with an extensive cross-inoculation program undertaken between selected accessions of *M. micrantha* (25) and rust pathotypes (9). These studies have been instrumental in the selection of the rust strain most suitable for the first target area of release (southeast India). An isolate of *P. spegazzinii* from Trinidad has been recommended for introduction. This will be the first fungal agent to be released against any weed in Southeast Asia and permission to import this rust into quarantine has been granted by the Indian Authorities. The anticipated success of this rust in relation to the results of the intraspecies specificity testing is discussed.

Keywords: intraspecies variation, invasive alien weed, *Mikania micrantha*, molecular techniques, rusts.

Introduction

*Mikania micrantha* Kunth. ex H.B.K. (Asteraceae) is a Neotropical invasive weed that can smother both agro-forestry and natural forest ecosystems, as well as many crops within home garden and plantation production systems in the tropical moist forest zones of Southeast Asia; tea and plantain are particularly severely affected (Holm *et al.* 1977, Waterhouse 1994). It was deliberately introduced into Asia, particularly for use as a cover crop in rubber (Wirjahrardja 1976), from as early as 1918 (Cock *et al.* 2000). It is regarded as a major weed in many countries, and is still in its invasive phase. Current control focuses on cultural (slashing) and chemical (herbicides) methods, but this is expensive, often ineffective, not sustainable and can be environmentally damaging (Palit 1981, Muniappan & Viraktamath 1993).
A collaborative project that ran between 1996 and 2000, to investigate an IPM approach for the control of the weed in the Western Ghats, India, was funded by the UK Department for International Development (DFID), through the Natural Resources Institute’s Crop Protection Program. The project involved three Indian organisations; Kerala Forest Research Institute (KFRI), Project Directorate of Biological Control (PDBC) and Assam Agricultural University (AAU), as well as CABI Bioscience (UK). It was concluded that classical biological control (CBC) was the most appropriate long-term solution for the control of this weed (Ellison 2001, Sankaran et al. 2001).

A broad range of fungal pathogens has been recorded on *M. micrantha* from its neotropical native range (Evans 1987, Barreto & Evans 1995). From this evaluation, three coevolved, autecious, microcyclic rust species were selected for further assessment as potential CBC agents against the weed in southern India. These rusts, *Dietelia portoricensis* (Whetzel & Olive) Buriticá & JF Hennen, *Dietelia mexicana* sp. nov. and *Puccinia spegazzinii* de Toni, are all highly damaging to their host in the field, causing leaf, petiole and stem infections leading to cankerling and whole plant death. None were found in the exotic range of the weed.

The nine pathotypes (seven of *P. spegazzinii* and one each of the two *Dietelia* spp.) were evaluated in the CABI Bioscience (UK) quarantine glasshouse, and an isolate of *P. spegazzinii* from Trinidad (W1761) was considered to be the prime candidate (Ellison 2001). This pathotype proved to be virulent against accessions collected from a wide range of Indian populations of the weed, and infected all of the accessions from the 10 populations sampled from the DFID target region of the Western Ghats.

A dossier was produced by CABI Bioscience for the Indian collaborators, containing detailed data on *P. spegazzinii*, following the FAO Code of Conduct (FAO 1996, Ellison & Murphy 2001). This was submitted to the India Directorate of Plant Quarantine & Storage by PDBC, together with a letter detailing that permission had been given by the Ministry of Agriculture Land and Marine Resources of Trinidad and Tobago for the use of their genetic resources, following the Convention on Biodiversity (http://www.biodiv.org/). Permission to import the rust into quarantine in India was granted in September 2002 and hand-carryage of the rust to quarantine facilities in Delhi was scheduled for mid-2003. Release in the Western Ghats and Assam was planned for the following year.

From this work it was apparent that these three, coevolved rusts demonstrate intraspecies specificity; each isolate only infecting a selected number of genotypes of its host. From field observations, considerable morphological variation, and hence, potential biotypic differentiation, is apparent within the *M. micrantha* species. This has ramifications for the potential success of CBC of this weed with the selected rust isolate. It is important to know how much genetic variation exists within the exotic range of the weed, and whether the Trinidad isolate has the inherent ability to be successful throughout the range of the weed. Consequently, a detailed cross-inoculation study was undertaken, whereby accessions of *M. micrantha* taken from populations in its native and exotic ranges, were challenged by a range of rust pathotypes. This was paralleled by a molecular analysis of these plant accessions. The preliminary conclusions are presented here.

**Materials and methods**

**Field collections**

Over the last decade, samples of living *M. micrantha* plants have been collected by CABI Bioscience personnel throughout both the Neotropics, and the paleotropical invasive range of the weed. These plant samples consisted of one or a few plant accessions, collected from within a population of the plant. Samples of rusts were also collected and brought back to the CABI Quarantine Unit (UK). Since these rusts do not survive drying, it was necessary to transport them on living plants. Each rust isolate was established in quarantine from a single pustule, assumed to have originated from a single basidiospore.

**Rust inoculation procedure**

Plants used for rust-inoculation studies were propagated from cuttings and grown in a 1:1 mixture of general purpose, peat-based potting compost and John Innes No. 2 soil-based compost. Pre- and post-inoculated plants were maintained in an air-conditioned, quarantine greenhouse chamber set at 22 ± 5ºC and with a humidity of between 50 and 80%. The chamber had a 12-hour light/dark cycle and was fitted with metal halide, full spectrum lamps, providing a light intensity ranging from 8000 to 13,000 Lux, depending on the ambient light. Vigorous test plants, with developing shoots or meristems, were mist sprayed with distilled water and then inoculated by suspending mature rust-infected material ca. 5 cm above the shoot apices, using plant ties attached to a wire frame. The plants were transferred to a dew chamber (Mercia Scientific, Birmingham, UK) set at 20ºC, for 24 hours. Under conditions of high humidity, basidiospores were shot-off from the teliospores (*P. spegazzinii*) or aecidial teliospores (*Dietelia* spp.) embedded in the plant tissue, and landed on the fresh host shoots, where they germinated and potentially infected. After removal from the dew chamber, inoculated plants were returned to the quarantine chamber for daily observation.

**Molecular characterisation**

More than 70 accessions of *M. micrantha* were collected throughout its native and introduced ranges
during the course of the study. A wide, representative selection of 51 accessions was included in the molecular characterization. Full site details are given in Ellison & Murphy (2001). Mikania micrantha can be an out-crossing species and thus the purity of each line is maintained by clonal propagation.

The genetic variability of weed samples was assessed by amplified fragment length polymorphism (AFLP). DNA was extracted from fresh leaf material using a Nucleon Phytopure DNA extraction kit (Tepnel Life Sciences, Manchester, UK). 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 help increase the yield and uniformity of the selective AFLP profiles. A total of five selective primers was used with the following selective nucleotides; AC, AG, CG, CT, and GT. The AFLP profiles were separated by electrophoresis through 1.5% (w/v) agarose gels (SeaKem LE, BMA, Wokingham, UK), which were run at 100V for 6 hours, stained with ethidium bromide and photographed. Gel photos were imported into GelCompar (Applied Maths, Kortrijk, Belgium) and a composite dendrogram of all five primers was produced using the unweighted pair group method using arithmetic averages (UPGMA) and derived with the Dice coefficient.

**Cross-inoculation studies**

A representative range of nine rust pathotypes was selected for this study, from the 16 that had been collected during the CABI surveys. Their site details are given in Ellison & Murphy (2001). All plants used were clonally propagated from original stock plants. Three plants were inoculated per individual cross-inoculation, and this was repeated at least twice following a fully susceptible response, and four times when no symptoms or a semi-resistant response was observed.

The following pathogenicity scores were used for the evaluation of the rusts:

0 No macroscopic symptoms.
1 Necrotic spots on inoculated vegetative parts — no sporulation.
2 Abnormal infection site: chlorotic patches on vegetative parts with very low teliospore or aecioid teliospore production around edges of chlorosis.
3 Abnormal infection site: pustules reduced in size with low teliospore or aecioid teliospore production in relation to compatible-host pathogen interaction.
4 Normal pustule formation, in relation to compatible-host pathogen interaction.

**Results and discussion**

**Distribution of rust pathogens**

The current records of the three rust species within the Neotropics are shown in Figure 1. *Puccinia spegazzinii* is the most widespread and was collected at altitudes ranging from near sea level to ~1200 m, whereas *D. portoricensis* appears to be restricted to Central America and *D. mexicana* sp. nov. has been recorded from Mexico only. All three species are highly damaging and appear to be restricted to their host. Due to the wide distribution of *P. spegazzinii*, suggesting a broad environmental adaptability, which is supported by the glasshouse data, this species was selected as the primary classical biological control agent for *M. micrantha* in its exotic range.

**Molecular characterisation**

The dendogram constructed from the *M. micrantha* accessions is given in Figure 2, and the following generalizations can be drawn from these data:

- The genetic diversity in the native range is greater than that in the exotic range.
- The results do not provide information on the origins of the exotic range weed populations; with the exception of one accession from Indonesia that appears to be similar to a genotype from Jamaica.
- With the exception of Jamaica, the accessions from the native range and the exotic range show a maximum of 67% similarity (between Australia and Brazil/Peru).
- There are numbers of genetic types which appear in more than one region in the exotic range, suggesting possible roots of distribution of the weed. Examples include the following: Sri Lanka and India; Nepal and India (Assam); PNG and Indonesia (West Java); Malaysia, Philippines and India.
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- Populations from Indonesia appear to be more genetically diverse than those from India, although both regions appear to have a similar number of separate introductions of the weed (5).

Cross-inoculation studies

The results are shown in Table 1. The following two overall generalisations can be made of these summarized data:

- The biotypes of *M. micrantha* within its native range are resistant to most of the rust pathotypes not present within their area of distribution.

- Most accessions of *M. micrantha* from its exotic range are susceptible or at least partially susceptible to all rust pathotypes.

The differential infection type responses occurring in particular host accession – pathogen isolate combinations indicate that qualitative resistance appears to be widespread in this interaction (Thrall & Burdon 2002). Most of the rust isolates that were studied in detail came from highly disparate populations of *M. micrantha*. Hence, it is not possible to draw conclusions about the size of each pathotype-susceptible population and whether more than one pathotype exists within a population; or indeed about the extent of variation within individual plant populations.

The absence of resistance to the rust pathotypes within the exotic range weed populations provides superficial support for the idea that resistance of this type has a metabolic cost that is selected against in the absence of the pathogen – as would occur when the plant is carried to a new environment (Thompson 1990). However, this possibility cannot be definitively concluded from these data, since the resistance status of the plants originally introduced into the Neotropics is unknown. It is conceivable that all introduced lines were from rust-susceptible Neotropical populations.

Exotic range populations of the weed were observed to have a vigorous growth form when compared with plants growing in their native range. All genotypes retained their field characteristics when grown under the same conditions in the glasshouse. This may suggest that gene-for-gene resistance, or perhaps a linked factor, carries a significant metabolic load. In addition, all three rusts, when inoculated onto fully susceptible populations of the plants in the exotic range, are highly aggressive (large pustules, plant death common). Conversely, these rust pathotypes are significantly less aggressive on their susceptible, native range biotypes (smaller pustules, less severe plant damage). However, again, without detailed knowledge of the plants that were originally introduced, this remains only an interesting, but unsubstantiated observation. Indeed, since the plants were originally introduced as a cover crop, it is likely that the most vigorously growing plants were selected.

The intermediate, semi-resistant rust pathogenicity could be governed by either gene-for-gene resistance, or pathotype-non-specific, multi-gene, horizontal resistance, though it would be expected that all the genotypes present in the exotic range would show a similar response to all the pathotypes if horizontal resistance was responsible (J.J. Burdon, pers. comm.). The intermediate pathogenicity reaction was equally expressed in both the native and exotic ranges of the plant, which may suggest that this type of resistance is not so readily lost from the exotic range populations as the gene(s) governing an immune response. However, again, lack of information on the resistance status of the plants originally introduced allows only speculation. If

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**Figure 2.** Dendogram of *Mikania micrantha* populations.
the semi-resistance response is governed by a number of genes, it could be argued that the horizontal resistance is gradually being eroded within the exotic range, but requires a longer period of evolution than the vertical resistance based on single genes. The metabolic cost of keeping these genes (or those linked to them) may not be as significant. Indeed, some populations of the weed are fully susceptible to all pathotypes of the rusts, and perhaps these populations originate from the earliest introductions of the weed. Alternatively, the multi-gene resistance may still be useful to the plant in reducing susceptibility to generalist pathogens in the exotic range.

**Conclusions**

Although the centres of origin of most of the exotic range population of the plant were not elucidated by the molecular characterisation, the results of the cross-inoculations did not suggest that it is necessary to obtain rust isolates from the specific area of origin of the weed genotype. The cross-inoculation studies indicated that most or possibly all the populations present in the exotic range of the weed are fully susceptible to one or more rust pathotypes. This may be because the original populations that were introduced into Asia were taken from populations of *M. micrantha* that were susceptible to the rust. Conversely, resistance may have been lost in the exotic range populations, isolated from their coevolved rusts. Nevertheless, the presence of a semi-resistance interaction necessitates the need for using the most virulent rust pathotype(s) for the genetic types of the weed present in a particular invaded region.

The relatively narrow genetic base of the weed in its palaetropical range, confirms the evidence in the literature of a small number of deliberate introductions of the plant (Wirjahardja 1976, Cock et al. 2000). This factor makes the concept of selecting different pathotypes for different target regions a feasible approach for CBC of the weed. It is proposed that a relatively quick and inexpensive DNA screen may facilitate this selection. For example, it is clear that the isolate of *P. spegazzinii* selected for use in the Western Ghats region of India is not the optimal pathotype match for the

| Table 1. Summary of intraspecies pathogenicity of *Puccinia spegazzinii* and *Dietelia portoricensis* isolates against world-wide populations of *Mikania micrantha*. |
|---|---|---|
| **Mikania collections**<sup>a,b</sup>/selected population | **Host reactions to rust isolates**<sup>c</sup> | **Dietelia portoricensis**<sup>a</sup> |
| | *Puccinia spegazzinii*<sup>a</sup> | | |
| | Argentina (1) | Peru (1) | Brazil (4) | Ecuador East (N) (1) | Ecuador West (Im) (1) | Trinidad (4) | Costa Rica [race] (1) | Mexico [race] (1) |
| **Argentina (1)** | ✔ | – | – | ✔ | ✔ | ✔ | ✔ | ✔ |
| **Peru (1)** | ✔ | ✔ | ✔ | ✗ | ✗ | ✗ | ✗ | ✗ |
| **Brazil (6)** | ✗ | ✔ | ✔ | ✗ | ✗ | ✗ | ✗ | ✗ |
| **Ecuador Napo (eastern)** | ✗ | ✗ | ✔ | ✔ | ✔ | ✔ | ✗ | ✗ |
| **Ecuador Imbabura (western)** | ✗ | ✗ | ✗ | ✗ | ✔ | ✔ | ✗ | ✗ |
| **Trinidad (4)** | ✗ | ✔ | ✔ | ✔ | ✔ | ✔ | ✗ | ✗ |
| **Jamaica (1)** | ✔ | ✗ | ✗ | ✔ | ✔ | ✔ | ✗ | ✗ |
| **Mexico W1904** | ✗ | ✗ | ✗ | ✗ | ✔ | ✔ | ✗ | ✗ |
| **Costa Rica 17–1** | ✗ | ✔ | ✔ | ✔ | ✔ | ✔ | ✗ | ✗ |
| **Nicaragua** | ✗ | ✔ | ✔ | ✔ | ✔ | ✔ | ✗ | ✗ |
| **Panama (2)** | ✗ | ✗ | ✗ | ✔ | ✔ | ✔ | ✗ | ✗ |
| **India south-west (10)** | ✗ | ✗ | ✔ | ✔ | ✔ | ✔ | ✗ | ✗ |
| **India north-east (7)** | ✔ | ✔ | ✔ | ✔ | ✔ | ✔ | ✗ | ✗ |
| **Nepal (1)** | ✔ | ✔ | ✔ | ✔ | ✔ | ✔ | ✗ | ✗ |
| **Sri Lanka (1)** | ✗ | ✗ | ✗ | ✗ | ✔ | ✔ | ✗ | ✗ |
| **Malaysia (2)** | ✗ | ✗ | ✗ | ✔ | ✔ | ✔ | ✗ | ✗ |
| **Philippines (1)** | ✗ | ✗ | ✗ | ✔ | ✔ | ✔ | ✗ | ✗ |
| **Indonesia (6)** | ✔ | ✔ | ✔ | ✔ | ✔ | ✔ | ✗ | ✗ |
| **Papua New Guinea (1)** | ✔ | ✔ | ✔ | ✔ | ✔ | ✔ | ✗ | ✗ |
| **Australia (1)** | ✔ | ✔ | ✔ | ✔ | ✔ | ✔ | ✗ | ✗ |

<sup>a</sup> Number of collections or isolates assessed.

<sup>b</sup> Not all combinations have been assessed. ? = Unclear result, confirmation still required.

<sup>c</sup> Host reactions: ✔ = fully compatible (pathogenicity score 1); ✔(+) = first choice if Trinidad pathotype not fully compatible; ✔(–) = Fully compatible, but number and size of pustules reduced in comparison to controls; ± = semi-resistance response (pathogenicity score 2/3); ✗ = not compatible (pathogenicity score 0/1); – = not tested;
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