Status Report

BIOLOGICAL CONTROL OF WEEDS USING FUNGAL NATURAL ENEMIES: A NEW TECHNOLOGY FOR WEED MANAGEMENT IN TEA?

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ABSTRACT

Tea (Camellia sinensis) is particularly susceptible to competition by weeds, and implementation of control practices requires considerable inputs from the farmer. Biological control (BC) using fungal natural enemies offers a sustainable, environmentally friendly and cost effective alternative strategy for the control of weeds. Two approaches can be considered in this strategy: classical biological control (CBC) of invasive alien weeds using their coevolved natural enemies, from the centre of origin of the weed; and inundative control using those pathogens that can be mass-produced, and which are already present in the country where the weed is a problem. The BC potential of 22 important weeds in tea in Asia is presented; it includes four weeds where CBC has already been successfully implemented somewhere in the world. A case study on the CBC of the invasive alien weed Mikania micrantha*, using the coevolved rust fungus* Puccinia spegazzinii *(from Latin America), in the Assam tea-growing region of India is discussed.*

Keywords : *Assam, tea; invasive alien weeds; classical biological control; bioherbicides; fungal natural enemies; Mikania micrantha, pathogens*

INTRODUCTION

Weeds can be a serious constraint to the production of tea (*Camellia sinensis* [L.] Kuntze), particularly during the first 3-4 year establishment phase. Once the canopy has formed, weed problems tend to diminish. Mechanical control (hoeing between the rows) can be used initially. But it becomes less feasible in many tea production areas as the plants establish, due to the risk of damage to the surface roots (Holm *et al.*, 1977). Cover cropping, such as inter-planting of legumes, is sometimes used to suppress weeds and reduce soil erosion during the establishment years (Holm *et al.*, 1977). It is important also to control weeds in the areas around the tea gardens to minimise re-infestation of the crop. This is usually achieved by slashing, which is labour intensive and requires to be repeated to control weed re-growth. The use of chemical herbicides is widely implemented (Chakravartee, 1994) depending on the availability and cost of labour and the prevailing value of the tea crop. New herbicide application regimes are being continually tested (Kabir *et al.*, 1991). However, there is emerging evidence of the environmentally damaging effects of herbicides (Phukan and George, 1991) and an increasing worry over herbicide residues in the processed tea leaf, resulting in human toxicity issues (Barooah, 1994) and spoilage of the processed tea leaf (MAFF, 1997). The EU has already rejected shipments of export tea due to high pesticide residues. In addition, with low/fluctuating tea prices, there is an increased economic pressure to produce quality tea for niche markets such as organic tea where the use of chemical herbicides is not permitted.

Taking these issues into account, there is a significant drive towards finding alternative strategies of weed control (as well as other pest problems). One solution lies in the development of biological control methods, which fits well into an integrated and biologically-based approach to pest

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management in agroecosystems (Charudattan, 2001). For the biological control of weeds using pathogens, two broad approaches can be considered: classical biological control (CBC) of exotic or alien weeds using co-evolved, obligate or biotrophic fungi, and inundative control. The latter is used against both native and exotic weeds, but typically exploits indigenous, non-obligate or necrotrophic pathogens, which are mass-produced and applied as formulated products (bioherbicides). This paper focuses on the important weeds of tea in Asia, and discusses the potential to manage them using biological control, particularly employing fungal natural enemies.

CONCEPTS AND PRINCIPLES IN BIOLOGICAL CONTROL

Classical Biological Control

This approach evokes the enemy release hypothesis (ERH), whereby plant populations, once freed of their natural enemy complexes, can expand rapidly and, therefore, become more competitive than those subject to natural control (Wilson, 1969; Mitchell and Power, 2003). Most introduced plant species do not become weedy, Once established in a new region, if environmental factors are favourable, then a population explosion may result with the subsequent development of weed invasions (Mack *et al.*, 2000). CBC aims to redress this imbalance by 'reuniting' a weed with selected, co-evolved natural enemies (plant pathogens, mainly fungi, and arthropods). The potential agents are collected from the centre of origin of the target weed, choosing those that appear to have the most impact on plant. After passing a comprehensive evaluation and screening programme the best (most specific and damaging) agent(s) is introduced and released in the exotic target area (FAO, 1996).

CBC is considered to be more effective in stable environments (Reznik, 1996), particularly when employing arthropod agents, and consequently has targeted mainly weeds of rangelands and natural habitats. However, there are a growing number of examples of CBC being used to control exotic weeds in agricultural systems (particularly with fungal natural enemies), including annual cropping ecosystems (Hasan and Wapshere, 1973; Chippendale, 1995; McFadyen, 1998, Reeder and Ellison, 1999). For example, the rust *Puccinia chondrillina* Bubak & Sydow was introduced into Australia and California to control skeleton weed (*Chondrilla juncea* L.) in both pasture and arable crops (wheat-fallow) (Hasan and Wapshere, 1973). The pathogen spread rapidly, and within a relatively short period of time the weed infestations had been reduced by 99% to a density approaching that recorded in its European native range (Cullen and Hasan, 1988). Increases in crop yield and saving due to a reduction in herbicide usage have been valued at over US$12 million each year (Mortensen, 1986) with a cost:benefit ratio of 1:112 (Marsden *et al.*, 1980).

Fungal pathogens are a relatively recent addition to the arsenal of natural enemies that can be exploited in CBC, the first agent was released in 1972. Arthropods have a history dating back over a century, with some spectacular success stories (Julien and Griffiths, 1998). However, pathogens are beginning to show their true value; over 25 introductions have now been made for the control of invasive alien weeds and a significant number are having a major impact on the weed populations, or are providing cause for optimism (Evans, 2002). For example, the gall-forming rust fungus, *Uromycladium tepperianum* (Sacc.) McAlpine was introduced into South Africa from Australia to control *Acacia saligna* (Labill.) Wendl. (Port Jackson willow), an invasive and damaging weed of the unique Fynbos ecosystem. After an 8-10 year lag phase, the rust is now responsible for a 90-95% reduction in the weed populations and the Fynbos is now in the process of recovery (Morris, 1997).

Inundative Biological Control (Bioherbicides)
This strategy normally exploits necrotrophic, or opportunistic fungal pathogens already present in the weedy range of the target plant, but for certain reasons fails to control the weed. The aim is to simulate early epiphytotics of the selected pathogen, within a population of the target weed species, which would naturally occur at the end of the season. Thus the weed is killed, or at least significantly reduced in competitive ability, sufficiently early to prevent crop losses. The pathogen is usually an easily-cultured, rapidly-sporulating fungus that can be bulked-up, formulated and sold as a 'product'. The bioherbicide is applied early in the season, and thus circumvents the time lag in the natural build-up of the pathogen, caused by poor inter-crop survival and inefficient dispersal. Unfortunately, these sound principles have been thwarted by economics, mainly governed by the technological constraints of formulation and the regulatory processes. Despite early optimism, and the genuinely high potential of a number of products, in practice progress has been slow and success limited (Auld and Morin, 1995; Greaves, 1996; Mortensen, 1997).

There has been some commercial success with mycoherbicides in North America; four products have reached the registration stage. Only two of these have been sold as products: Collego® based on *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. f.sp. *aeschynomene* for the control of northern jointvetch (*Aeschynomene virginica* (L.) B.S.P.: Leguminosae) in the southern USA, and DeVine® based on fresh-rather than formulated preparations of *Phytophthora palmivora* (Butler) Butler for use against stranglervine (*Morrenia odorata* (H.&A.) Lindl.: Cucurbitaceae) in orchard crops. Unfortunately, none of the products are currently available (as of 2004) mainly due to small market size resulting in limited profitability, compounded by registration and production costs. In China, another product, Lubao (*C. gloeosporioides* f. sp. *cuscutae*) has been produced and used locally at the cottage-industry level to control dodder (*Cuscuta* spp.). The current status of the product is unknown, but lack of quality control has been a problem (Evans *at al.*, 2001b). In Japan, a product Camperico® has been successfully marketed for the control of annual bluegrass (*Poa annua* L.) in golf course green, for which effective herbicides are not available. The product is based on a highly specific, bacterial endophyte, *Xanthomonas campestris* pv. *poae* Egli & Schmidt, that is applied to the cut surface of the greens but only infects the target bluegrass (Imaizumi and Fujimori, 1998). Its commercial success lies in value of the market: golf course consortia are prepared to pay astronomical amount for the product.

The specificity of bioherbicides is considered to be a positive attribute, but there is also a serious downside. Agriculturists like to be able to apply one herbicide that deals with their entire weed problems, a bioherbicide will usually only deal with one of them. In the Philippines, Eusebio and Watson (2000) wesre developing a potential product composed of a mixture of pathogens, each specific to a different weed species or genus within the rice weed complex. In the USA a similar multipathogen mycoherbicide is about to be marketed (Chandramohan and Charudattan, 2003). The success of such products is yet to be put to the test, but there is certainly cause for optimism.

A final area worthy of comment is the recent success that mycoherbicides have been having as formulated produces for the treatment of cut stumps of woody weeds to prevent re-growth. The fungi involved are plurivourous pathogens (attack a range of host), but their use has been sanctioned in places where susceptible non-target crops are out of the range of the spread of the applied inoculum (Evans *et al.*, 2001a; de Jong *et al.*, 1990). For example, alien wattle trees (*Acacia* spp. : Mimosaceae) are successfully controlled in South Africa by the wood rot fungus, *Cylindrobasidium laeve* (Pers.: Fr.) Chmuris (Morris *et al.*, 1998; Morris *et al.*, 1999). The product Stumpout® is produced at the cottage-industry level employing a relatively simple formulation technology, and it has been highly successful.

IMPORTANT WEEDS OF TEA

Table 1 lists 22 of the most important weeds in tea in Asia. Holm *et al.* (1977) consider the four most serious and widespread of these to be *Imperata cylindrica, Ageratum conyzoides, Paspalum conjugatum* and *Cynodon dactylon.* In addition, *Saccharum spontaneum*, *Digitaria adscendens*, *Panicum repens* and *Mikania micrantha* are also regarded to be of major importance in one or more tea growing areas in Asia. It is apparent from this list that perennial stoloniferous and rhizomatous grasses cause the most concern in tea. They spread easily under and between the bushes, and only small fragments of these perennating organs, readily produced during cultivation, are needed to generate a new plant. Unfortunately, this group of weeds is notoriously the most difficult to control using biological methods, due both to their habit (protected meristem and ability to outgrow infection) and evolutionary closeness to major graminaceous crops. In list given by Julien and Griffiths (1998) there are no examples of arthropod species having been released against grass targets as classical biological control agents, since they tend not to be specific to a single grass species (Evans, 1991). Many co-evolved pathogens, however, have a highly restricted host range, attacking a single grass species or even a biotype, and would appear to be the most appropriate agents to consider as natural enemies for weeds within the *Poaceae*.

In additions to these factors, many grasses have some economic value, which can preclude from consideration as potential CBC targets. In Table 1 four species (*Leersia hexandra, Cynodon dactylon, Pennisetum clandestinum* and *Saccharum spontaneum*) could not be targeted, and even the highly noxious *Imperata cylindrica* may be considered too valuable as a source of thatch. However, with this later species, two seed infecting smuts are recorded (Evans, 1987) this would have the advantage of reducing spread and re-infestation of a cleared area by affecting seed production, but still allow the survival of desirable stands of the grass.

There are three species listed (*Covolvulus arvensis, I. cylindrical* and *P. repens*) that have mycoherbicides under development for their control outside Asia, although no products are currently on the market (Chandramohan and Charudattan, 2003; Defago *et al.*, 2001; El Sayed *et al.*, 2001). Others such as *Commelina benghalensis, Bidens pilosa* and *Pennisetum polystachion* being pathogens potentially suitable for mycoherbicide development have been identified. For weeds with significant economic or cultural value, mycoherbicides may be the only biological method of control that can be considered.

Table 1 lists at least four serious (or significant) weeds of tea that have been successfully controlled by the introduction of their natural enemies somewhere in the world (in these cases all are insects agents); these are *Chromolaena odorata, Lantana camara, Mimosa invisa* and *Sida acuta*. In addition, *Mikania micrantha* is close to having a rust agent released in 2004 in India, and is presented as a case study in the next section below. Clearly, these above examples should be the initial focus of a programme to tackle tea weeds since they are likely to yield significant returns for minimal inputs. Little or no information is available concerning the natural enemies of eight of the weed species listed, but for many of them nobody has actually looked and a number of them warrant some inputs.

Table 1. Major weeds of tea in Asia and their biological control potential (Crop Protection Compendium, 2001; Holm *et al.*, 1977; http://www.cabdirect.org).

Weed species, family, common name, botanical details	**Region in Asia where weed is a major problem in tea**	**Origin**	**BC potential, notes and references**
Ageratum conyzoides L. *(Asteraceae)*, goat weed, annual herb, seed	Sri Lanka, N.E. India, Japan, Taiwan, Malaysia, Indonesia	Tropical America	Scant information on natural enemies in native range, but it is not a serious weed there suggesting it may be a potentially good target for CBC (Holm *et al.*, 1977; Waterhouse, 1994).
Bidens pilosa L. *(Asteraceae)*, Cobbler's pegs, annual herb, seed	S. India	Tropical America	Leaves sometimes eaten by humans. Has been assessed for BC potential with pathogens by Evans (1987). A number of interesting species have been recorded particularly in its centre of origin. Unfortunately, its weedy status in native range suggests it may not be a good target for CBC (Holm *et al.*, 1977).
Chromolaena odorata (L.) R.M. King & H. Robinson *(Asteraceae)* archangel, perennial scrambling shrub	India (not a major weed)	Central to South America	Used for fallow cover. CBC successfully undertaken in Indonesia (McFadyen *et al*, 1999; Waterhouse, 1994). Although not a major tea weed, CBC is a proven control option. Pathogens have also been assessed (Elango *et al.*, 1993).
Commelina benghalensis L. (*Commelinaceae*)Wandering Jew, benghal dayflower, annual or perennial herb, seeds and stolons	India (not a major weed)	Old World tropics (possibly India or East Africa)	Poor fodder. Potential pathogens for CBC identified (Holm *et al.*, 1977; Evans, 1987; Waterhouse, 1994; Ellison and Barreto, 2004). Although not a major weed in tea, a rich mycobiota has been recorded from this species, worthy of further study.
Convolvulus arvensis L. (*Convolvulaceae*), bindweed, climbing perennial herb, shoots from stems, seed	Sri Lanka	Europe	CBC undertaken in North America with insects, control not achieved (Julien and Griffiths, 1998). Considerable work on mycoherbicides in Europe (Defago *et al.*, 2001; El Sayed *et al.*, 2001). The value of this technology for application in Sri Lanka may be worth investigating.
Conyza sumatrensis (Retz.) E. Walker (= *Erigeron sumatrensis* Retz.) (Asteraceae), fleabane, annual/biennial herb, seed	Sri Lanka, S. India	Brazil	Not successfully controlled by paraquat therefore, increasing problem in tea (Luxmei de Silva and Ranamukaarachchi, 1994). No investigation of biological control potential has been made.
Crassocephalum crepidioides (Benth.) S. Moore, (*Asteraceae*) redflower ragleaf, annual herb, seed	Sri Lanka, S. India	Madagascar, tropical Africa	Not successfully controlled by paraquat; therefore, increasing problem in tea (Luxmei de Silva and Ranamukaarachchi, 1994). No investigation of biological control potential has been made.
Cynodon dactylon (L.) Pers. (Poaceae), Bermuda grass, perennial rhizomatous grass	Indonesia, India, Sri Lanka	Tropical Africa, or Indo-Malaysia	Important economic value (fodder, lawns, playing fields, soil stabilization). Therefore, potential conflicts of interest with CBC. None have been attempted (Holm *et al.*, 1977; Waterhouse, 1994.)

Weed species, family, common name, botanical details	Region in Asia where weed is a major problem in tea	Origin	BC potential, notes and references
Digitaria adscendens (HBK) Henr. (= Digitaria ciliaris [Retz.] Koch), (*Poaceae*), summer grass (suruwari-India), tufted usually annual grass, seed	Taiwan, S. India, Sri Lanka, Japan	Probably Taiwan	Minor value as fodder. Not assessed for BC. Weedy status in native range suggests not a good target for CBC. Although may suggest native range incorrectly identified, may be worth preliminary study (Holm *et al.*, 1977).
Imperata cylindrica (L.) Beauv. (*Poaceae*), cogon grass, alang-alang, thatch perennial rhizomatous grass, seed	N.E. India, Malaysia, Indonesia, Sri Lanka, Japan, Taiwan	Old World, possibly Nepal	Paper making, palatable to stock when young, widely used for thatching (Holm *et al.*, 1977). Conflicts of interest with CBC, although a study by Evans (1987) revealed a number of interesting fungal natural enemies. Mycoherbicides have been investigated in Malaysia (Caunter and Wong, 1988; Yandoc *et al.*, 1999) and there is an ongoing collaborative programme between USA and Africa (Chandramohan and Charudattan, 2003; Charudattan, 2003).
Lantana camara L. (Verbenaceae), lantana, white sage, tick berry, scrambling shrub	Indonesia , India (important but not major)	C. and S. America	Good potential for successful CBC. Extensive CBC programmes implemented throughout exotic range since 1902 and 39 agents released (ex S. America), including three pathogens (Thomas and Elliso, 2000). Nine insects have had significant local impact (Broughton, 2000).
Leersia hexandra Sw. (*Poaceae*), southern cut grass, tall perennial, creeping rhizomatous grass, seed	Indonesia (serious)	Tropical America	Important fodder grass; therefore, potential conflicts of interest with CBC. None have been attempted (Holm *et al.*, 1977).
Mikania micrantha Kunth. ex HKB (*Asteraceae*), mile-a-minute, herbaceous/woody vine, stem node rooting and seed	Assam (serious)	Tropical America	Good potential for CBC, see case study later in the text.
Mimosa invisa Mart. ex Colla. (*Leguminosae*), creeping sensitive plant, spreading biennial/perennial shrub, seed	Indonesia	Brazil	CBC successfully implemented in Australia and PNG using insect agent (psyllid) (Julien and Griffiths, 1998; Kuniata and Korowi, 2001). A reputedly specific isolate of the fungus (*Corynespora cassiicola*) has been recorded from Australia, PNG and Western Samoa, where it causes significant damage under certain conditions (Waterhouse, 1994). It may have potential as a mycoherbicide.
Mimosa pudica L., (*Leguminosae*), sensitive plant, trailing perennial shrub, seed	Indonesia	Tropical America	Very little is known about the natural enemies on this plant in its native range (Evans, 1987). It is certainly worthy of further study (Waterhouse, 1994).
Oxalis corniculata (*Oxalidaceae*), creeping woodsorrel (USA), creeping stoloniferous perennial, seed	India (principal), Japan, Sri Lanka, Indonesia. Taiwan	Europe and America	Cosmopolitan weed. There is very little information on the natural enemies of this weed, although a rust has recently been recorded from China, and a powdery mildew from Korea. The cosmopolitan status on this plant probably precludes it from CBC, but mycoherbicides may we worth investigating (Holm *et al.*, 1977).

Weed species, family, common name, botanical details	Region in Asia where weed is a major problem in tea	Origin	BC potential, notes and references
Panicum repens L. (*Poaceae*), torpedo grass, perennial rhizomatous grass, seed	Sri Lanka, Indonesia, India, Taiwan,	Old world, probably African/Mediterranean origin	Poor fodder value. Very few natural enemies have been recorded, but the plant is not considered to be a weed in its purported native range. Therefore, preliminary surveys are required before a realistic evaluation of the prospects for biological control can be made (Waterhouse, 1994; Holm *et al.*, 1977). Due to a number of economically important species in the genus, pathogens should be the initial focus of surveys. This weed is included in a mycoherbicide that targets a number of pathogens that is currently under development in the U.S.A. (Chandramohan and Charudattan, 2003).
Paspalum conjugatum Berg.(*Poaceae*), sour grass, perennial, stoloniferous grass, seed	Taiwan, India, Sri Lanka, Indonesia	Tropical America	Poor fodder value, suitable only when young. Very few natural enemies have been recorded, but the plant is considered to be an important weed in the Caribbean area, which lessens the CBC potential. However, regions outside the Caribbean could be surveyed (Waterhouse, 1994; Holm *et al.*, 1977).
Pennisetum clandestinum Hochst. (*Poaceae*), Kikuyu grass, perennial, rhizomatous grass	India, Sri Lanka	Tropical eastern Africa	Used as pasture grass and soil stabilizer; therefore, potential conflicts of interest with CBC (Holm *et al.*, 1977).
Pennisetum polystachion (L.) Schultes (*Poaceae*), mission grass, tufted annual or perennial grass, seed	Sri Lanka (locally important)	Tropical Africa	Minor value as fodder crop. There is almost nothing known about the insect natural enemies on this weed (Waterhouse, 1994). However, a significant number of pathogens have been recorded (Herb. IMI, CABI Bioscience, UK), and deserve further investigation.
Saccharum spontaneum L. (Poaceae), sungrass perennial, rhizomatous grass, seed	N.E India, Indonesia	India	Cattle fodder (poor); soil mulch, roof thatching, rope and mat making (Asia); medicinal properties (Philippines); young shoots eaten (Indonesia); used in breeding programmes (believed to be an ancestor of *S. officinarum* [commercial sugar cane]). Conflicts of interest likely to prevent CBC, and in India it is a native plant.
Sida acuta Burman f. (*Malvaceae*), spinyhead sida, perennial shrub or herb, seed	Taiwan (common), Sri Lanka	Central America	Some medicinal value used as a minor source of fibre. CBC successfully implemented in Australia using beetle (Julien and Griffiths, 1998). Evans (1987) assessed the potential of pathogens recorded from this host and concluded that *Mycovellosiella sidarum* recorded from Dominica may have biological control potential.

INVITATION

FOR AUTHORS FOR SUBSEQUENT ENLARGED ISSUE OF IJTS

SPECIAL ISSUE OF IJTS BIOLOGICAL CONTROL OF TEA PESTS

The current issue on Biopesticides contains five papers written by experts. However, it leaves many other biological control measures untouched. This gap necessitates compiling another special issue of the International Journal of Tea Science on biocides for tea. Some of the gaps identified are as follows:

Other Biopesticides for Tea

- NPV and Granulose virus for tea pest control
- BT-based biocides for tea pests
- Fungal antagonists for control of tea diseases
- Entomopathogenic / Entmophagous fingi
- Entomogenous Nematodes
- Parasites for control of tea pests
- Pheromones / sex hormones
- Arthopods for control of tea weeds
- Role of Rhizosphere microbes
- Role of phylloplane microbes in tea pest control
- Botanical pesticides (other than neem)
- Volume of pesticides used in tea-quantification
- Role of nutrients on pest populations
- Environmental factors on pest population-shade, pH, moisture and C
- Allelopathic cover crops to supress succession of weeds in young tea plantations
- Organic tea: chemical-less tea cultivation

The editorial Board of the International Jounal of Tea Science invites authors on biological control in tea to write a comprehensive paper on the subject of their specialisation for this special issue of the International Journal of Tea Science. Intending authors may please contact the Resident editor on E-mail:

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N.K. Jain
Resident Editor

Fig. 1 : a) *Mikania micrantha* smothering tea lawn in Assam. Arrow indicates the vine whips growing above the tea shoots that are ready to harvest; b) the neotropical rust fungus *Puccinia spegazzinii*, infecting *M. micrantha* (ex Assam). Note that the teliospore infections are on all aerial parts of the plant.

CASE STUDY : CLASSICAL BIOLOGICAL CONTROL OF *MIKANIA MICRANTHA* (MILE-A-MINUTE WEED)

Introduction

Mikania micrantha is an extremely serious weed of tea in Assam, where it is causing devastating economic losses by smothering the tea gardens: it reduces yield and seriously interferes with leaf plucking (Figure 1a). It is a perennial, creeping vine that is able to propagate via seeds and can root at the nodes to form new plants. It has an exceptionally fast growth rate, 8-9cm per day has been recorded (Choudhury, 1972). In Assam it is referred to as a 'green tidal wave', invading the tea from the forests as the rains begin. However, it is not a problem in any other tea-growing region, largely because most tea is grown at higher altitudes than in Assam where *M. micrantha* is not able to tolerate the low temperatures. *M. micrantha* is also invading a wide range of other agroecosystems and natural forests over huge areas of the moist tropical zones of Asia (Holm *et al.*, 1977; Waterhouse, 1994). It is still in its invasive phase, for example, in China where it is a serious problem in National Conservation Areas within Guangdong Province and poses a threat to the highly biodiverse region of Hainan Island (Feng *et al.*, 2002; J. Ding, pers. comm., 2001).

Mikania micrantha has a Latin American centre of origin and was deliberately introduced into the old world, particularly for use as a cover crop, probably on several occasions from as early as 1918 (Cock *et al.*, 2000). In northeast India anecdotal evidence suggests that it was used for airfield camouflage in Assam during World War II (Barbora, A.C., pers. comm., 1999). It has certainly been an on-going problem in this region since the 1940s, but its importance has escalated in recent decades due to large-scale degradation of natural forests, from which stronghold *M. micrantha* can invade the tea gardens. This has led to a massive increase in herbicide use which is proving to be detrimental to the marketing of the tea crop, particularly in the EU, due to herbicide residues. Current control focuses on cultural (slashing) and chemical (herbicides) methods that are expensive, often ineffective, not sustainable, and can be environmentally damaging (Sen Sarma and Mishra, 1986; Muniappan and Viraktamath, 1993; Palit, 1981). This led to the development of a project to investigate alternative control options for the weed.

Background To The Programme

Classical biological control of *M. micrantha* was first initiated in 1978, concentrating on insect agents for use in Malaysia and the Solomon Islands. Unfortunately, failure of the first released agent (*Liothrips mikaniae* [Thripidae]) to establish discouraged further investment (Cock *et al.*, 2000). Nevertheless, the weed problem continued to increase and this time Indian scientists raised the issue of the need for a sustainable method of control. A collaborative project was established, that ran between 1996 and 2000, to investigate an IPM approach for the control of the weed in the Western Ghats and Assam. It was funded by the UK Department for International Development (DFID), through the Natural Resources Institute's Crop Protection Programme. 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. The work included mapping the distribution and monitoring the spread of the weed assessing its socio-economic impact on homegarden subsistence agriculture investigating control methods and evaluating fungal pathogens as biological control agents (mycoherbicides and classical introductions) (Sankaran *et al.*, 2001; Ellison, 2001). Surveys in India have revealed no indigenous natural enemies that could effectively be used to control *M. micrantha*. It was concluded that classical biological control (CBC) using co-evolved natural enemies was the most appropriate long-term solution for the control of this weed. It is rarely a weed in its native range in the America and natural enemies are seen to exert a significant pressure on the occurrence and abundance of the species (Parker, 1972; Cock *et al.*, 2000).

Implementation Of The Programme

The project evaluated a broad range of fungal pathogens that occur on *M. micrantha* in its neotropical native range (Barreto and Evans, 1995). From this evaluation a co-evolved, autoecious (does not require a secondary host), microcyclic (reduced number of spore stages in the life-cycle) rust species, *Puccinia spegazzinii* de Toni, was selected for further assessment as a potential CBC agent. It was highly damaging to its host in the field causing leaf, petiole and stem infections leading to cankering and whole plant death, and was not found in the exotic range of the weed (Figure 1b.). This rust is widely distributed on *M. micrantha* throughout the native range of the plant, including at different altitudes (from near sea level to ~1200m), which suggested that the pathogen has a broad environmental tolerance.

Isolates of *P. spegazzinii* from 6 countries (Trinidad, Costa Rica, Peru, Brazil, Argentina and Ecuador) were evaluated in the CABI Bioscience UK intermediate, quarantine glasshouse, for their pathogenicity towards accessions of *M. micrantha* collected from all over the invasive range of the species in Asia. This work is on-going as more countries consider the rust for introduction. A representative selection of the rust isolates (7) is continuously maintained at CABI Bioscience for pathogenicity screening of new accessions of the weed. For India, an isolate of *P. spegazzinii* from Trinidad was considered to be the prime candidate for introduction (Ellison, 2001). This pathotype proved to be virulent against a wide range of Indian isolates of the weed. A full evaluation of the pathotype was undertaken focusing on host specificity testing and evaluation of biological parameters, following the guidelines set out in the FAO Code of Conduct (FAO, 1996).

The host specificity testing forms the basis of the risk analysis that is undertaken before any CBC agent is released in a new environment. The centrifugal, phylogenetic testing sequence was used to select the test species (Wapshere, 1974). This tried and tested procedure selects plants based on their taxonomic relatedness to the target species, selecting example species from the same genus, tribe, other tribes in the same family, etc. This works particularly well for co-evolved pathogens, since their reliance on their host plant has evolved over millennia, involving many genes, and enabling them to survive on only one or a limited number of predictable, closely related species. Thus, it is logical to test these first, although, to allay the understandable fears of the public, a much broader range of species, including crop plants are also included in the screening. For *P. spegazzinii,* more than 60 non-target plants have been challenged with the rust, including seven other *Mikania* species; none of these became infected.

In addition to confirming the life-cycle and infection process of *P. spegazzinii,* the interaction of the rust to key environmental parameters were also investigated, including temperature tolerance and dew period requirement. The latter is the length of time that free water (either rain or dew) is required for the rust infective spores (basidiospores) to infect the plant. It was found that the rust was able to infect at temperatures ranging between 15 and 25°C and also after less than 10 hours of dew (Ellison, 2001). Both of these are compatible with the prevailing environmental conditions in the Asian invasive range of *M. micrantha.*

The above data formed the core of the dossier that was produced by CABI Bioscience for the Indian collaborators (Ellison and Murphy, 2001). This was submitted to the India Directorate of Plant Quarantine and Storage (IDPQS) by PDBC. A letter was included 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-carriage of the rust to the National Bureau of Plant Genetic Resources, New Delhi was undertaken in September 2003, where it was established. The rust is currently being checked for purity and final host range testing is being undertaken under quarantine, before a release permit can be issued by the IDPQS. Release in the Western Ghats and Assam is planned for the start of the rains in 2004. A similar implementation programme commenced in October 2003, for China, funded by the UK-DEFRA Darwin Initiative.

Potential Field Efficacy

From extensive field observations within both the native and exotic ranges of *M. micrantha,* and investigations under quarantine in the UK, the potential of *P. spegazzinii* to have a significant effect on this weed in its invasive range is high. Although a suite of natural enemies may be required to achieve significant control of some invasive alien weeds, *P. spegazzinii* may well prove to be the 'silver-bullet'. It is predicted that, if released in the moist forest regions of India, the rust would establish and spread rapidly under the prevailing environmental conditions. It should exert unilaterally a significant effect on the abundance and spread of the *M. micrantha* populations within a few growing seasons. In the long term, it is predicted that the growth and fecundity of *M. micrantha* would be severely affected over its entire range, reducing the weed population to a level close to that in its native range: a minor component of the flora. Thus, the weed should no longer pose a threat to the agricultural economy of the affected regions, nor to natural forest ecosystems (Ellison and Murphy, 2001).

Release Strategy

Puccinia spegazzinii is a biotrophic pathogen: it is not able to survive without its host and thus cannot be produced *in vitro*. There are two spore stages; teliospores that are embedded as pustules in the host plant tissue and basidiospores, which are produced and released from the teliospores under conditions of high humidity. It is the basidiospores that infect new susceptible host material and from which teliospores are formed within the host tissue. Consequently, release strategies must involve living infected plants.

Inoculative Strategy

The simplest low-tech strategy involves placing the pots of rust infected plants with young pustules at strategic points within severely weed-infested areas as foci of infection. It is important to give the rust optimum conditions to encourage infection and establishment: infected plants should be placed in cool, shaded positions within the weed canopy (amongst plants with fresh new growth) in the evening, ideally shortly after rainfall. The rust infected plants can even be planted in the soil to minimise the risk of the soil in the pot drying-out. This will enable survival of the inoculum until suitable conditions for infection occur, since it is difficult to predict the weather conditions. This approach requires minimum human input and is suitable for initiating epiphytotics within large weed infestations in forestry and natural habitats. However, due to the nature of CBC, a significant impact on the weed populations is unlikely to be observed for a number of years, usually between five and ten.

Inundative strategy

To encourage a quicker impact, for example in high maintenance agriculture situations such as tea plantations, an inundative strategy is proposed employing the same basic principal as the inoculative strategy. Large numbers of infected plants will be placed within the new flushes of *M. micrantha* at the start of the rains, at optimum intervals to establish an early season epiphytotic. This will help mitigate the natural lag phase before the impact of a CBC is realised, and thereby avoid the naturally slow build-up of the pathogen in the *M. micrantha* populations at the start of the rainy season. Infected plants can be produced en masse in purpose built/dedicated propagation units and distributed in the plantations. It is considerably more labour intensive than an inoculative strategy, but will pay dividends through reduction in labour requirements throughout the season preventing contamination of the tea directly by the *Mikania* leaf and potentially reducing herbicide usage. A prototype rust production unit is currently under construction at Assam Agricultural University, India (contact: Dr. K.C. Puzari). It is envisaged that this approach will prevent *M. micrantha* from fully establishing in the crop, and that the rust will then maintain the weed at low levels throughout the rest of the season by natural cycling. In the long-term it may prove unnecessary to continue to adopt this inundative approach since, as discussed above, weed populations should substantially decline throughout its exotic range and the weed should no longer be considered invasive.

DISCUSSION AND CONCLUSIONS

Plant pathogens are potentially valuable additions to the arsenal of weapons for use against weeds in tea. Invasive alien weeds make up the majority of the major tea weeds in Asia, and CBC offers a cheap, pollution free, proven and sustainable method for their management. However, fungal natural enemies have only been considered as potential agents for a relatively short period of the history of CBC, there have been significant successes with the 25 or so exotic fungal pathogens that have been exploited (Evans, 2002). A number of these have been responsible for spectacular financial and environmental returns. *Mikania micrantha* is an excellent example where this new generation of classical biological control agents, namely fungal pathogens, can make a real difference to the lives of farmers since, even with no inputs, it is anticipated that significant control will be achieved by the release of the rust fungus *Puccinia spegazzinii.*

CBC, as a technology, is usually developed by governments and aid agencies. Although the benefits of this approach can be strikingly high for agriculturalists (as well as the natural environment), there are no traditional profits to be made (CBC is self perpetuating, and thus free at the 'user' level) which prevents company interest. However, in Australia, for example, CBC of rangeland weeds is financially supported by Cattle and Sheep Ranchers Associations, as well as the Wool and Meat Industries of the affected states. In Asia, it may be feasible for a number of large tea estates in a region to form a consortium and fund a BC programme against their most important weed (i.e. one that is not successfully controlled by conventional methods). Or perhaps the local university may consider applying for inter-government aid such as from the Asian Development Bank (ADB). Small scale funding could initially be sought for a weed assessment study including a socio-economic analysis. This would enable the correct target selection and approach to be established before the funding of a full programme. In a turbulent market, tea grown for high returns, such as organic tea, may encourage the development of natural solutions to weed problems, not only because of the expense of other control methods, but because chemical herbicides may be prohibited.

CBC is sometimes the 'silver bullet' needed for the control of an alien weed, but more often this approach should be considered as part of a integrated pest management strategy (Altieri and Doll, 1978; Labrada, 1996). For example, judicious use of pesticides at critical times can improve the efficacy of a CBC agent, similarly augmenting an agent or manually reducing a weed at the critical point of the season may eliminate the need for further punitive action for the rest of the season. Indeed in developing countries, CBC may prove to be the only sustainable method of controlling the exotic agricultural weed flora: however, more research effort is required to realise the full potential of this method as part of an IPM approach in cropping situations.

Bioherbicides technology is still very much in its infancy and has not had the impact on agricultural weed control, as was perhaps anticipated from the initial successes (Evans *et al.*, 2001a). This does not mean that some excellent products may not become available in the future for weed control in tea. There is a growing public drive to reduce reliance on chemical herbicides and this should increase that amount of investment in the development of natural products throughout the world. Hopefully, this will enable the well-documented constraints in formulation, production and marketing of bioherbicides to be overcome. On large tea estates, which regularly use chemical herbicides to control one or a few dominant weeds, that could not be controlled using CBC due to conflicts of interest, the development of a mycoherbicide may be worth considering. However, resource poor farmers are unlikely to adopt this technology, particularly if chemical herbicides do not form part of their current weed control practices.

Economic constraints (rejection of tea with herbicide residues) increase the value of developing BC programmes against weeds of tea. Increasing incidences of herbicide resistance in weed populations, together with the all too apparent ecological damage and human risk caused by the indiscriminate use of chemical herbicides, emphasise the need to explore and exploit all alternative methods of weed control such as the use of fungal natural enemies.

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