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Fungal Biocontrol Agents of Weeds

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Introduction

Weeds are an ever-present and increasingly significant constraint to agricultural production worldwide. Ironically, more 'advanced' and changing cultivation practices have led to weeds becoming more rather than less problematic, especially in the major cereal food crops. For example, in rice (*Oryza sativa* L.), labour shortages and rising costs have resulted in a widespread shift from the traditional transplanting culture to direct seeding, whilst the development of hardy upland rice varieties has meant that cultivation is expanding into drier ecosystems, where a radically different weed biota has to be overcome. Such factors, coupled with water shortages in irrigated rice systems, have resulted in increased weed pressures, particularly from grassy weeds (Baker and Terry, 1991). Similar problems are mirrored in other crop systems.

The overall impact of weeds on crop production can, at best, only be crudely calculated, with average losses varying from 10 to 20%. In the USA alone, it has been estimated that some US$15 billion (thousand million) are lost annually due to weeds (Bridges, 1994). In such highly developed, agriculturally mechanized countries, with extensive crop monocultures, production levels can only be maintained through the regular and wholesale application of pesticides, particularly of chemical herbicides, which account for almost 50% of the agrochemical market (Woodburn, 1995). However, the use of herbicides as a management tool is becoming more problematic due to the increasing occurrence of herbicide-resistant weed populations, especially in wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) (LeBaron and Gressel, 1982). Thus, new strategies for the long-term management of agricultural weeds need to be developed: or 'new tools for the toolkit', which at present comprises only chemical herbicides and cultural–mechanical options. In developing countries, however, the economics and logistics inherent in applying pesticides often dictate against their use, most notably in Africa, where manual weeding, predominantly by women, is the norm. In terms of human resources alone, which could be better invested in

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other more meaningful pursuits, such social costs are a continual hindrance to development.

As international travel and global trade increase, more plant species, with potentially weedy traits, are being moved around the world, giving rise to new, invasive weed problems, particularly in non-agricultural or natural ecosystems (Mack *et al.*, 2000). In these situations, alien or exotic weeds now contribute the major threat to biodiversity after habitat destruction (Bell, 1983; Heywood, 1989; Cronk and Fuller, 1995; Binggeli, 1996). Moreover, weed control is rarely practised, either because it is environmentally hazardous, as in the case of chemical herbicides, or prohibitively expensive, as with cultural–mechanical techniques, or simply impractical over extensively infested areas – although, more typically, it is a combination of all these factors. Thus, once again, alternative strategies need to be resourced and integrated into a management plan if the ongoing threat from invasive weeds is to be met.

In addition to weed infestations, crops are also subject to attacks from and are frequently devastated by fungal pathogens. In particular, exotic diseases have been and continue to be a significant threat to agriculture and have the potential to destabilize economies on a local as well as on a global scale, with serious socio-economic consequences (Large, 1940; Kingsolver *et al.*, 1983). Can this destructive power of plant pathogens be harnessed for humans' advantage and directed towards and integrated within an effective weed management strategy? The aim of the chapter is to try to answer this question by: monitoring the progress to date; analysing the problems involved; and assessing the long-term potential of biocontrol of weeds using fungal agents.

Fungal Biological Control Agents

Fungal biological control agents (BCAs) can be exploited for weed management using two seemingly distinct strategies: the inoculative or classical approach, and the inundative or mycoherbicidal approach. However, as will be shown, these need not necessarily be mutually exclusive.

The inoculative approach

Concepts

The inoculative or classical approach involves the use of host-specific or co-evolved fungal pathogens to control alien, highly aggressive and invasive weeds for which cultural and chemical methods have either failed or are inappropriate for economic or environmental reasons. The principles and protocols involved have been developed and employed by entomologists with considerable success since the early 1900s (Julien and Griffiths, 1998; McFadyen, 1998). The philosophy underpinning this approach is simple and is based on the assumption that, within natural or primary ecosystems, the component organisms are in a dynamic equilibrium, and that natural enemies play a pivotal role in the regulation of the plant or animal populations. When organisms are freed of these constraints, such as occurs after they are deliberately or accidentally introduced by humans into new or exotic ecosystems, then their fitness increases in relation to that of the indigenous flora or fauna. Thus, given favourable conditions for growth and reproduction, their populations can increase unchecked and, eventually, the alien organism may reach pest status. Characteristically, many of the alien weeds which are now problematic in agricultural ecosystems are only minor weeds in their native ranges (Barreto and Evans, 1997). Those affecting natural ecosystems are rarely weeds in their centres of origin and may even be economically useful or ecologically important members of the indigenous flora (Barreto and Evans, 1988; Cronk and Fuller, 1995).

Progress

Despite the fact that insect natural enemies have been evaluated as BCAs of alien, invasive weeds and employed against them for more than a century, the exploitation of fungal BCAs has a relatively short history, dating back to only the 1970s. The first pioneering projects – one using a rust fungus (*Puccinia chondrillina* Bubak & Sydenham) against a weed (*Chondrilla juncea* L.) of Mediterranean origin in southeast Australia, the other involving the importation of a white smut (*Entyloma ageratinae* Barreto & Evans) into Hawaii for control of a rare Mexican plant (*Ageratina riparia* (Regel) K. & R.), which was invading upland pastures and natural forest ecosystems – were spectacularly successful (Fig. 6.1). In the case of *C. juncea* (skeleton weed),

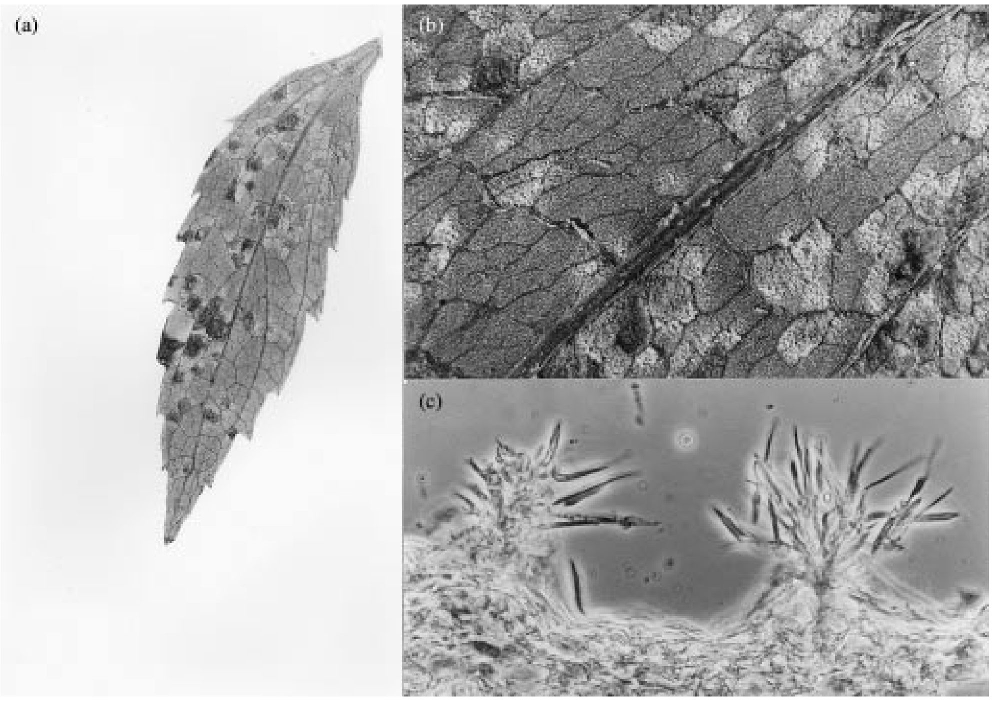

Fig. 6.1. White smut, *Entyloma ageratinae*, on mist-flower, *Ageratina riparia*. (a) Symptoms on mist-flower leaf (lower surface), collected in Veracruz, Mexico, showing pale green and necrotic lesions. (b) Close-up of above showing aggregated, white fructifications (caespituli). (c) Section through caespituli showing sporidial production; the spores are aseptate and thus distinguished from the cercosporoid group of fungi, in which the pathogen was initially classified following its release in Hawaii in 1975. The white smut achieved substantial to complete control of the invasive target weed and was later successfully introduced into South Africa in 1989 (Morris, 1991). It has recently been released in New Zealand (1998), where it has readily established and even exceeded its predicted impact on weed populations (Anon., 1999).

infestations in wheat were reduced by more than 99% to densities approaching those in the native range, and with benefits estimated at *c.* US$15 million per annum (Burdon *et al.*, 1981; Cullen and Hasan, 1988; Tisdell, 1990). For *A. riparia* (mist-flower) in Hawaii, agricultural land has been rehabilitated and natural ecosystems have been reclaimed and protected from further invasion (Trujillo, 1985; Davis *et al.*, 1992).

The question could be asked as to why the inoculative approach using fungal pathogens for biocontrol of weeds was not adopted earlier and, paradoxically, not rigorously pursued, since these initial successes, by biocontrol practitioners in general and plant pathologists in particular. There seems to be little doubt that this was and still is due mainly to concern about the safety aspects of transferring exotic plant pathogens between countries or geographic regions, especially by regulatory authorities. Indeed, many countries, including the UK, still have no specific protocol relating to the importation of fungal pathogens for biological control (Tatchell, 1996). The risks involved and the protocols developed to assess, minimize and predict these are dealt with in detail elsewhere in this book (see Goettel *et al.,* Chapter 13). Suffice it to say that extensive host-range testing and fundamental studies on the biology and ecology of the potential agents are essential prerequisites before any exotic fungal pathogen can be proposed for introduction into the target country (Adams, 1988; Weidemann and TeBeest, 1990; Watson, 1991; Weidemann, 1991). Such proposals are also subject to vetting by the in-country quarantine or legislative body, usually empowered with keeping out rather than bringing in exotic plant pathogens, before the selected agent can finally be approved for release.

Historically, Australia has played a key role in developing the protocols and in funding the majority of projects for the inoculative or classical use of fungal BCAs, and this recent history has been thoroughly reviewed (Wapshere, 1975, 1989; Hasan, 1980; Cullen and Hasan, 1988; McRae, 1988; Wapshere *et al.*, 1989; Evans and Ellison, 1990; Hasan and Ayres, 1990; TeBeest *et al.*, 1992; TeBeest, 1993; Mortensen, 1997; McFadyen, 1988; Evans, 2000). These should be consulted for a more comprehensive coverage of the subject. This review will concentrate on the present history, as well as on the long-term future of the inoculative approach.

Thus far, over 20 exotic fungal pathogens have been deliberately imported and released as classical BCAs of weeds worldwide, nearly half of these within the last 4–5 years (Julien and Griffiths, 1998), and a number of others are waiting in the wings (Evans, 2000). Most are obligate biotrophs, with the majority pertaining to the rust fungi (*Uredinales*), chosen because of their proved host specificity, their ability to disperse rapidly and efficiently over vast areas and their destructive powers, as evidenced by their impacts on cultivated crops. Several others have been illegally introduced, most notably blackberry rust, *Phragmidium violaceum* (Schulz.) Wint., in Australia in 1984, probably by farmers frustrated by the slow progress of the government-sponsored efforts (Marks *et al.*, 1984; Field and Bruzzese, 1985). Although none of the subsequent introductions of exotic fungal BCAs have yet to achieve the spectacular successes of the first releases, there is increasing evidence that there may be extended lag phases before the pathogens 'kick in' and begin to have a significant impact on weed populations. For example, an Australian gall-forming rust fungus, *Uromycladium tepperianum* (Sacc.) McAlpine, was introduced into South Africa in 1987 as a potential BCA of Port Jackson willow, *Acacia saligna* (Lab.) Wend., one of many *Acacia* spp. invading natural ecosystems in that country. However, the initial results were disappointing (Morris, 1991), and it is only within the last few years that the rust has built up to epiphytotic levels, with as many as 1500 galls developing on each tree. The continual pressure on the growing points effectively kills the trees and weed populations have been reduced by 90–95%, with a corresponding increase in the native vegetation (Morris, 1997, 1999).

Another rust BCA that is now beginning to have an impact on its target weed host is *Maravalia cryptostegiae* (Cummins) Ono (Fig. 6.2), closely related to coffee-leaf rust, which was released in Queensland, Australia, in 1994, in an attempt to halt the spread of rubber-vine weed, *Cryptostegia grandiflora* Roxb. ex R. Br., originally imported from Madagascar as an ornamental (Evans, 1993; Evans and Tomley, 1994). This asclepiadaceous woody climber has been described as the single biggest threat to biodiversity in tropical Australia (McFadyen and Harvey, 1990). Because of the vast areas involved and their remoteness, helicopters have been used to disperse rust inoculum

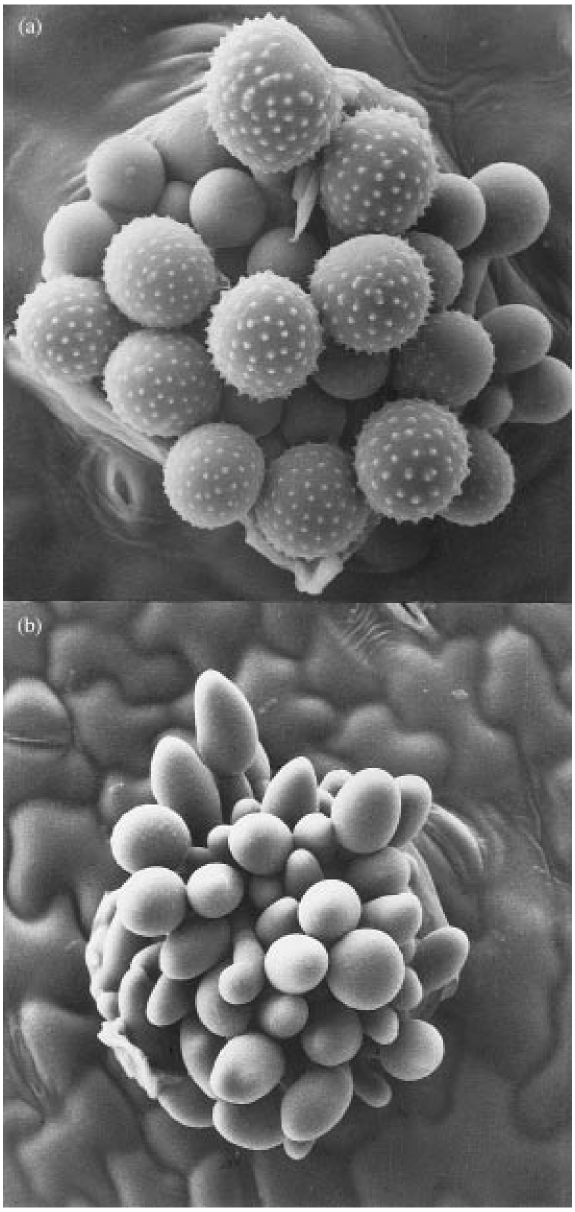

Fig. 6.2. The rust, *Maravalia cryptostegiae*, a biological control agent of rubber-vine in Australia. (a) SEM of uredinioid teliospores bursting through the lower leaf surface of *Cryptostegia grandiflora*. These spores are multifunctional fulfilling dispersal, survival and sexual roles (Evans, 1993). (b) SEM of thin-walled, non-resting telioid teliospores produced during sustained periods of high humidity but which appear to be non-functional in the partially expanded life cycle (Evans, 1993). The rust was released in Queensland in 1994 and rapidly caused severe and repeated defoliation of rubber-vine weed infestations (Tomley and Hardwick, 1996), which has been sustained leading to the resurgence of the native vegetation (A.J. Tomley, personal communication).

– initially, crudely, in the form of infected tissues, latterly by spraying spore suspensions with motorized mist-blowers into the forest canopies. Thus, the inoculative approach is being 'fast-tracked' by an inundative application strategy. A similar combination of strategies is also being exploited in the Northern Territory of Australia to combat the invasive, woody weed *Mimosa pigra* L. (or giant sensitive plant), which currently occupies large tracts of floodplains in this region and poses a potential threat to its prestigious national parks. In addition to a suite or guild of insect BCAs, two fungal pathogens have been introduced from Mexico (Fig. 6.3). The monotypic rust, *Diabole cubensis* (Arth. & John.) Arth., which causes defoliation, is mainly active during the dry season, whilst the hemibiotrophic ascomycete, *Sphaerulina mimosae-pigrae* Evans & Carrion (anamorph: *Phloeospora mimosae-pigrae* (Evans & Carrion), is a wet-season pathogen causing petiole and stem cankers (Evans *et al.*, 1995). It is anticipated that the two fungal BCAs will complement each other to exert pressure on the impenetrable weed thickets over the whole year. As an added bonus, it was later discovered that the anamorph is capable of yeast-like growth *in vitro*, producing a succession of viable and infective conidia by budding. These are now being formulated in methyl cellulose, and applied as a mycoherbicide with a knapsack sprayer (Forno *et al.*, 1996). Once again, these two seemingly distinct approaches to weed management have led to a common implementation strategy.

From an analysis of all the classical weed biocontrol projects which have reached this implementation phase, it becomes clear that, for certain invasive weeds, there is a 'silver bullet' solution. Notably, skeleton weed in Australia, mist-flower in Hawaii and now Port Jackson willow in South Africa have all been brought under complete control: and a single, classically released fungal BCA has been the principal cause of their demise. There is recent evidence to indicate that the same may apply for the rubber-vine weed in Queensland (A.J. Tomley, personal communication). Some outstanding examples can also be drawn from the entomological literature on the classical biocontrol of weeds (McFadyen, 1998). For other invasive weeds, however, there is no such simple and elegant remedy, and a guild of natural enemies (both insect and fungi) may be required to achieve the desired level of control, often integrated with more traditional management practices. Such will probably be the case with the giant sensitive plant in the Northern Territory and also certainly the same situation exists with parthenium weed (*Parthenium hysterophorus* L.) in Queensland, Australia. This composite weed of neotropical origin, which was accidentally introduced into both Australia and India within the last 30–40 years, is now the number one terrestrial weed target in the latter country, as well as in Queensland, where it not only constitutes an agricultural problem, invading both crops and grazing land, but also poses a serious health hazard due to its many allergenic properties (Evans, 1997). Biocontrol has been a major component of its management strategy in Australia, where, in addition to eight insect BCAs, two rust fungi from its Mexican native range have recently been introduced. The weed appears to have a significantly greater geographic range than any of its natural enemies, thus complicating the control strategy and necessitating a multi-release inoculative approach. One of these rust species, *Puccinia abrupta* Diet. & Holw. var. *partheniicola* (Jackson) Parmelee, has a predominantly subtropical, semi-arid distribution in Mexico, whilst the other (*Puccinia melampodii* Diet. & Holw.) is mainly restricted to the humid-tropical zones. Therefore, as the weed continues to invade other ecosystems in eastern Australia, spreading from its semi-arid rangeland foothold, a range of ecologically adapted BCAs will need to be deployed.

An analysis of the current work in exploring for and the exploitation of fungal

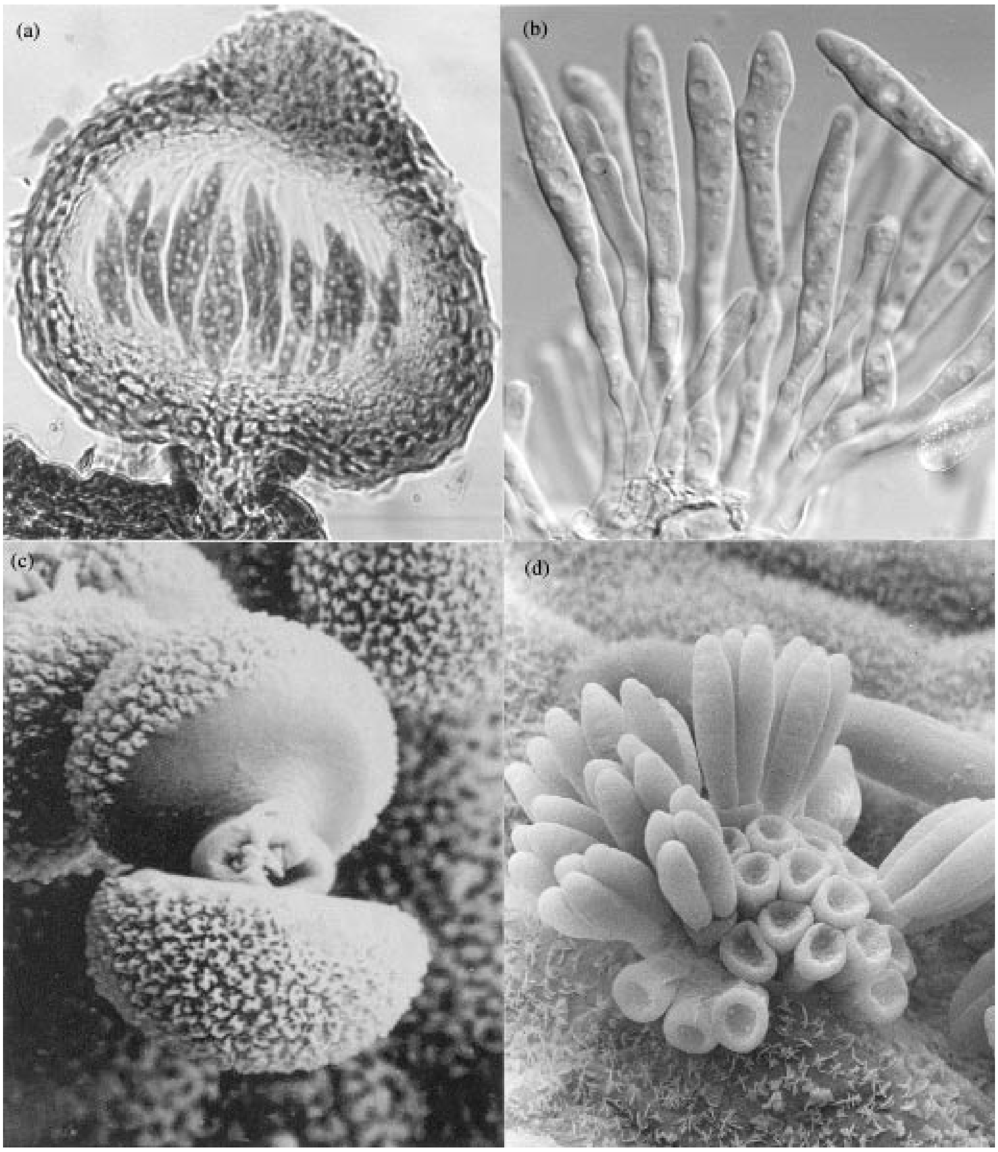

Fig. 6.3. Potential fungal biocontrol agents of *Mimosa pigra*. *Sphaerulina mimosae-pigrae* (a) and its anamorph *Phloeospora mimosae-pigrae* (b), released in the Northern Territory in 1994; both stages can be produced on still-green host tissues prior to necrosis and thus the pathogen can be classified as a hemibiotroph. (c) *Diabole cubensis*, a monotypic rust showing the highly distinctive unicellular, paired teliospores, which was introduced into Australia in 1996. (d) Hypophyllous fructification (basidioma) of *Microstroma ruizii-belinii* Evans & Carrion (*Exobasidiales*). Relatively little is known about the biology and taxonomic affinities of these basidiomycetes; hence it has not been evaluated further as a BCA. Note the production of four basidiospores from each basidial cup in the basidioma, and four sterigmatal scars at the base of empty cups.

pathogens for classical biocontrol of weeds shows that most is being directed towards subtropical and tropical ecosystems. Evans (1987) discussed the possibilities of applying the inoculative approach to a number of invasive tropical weeds; amongst them, *Lantana camara* L., *Rottboellia cochinchinensis* (Lour.) Clayton and *Mikania micrantha* H.B.K. were targeted. Since then, funding has been realized to explore the potential of exotic fungal pathogens for their control (Evans and Ellison, 1990; Evans, 1995a). In view of the fact that all these projects are now nearing completion, it is considered relevant here to highlight the problems involved and to detail the progress to date.

LANTANA CAMARA. Over the past century, this neotropical verbenaceous shrub has become a serious pan-tropical invasive weed of both agricultural and conservation land in Africa, Asia, Australasia and Oceania and is of particular ecological importance in small island ecosystems (Holm *et al.*, 1977; Cronk and Fuller, 1995). In Australia, for example, almost 4 million ha are now infested by the weed, with an estimated Aus$17 million being spent annually in Queensland state alone on clearing operations (Tomley and Evans, 1995). This weed occupies a seminal position in biocontrol history since this was one of the first pests to be targeted for the inoculative approach, dating back to the 1890s (Funasaki *et al.*, 1989). Of the many agents released over this period, all, until very recently, have been insect natural enemies (Julien and Griffiths, 1998). However, McFadyen (1998) has concluded that the biocontrol programmes in most of the target areas, perhaps with the exception of Hawaii, should be viewed as failures.

The extreme plasticity of *L. camara*, due to natural and human-mediated hybridization, makes this a difficult target, but two rusts, amongst other potential pathogens (Barreto *et al.*, 1995), are now showing promise since they both attack the major weed biotype in Australia. A strain of a leaf rust, *Prospodium tuberculatum* (Speg.) Arth. from Brazil, will probably be released in 2001, after its importation is approved by Australian Quarantine and Inspection Services. From greenhouse studies of its infection parameters and host range, it is expected to exert some pressure on the weed, at least in subtropical regions. Recently, an extremely virulent isolate of a microcyclic rust from the Peruvian Amazon, *Puccinia lantanae* Farlow, has been evaluated and, because it can attack both petioles and stems, as well as leaves, causing cankering and girdling, it is predicted that this rust strain could have a considerable impact on weed populations in tropical Australia. The importance of strain selection cannot be over-stressed, and this Amazonian isolate is the only one of many observed in the field in South and Central America that consistently invades and kills stem tissue.

ROTTBOELLIA COCHINCHINENSIS. *R. cochinchinensis* or itch grass is a plant of Afro-Asian origin which is now a major invasive weed in Latin America, especially in graminaceous crops (Evans, 1991; Ellison and Evans, 1995). The initial strategy was to develop a mycoherbicide based on a co-evolved necrotrophic fungal pathogen rather than a biotroph, this being the first deliberate attempt to combine both inoculative and inundative approaches. However, despite the fact that the *Colletotrichum* sp. selected and field-evaluated in Thailand proved to be undescribed and specific to the weed – too specific in fact, since it only infected a single biotype – it was adjudged to be politically sensitive to introduce an alien species of *Colletotrichum* into the New World. Two biotrophic fungi, the rust *Puccinia rottboelliae* P. & H. Sydow and the smut *Sporisorium ophiuri* (P. Henn.) Vanky, were considered to offer potential as inoculative agents. Following preliminary greenhouse evaluation, a strain of *S. ophiuri* from Madagascar was selected for further specificity screening. This head smut systemically infects weed seedlings in the soil and completely replaces all the inflorescences. Since seeds are the only means of propagation and perennation and the seed bank is short-lived, the smut appears to have the potential to significantly affect weed populations (Smith *et al.*, 1997). A proposal and protocol for importing the smut into Costa Rica, based on Food and Agriculture Organisation (FAO) guidelines (FAO, 1996), have now been accepted by the quarantine authorities of that country (Reeder and Ellison, 1999), and it is expected that this BCA will be released within the year. This project is breaking new ground since it is the first time that a true smut has been exploited as a classical BCA, the first time that a grass weed has been targeted for the inoculative approach (Evans, 1991) and the first time that any exotic plant pathogen has been introduced into Central America for weed control. Once released, of course, the smut will eventually disperse to all the countries in the region where the weed is present, including the southern USA. Significantly, perhaps, international quarantine legislation does not cover such movement of fungal pathogens, and Costa Rica is under no legal obligation to inform its neighbours of the importation.

MIKANIA MICRANTHA. This rampant climbing plant, one of several to share the epithet mile-a-minute weed, is a neotropical composite species with a native range extending from southern Brazil to Mexico. It has been a long-standing problem in plantation crops in Malaysia (Waterhouse, 1994) and northeast India (Parker, 1972), but is only a relatively recent arrival in the western Indian states, where it invades and smothers both natural and managed forests, as well as crops at the forest interface. In this biodiverse forest region, the weed has been the target of a classical biocontrol project over the last 4 years and an assessment of a microcyclic rust fungus, *Puccinia spegazzinii* de Toni, orginally highlighted as a potential inoculative BCA by Evans (1987) and Barreto and Evans (1995), has recently been completed. Rust strains were collected throughout Latin America, including the Caribbean. Eleven of these have now been screened against a comprehensive range of plant biotypes, which were collected and then characterized molecularly not only in India but in all the palaeotropic countries where it has become an invasive weed problem. A rust strain from Trinidad has proved to be highly pathogenic to all the major weed biotypes and is specific to *M. micrantha*, attacking not only the leaves but also the petioles and stems, leading to ring-barking and death. Other potential agents, identified during the initial field surveys (Barreto and Evans, 1995), also cause severe leaf damage but it is this ability of *P. spegazzinii* to kill the stems, and thus prevent further regrowth, which suggests that this pathogen will provide the 'silver bullet' solution. This will be an important test case for India since the inoculative approach has rarely been used as a weed management strategy. Thus, the in-country quarantine authorities will be on a learning curve regarding exotic plant pathogens, and great caution will need to be exercised in developing the protocol for importation.

Potential

There seems to be no doubt that the classical or inoculative approach can be an extremely effective, economic, sustainable and environmentally desirable strategy for the management of alien invasive weeds, many of which appear to be ideal targets. Indeed, with the burgeoning international trade in plants and plant produce, more exotic species, with actual or potential weedy traits, will be moved around the world, either deliberately for amenity purposes or accidentally as contaminants, and thus the problem of invasives will only increase. Fragile island ecosystems are especially under threat but many other ecosystems, both natural and agricultural, can also be overrun by alien weeds (Cronk and Fuller, 1995). Of the relatively few fungal pathogens that have been exploited so far as classical BCAs of weeds, a significant proportion have shown or are showing great potential. Paradoxically, despite this obvious potential and the fact that significantly more insect natural enemies have been and are being considered as classical BCAs (McFadyen, 1998), there is still an illogical fear, or pathophobia (Freeman and Charudattan, 1985), surrounding the use of exotic plant pathogens for weed control. These ill-conceived and usually misinformed objections or reservations concerning the introduction of fungal BCAs invariably embrace the concepts of mutation and host-range extension, in spite of the rarity of the former and the certainty that the latter will be picked up in the centrifugal, phylogenetic host-range screens now routinely followed in pest-risk analysis (Wapshere, 1975). Indeed, these tests have since been shown to be too rigorous, with the possibility of rejection of potentially useful BCAs (Wapshere, 1989; Evans, 1995b, 1998). Moreover, one of the critical advantages of classical biocontrol over chemical pesticides is now considered to be its evolutionary stability. In a co-evolved association, the natural enemy adapts to genetic changes in the host but is genetically stable outside it. In contrast, chemical pesticides have been described as evolutionarily evanescent and hence the development of herbicide resistance in weeds is inevitable (Holt and Hochberg, 1997).

This inherent specificity of classical BCAs restricts their use to a single weed; in addition there is no saleable product and therefore there are no direct profits to an investor. Hence, the financing of inoculative biocontrol projects is and will continue to be problematic. Moreover, such projects can only be implemented as a matter of public interest since, once released, the exotic agents cannot be restricted to individual properties. This creates further problems if the alien weed is perceived as having some value, economic or otherwise, even if this is by only a tiny majority of the population in the target country. Thus, ‘rule of law’ legislation may need to be in place in order to avoid conflicts of interest (Harris, 1985). For example, one contentious dispute with bee-keepers in Australia delayed the implementation of a weed biocontrol project by up to 10 years, and resulted in the first specific Biological Control Act in 1984, which provided a legal basis for the introduction of weed BCAs (Cullen and Delfosse, 1985).

Whilst most northern-hemisphere countries have yet to approve or even to initiate the legislation for the introduction of exotic BCAs (Tatchell, 1996), particularly in Europe, where there are a number of well-documented, environmentally problematic alien weeds, the appropriate technologies and legislative issues have been developed, refined and promulgated for some considerable time by southern-hemisphere countries, such as Australia, New Zealand and South Africa. Although guidelines have been produced for the introduction of exotic BCAs of weeds in the USA (Klingman and Coulson, 1982), where half of the weeds and 13 of the top 15 weeds are alien species (Watson, 1991), very few fungal pathogens have been released so far.

It is not just a question of educating and lobbying individual countries, in order to change their pathophobic mentality, but also those international agencies concerned with protecting the environment, such as the World Wildlife Fund (WWF) and the International Union for the Conservation of Nature (IUCN), both of which maintain a policy whereby exotic organisms of any description are prohibited from being moved between countries or geographic regions. In effect, therefore, such bodies legislate against or prevent the adoption of the inoculative biocontrol approach for invasive weeds.

In conclusion, massive infestations of alien weeds, especially over vast areas and/or in fragile or ecologically sensitive ecosystems, dictate that conventional control practices are impractical, uneconomic or environmentally undesirable. In such situations, classical biocontrol offers the only economically suitable method for the long-term management of these weeds. For other alien weeds, particularly those of agricultural importance, the solution lies in developing an integrated pest management strategy, but one in which the inoculative approach, or, indeed, the inundative approach, as will be discussed below, could underpin more traditional control measures.

The inundative approach

Concepts

In this approach, pathogens of weeds are typically used as an inundative inoculum to incite sufficient disease to provide weed control, typically on a seasonal basis, with a need for annual repeated applications

Progress

Augmentation of natural populations of indigenous fungal pathogens has had some commercial success. DeVine™, a liquid formulation of *Phytophthora palmivora* (Butler) Butler, is used for control of *Morrenia odorata* (H. & A.) Lindl. (strangler vine) in Florida citrus groves. DeVine was first registered in 1981 and continues to be available from Abbott Laboratories on a pre-order basis for a very restricted market (Charudattan, 1991). Collego™, a dry powdered formulation of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. f. sp. *aeschynomene*, is used for the control of *Aeschynomene virginica* (L.) B.S.P. (northern joint-vetch) in rice and soybeans in the southeastern USA. Collego was marketed from 1982 to 1992, with approximately 2500 ha treated annually (Templeton, 1992). Environmental Protection Agency (EPA) reregistration was not pursued until 1997 and the product is once again available, with approximately 5000 ha treated in 1998 (D. Johnson, personal communication). BioMal™ is a dry formulation of *C. gloeosporioides* f. sp. *malvae* and, although registered in Canada for the control of round-leaved mallow (*Malva pusilla* Sm.), it has not been marketed because of high production costs (K. Mortensen, personal communication). Another product, Lubao (*C. gloeosporioides* f. sp. *cuscutae*) has been used in China since 1966 for the control of dodder (*Cuscuta chinensis* Lam. and *Cuscuta australis* R. Br.) in soybeans (Gao and Gan, 1992), with inoculum being produced and distributed locally at the cottage-industry level. The present status of Lubao in China is unknown, but the lack of quality control has frequently resulted in the loss of culture virulence (R. Wang, personal communication).

Problems

To date, there have been no real commercial successes with mycoherbicides. Both DeVine and Collego have provided good to excellent weed control, but, since the market sizes are so small, profit margins are slight, if any. Commercial development models for mycoherbicides have been based on the premise that mycoherbicides had to be ‘like a herbicide’ as a ‘stand-alone’ product. A mycoherbicide must provide complete kill, have a long shelf-life and compete economically with chemical herbicides. Numerous linkages were forged between public institutions and private industry to facilitate mycoherbicide development, but for the most part these have failed, mainly due to the ‘like a herbicide’ paradigm. Most candidate mycoherbicides have failed due to biological, technological or commercial constraints impeding their commercial development (Auld and Morin, 1995). Extended dew requirements, low fecundity, low virulence, minimal shelf-life and restricted niche markets are common faults impeding commercial mycoherbicide development. Biological and technological constraints are being actively researched in a number of model systems, but scientists have limited opportunity to address commercial constraints other than choice of target and to shift away from the ‘agrochemical industrial partner’ development model.

Many pathogens with mycoherbicide potential have been discovered, but most lack sufficient aggressiveness to overcome weed defences and achieve adequate control (Gressel *et al.*, 1996). Improved understanding of mechanisms involved in determining virulence would assist in the selection of preferred isolates as biological weed control agents. Luo and TeBeest (1998) have developed an infection component analysis to aid in the assessment of best overall relative fitness of isolates of *C. gloeosporioides* f. sp. *aeschynomene*, which is applicable to most other mycoherbicide candidates.

Weed defences can be suppressed and mycoherbicide efficacy improved by chemical and biological synergy (Gressel *et al.*, 1996). The chemical synergist can be a compound that specifically overcomes one of the host responses to pathogen attack (Sharon *et al.*, 1992) or a chemical that otherwise weakens or wounds the weed, facilitating pathogenicity. Biological synergy is the use of other organisms to enhance dispersal or infection.

Fungi can be divided into those that readily sporulate in liquid culture and those that do not (Stowell, 1991). Commercial development requires low-cost production methods, and the use of liquid-culture fermentation is thought to be necessary to achieve this goal. Much of the success of Collego was due to the mass production of spores and preservation by drying being achieved in commercial-scale facilities. However, this has been elusive not only with other *Colletotrichum* species but with other fungal genera. Submerged liquid production systems are preferred for mass production of mycoherbicides (Churchill, 1982; Stowell, 1991), but many fungi being evaluated as prospective mycoherbicides do not sporulate in liquid culture. Nevertheless, they are generally very adaptable to solid-substrate fermentation on agriculturally based products and this method has been used to produce inoculum of several fungi currently being evaluated as mycoherbicides (Morin *et al.*, 1989; Watson *et al.*, 1997; Zhang and Watson, 1997).

Despite a wealth of research over the last four decades, which has resulted in a formidable list of candidates for further development, there has been relatively little progress in translating potential efficacy as a mycoherbicide into practical reality. The failure of candidate organisms to achieve product development status may be due to a number of reasons, several of which have already been touched upon. It is only too obvious from the literature that poor strain selection, strain instability and poor target selection are all important contributors to that failure. Far more often, however, it is apparent that poor or unreliable performance in field conditions is responsible for development being abandoned.

Inconsistent field performance may itself have several causes. Production difficulties can affect product reliability and thus the organism may deteriorate in storage, poor timing of application may seriously reduce efficacy and environmental constraints may influence the outcome (Greaves *et al.*, 1998). Storage factors and environmental constraints are usually cited as the most common causes of unreliability and these are the two factors most likely to respond to improved formulation. Unfortunately, the majority of publications about mycoherbicides show a woeful ignorance of the need for and the provision of a suitable formulation. It seems to be accepted that a spore suspension in water containing a little surfactant (to ensure even suspension of the hydrophobic spores) is adequate. In many cases, it does appear to be sufficient, but only because the suspension contains a massive concentration of spores and is applied at high volume rates (up to 3000 l ha^{-1}). Such sprays run off the plant target area, distributing spores to all the most vulnerable sites, such as axillary meristems and stem bases. Subsequently, the plants are usually exposed to a long (up to 24 h) dew period, so ensuring that spore germination, infection of the plant and disease expression are all maximized.

While this procedure is academically satisfying, in that it provides positive results, it completely misrepresents the practical realities of applying such living products and is responsible for many organisms being wrongly identified as potential microbial herbicides and for much time being wasted in unproductive development research. The goal of developing robust and reliable products will only be achieved via selection of aggressive, specific and stable agents, properly formulated to withstand storage and environmental constraints and to allow for application at reasonable volumes using conventional spray equipment. It is not really possible or appropriate to totally separate application and formulation, as each interacts dynamically with the other. However, within the space constraints of this chapter, we shall focus on potential solutions to improve the efficiency of fungal BCAs exploited as mycoherbicides.

Solutions

APPLICATION FACTORS. Formulation must be integrated carefully with the application equipment and system to be used. Current trends in agriculture are to minimize the application volume used and to deliver it so as to minimize drift. This latter objective requires attention to be paid to the droplet spectrum generated by the sprayer so as to avoid producing small, drift-prone droplets. Droplet size can be markedly affected by formulation and, in turn, can affect the capacity of the droplet to carry spores. Many of the aspects relevant to these processes are described by Greaves *et al.* (1998) and are summarized as diagrams that relate formulation to the unit processes involved: droplet formation; application; impaction and retention on the target; and deposit formation, leading subsequently to infection and disease expression. It is worth emphasizing that retention and distribution are affected by the nature of the plant. Generally, a determinate growth habit allows better chances of successful control than an indeterminate habit, which favours survival from structures such as rhizomes. Similarly, plants with highly hydrophobic surfaces are less prone to infection from foliar applications. In this latter case, formulation can overcome the problem to a large degree. Although it is beyond the scope of this chapter to review spray application factors, it is important to reiterate that spray application volume and spray distribution on the target are critically important for the efficacy of mycoherbicides.

FORMULATION. Formulation is the combination of specific ingredients (additives and adjuvants) to provide a practical crop protection product. Additives are used to maintain the long-term viability of the agent's propagules and to facilitate ease of handling, storage and application. Adjuvants are proprietary products added to improve spray delivery of the product or to enhance the pathological impact of the propagules.

To enable proper formulation of any mycoherbicide, it is essential to understand fully those factors which affect efficacy. Each factor may vary quantitatively and qualitatively for different agents and, therefore, may require different formulation inputs to allow for maximum effect. In practice, of course, it is unlikely that all formulation demands can be met in full and an informed compromise will be required. The following will deal with formulation requirements in relation to specific important functions that must be aided.

Distribution of inoculum on the target

This is probably the most important area where formulation can improve efficacy; however, despite its importance, this area has been greatly under-researched. Most plants can withstand a high degree of defoliation and, although this may initially check growth, subsequent growth can be accelerated, often producing multiple stems, so overcompensating for the previous check. Thus, the target plant may be even more vigorous after treatment than before. The most useful target sites are the meristems, from which regrowth occurs after defoliation, and, preferably, the stem below the lowest meristems, at the cotyledonary node. Girdling of the stem with disease lesions at this level cannot be overcome by the host and death is inevitable and quick. Unfortunately, these preferred target sites are often shielded from spray by the foliage of the target weed and of the neighbouring crop. This shielding effect is, of course, readily overcome in laboratory experiments by the routine use of high application volumes that lead to mass flow of the applied suspension of spores across plant surfaces, so redistributing the spores to the favoured sites. In practical field situations, however, this is more difficult to achieve. Addition of powerful surfactants can aid movement of deposited spray, especially down steeply angled surfaces, such as stems. In this case, the difficulty is in stopping the movement at the desired point. As the meristems are in the angle between stem and leaf petiole, there is a good chance that spray will be trapped and retained, although there is less chance of this happening on the open stem. Perhaps, if spores are trapped at the intersection of stem and soil surface, they may cause sufficient infection to girdle the stem. Whilst this specific phenomenon has not been investigated, it has been noted that the majority of foliar pathogens will not function effectively when applied as a soil drench.

It is beyond the scope of this short review to deal with the subject in detail. Nevertheless, one possibility for improving the situation is to manipulate the spraying system in order to maximize deposition on the preferred target sites on the weed. This may involve changing either the nozzle type, so as to alter the droplet size spectrum, or the nozzle position relative to the crop. The use of angled spray nozzles, for example, may be advantageous. Preliminary results suggest that spray deposition on the lower stem of the target weed can be significantly increased using an 'Evenspray' nozzle angled at 45° and positioned 40 cm above the plant (J. Lawrie and M.P. Greaves, unpublished data). This seems to be a better option than using a drop-leg sprayer to apply a sticky formulation from close to the soil surface. Boyette *et al.* (1996) and Greaves *et al.* (1998) have recently reviewed formulation and application of mycoherbicides.

Whatever spraying system is adopted, there will still be a need for formulation to ensure good infection at the site of retention and deposit formation. Surfactants, used to aid the spread of spray deposit across the plant surface, also increase evaporation and so reduce the persistence of the aqueous component of the spray. This will reduce the chances of germination of the spores and will need to be prevented by the use of an adjuvant such as a humectant. Alternatively, an oil-based formulation, in which water droplets become entrained (Boyette, 1994; Greaves *et al.*, 1998), or an invert emulsion (Quimby *et al.*, 1988; Amsellem *et al.*, 1990) can be used. These will be effective at moving across hydrophobic plant surfaces and are less volatile than water, so avoiding the clumping of spores that may occur when aqueous deposits dry out (Potyka, 1996).

Factors affecting efficacy

Mycoherbicides applied to the aerial parts of plants are severely affected by lack of free water to promote spore germination, growth and infection. Dew periods are generally short (> 6 h) and irregular in occurrence. Fungal pathogens on the other hand, usually require long periods of exposure to free water (> 12 h) relatively soon after application (Auld *et al.*, 1988; Say, 1990; Boyette, 1994; Auld and Morin, 1995). Timing the application to match the occurrence of rain or, especially, dew is not easy as forecasting these events is not a precise science and they may not occur when weed control is required. Although the formulation of herbicides has advanced our ability to significantly reduce the drying rates of deposits, it is not so easy to achieve when the deposit contains a living fungus. The adjuvants used to reduce drying may be directly toxic to the fungus or may induce osmotic shock (Potyka, 1996). Recent work (G. Dutton, J. Lawrie and M.P. Greaves, unpublished data) has shown that polymers such as polyvinyl alcohols or plant mucilloids can be effective in reducing dependency on dew and have no undesirable effects, at least on the fungi tested so far. It has to be stressed that non-toxicity to one fungus does not necessarily mean that all fungi will be equally unaffected. In general, it has to be assumed that each candidate mycoherbicide will require a custom-made formulation. Equally, it has to be recognized that, despite the recent advances in research on formulation in order to overcome dew dependency, additional research into this subject is still urgently required (Greaves *et al.*, 1998).

Toxicity and synergism from additives

As mentioned above, it is axiomatic that any formulation additive must be non-toxic to the candidate agent. Preferably, it should enhance action in a synergistic way. Unfortunately, the literature rarely presents records of additives that have been found to be toxic to a range of organisms, suggesting that such tests have not been done. Where such data are presented, generally, they are only relevant to spore germination. This reliance on germination data to assess 'toxicity' may be misleading. Inhibition of germination can be more than compensated for if appressorium formation is stimulated (Potyka, 1996), and infection may then be unimpaired or even enhanced. To be sure that the most appropriate formulation is achieved, it is necessary to test each proposed formulation component against the candidate organism in comprehensive laboratory tests of toxicity and synergism. The results of such laboratory tests should be confirmed, for short-listed components, in greenhouse tests, using the living plant target to assess infection efficacy.

The inherent specificity of mycoherbicides is highly desirable from the point of view of environmental safety, although recent trends show that this is not always necessary, as will be shown later. At the same time, it can be a disadvantage from a commercial aspect. Farmers rarely have a monospecies weed problem and therefore those relatively rare niche markets where a monospecific product can be sold must be identified. Alternatively, the range of weeds controlled by the product can be increased. The obvious tactic of mixing spores of different fungi, each effective against a different weed has a clear limit. A minimum spore concentration in each spray droplet is required to achieve infection and, as spores have a fixed volume, only so many can be packed into each droplet. Thus, in practice, it is likely to be possible to mix no more than two or three fungi in one formulation. This assumes, of course, that all the fungi are compatible with the formulation components. Specificity may also be manipulated in one fungus by growing it on particular substrates (Boyette and Abbas, 1994). However, a more feasible way of extending the range of weeds infected is to adjust the chemical components of the formulation. Amsellem *et al.* (1991) have demonstrated such abolition of specificity using an invert emulsion. It would be reasonable to suppose that tank mixing with a low dose of chemical herbicide might easily achieve the same objective and, indeed, this has received significant attention. A second attractive benefit of this approach is the possibility of achieving significant reduction of herbicide input to the environment. Beneficial effects of mixing chemical herbicides and mycoherbicides have been reported to be both additive and synergistic (Scheepens, 1987; Wymore *et al.*, 1987; Wymore and Watson, 1989; Grant *et al.*, 1990; Gohbara and Yamaguchi, 1993). As pointed out by Hoagland (1996), iatrogenic disease, arising from the use of pesticides, is common and is a powerful argument for focusing significant effort in order to exploit the phenomenon in the context of mycoherbicides.

There is a wide range of adjuvants available to formulate mycoherbicides. The toxic and synergistic attributes of these are reviewed by Greaves *et al.* (1998) and need not be repeated here. However, it is worth emphasizing that the effects of each surfactant, humectant, thickener, skinning agent or adhesive can be different with each fungus requiring formulation. There is no alternative to the tedium of individually testing each adjuvant in both *in vitro* and *in vivo* tests. Failure to do so will inevitably lead to unreliable performance of the product in the field and to its withdrawal from commercial development.

Storage

Mycoherbicides are most commonly applied as a liquid containing suspended spores. Clearly, this is not the most appropriate form in which to store the product. Not only is it bulky and heavy but the presence of water will permit the spores to germinate, after which they will rapidly die if they do not contact their host weed. Consequently, the favoured initial formulation is a wettable powder comprising a hydrophilic carrier mixed with the hydrophobic spores. In this state, many spores have been found to survive for up to 2 years without unacceptable loss of viability and efficacy (Mortensen, 1988), although difficulties have been encountered with some fungi (Jackson *et al.*, 1996). The ability to survive long-term storage is highly desirable in a mycoherbicide since it allows the passage of the product through the production, packaging, distribution and sales process without loss of efficacy. However, at least one product (DeVine) is made to order and supplied to the grower for immediate use. It is unlikely that such a system will be used as a commercial norm and its economic value must be questionable.

Solid formulations

A wide range of solid formulations has been developed and several have been applied in practice. These range from products based on plant residues, such as wheat bran

(Morris, 1989) and sorghum straw (Ciotola *et al.*, 1995) to those based on agar (Scheepens, 1987, 1990). Although these formulations are effective, they are also bulky, often inconsistent in quality and expensive to apply. The first uniform, reliable, solid carriers were mineral-based granules (Walker, 1981), but subsequently alginate-based granules (Walker and Connick, 1983; Fravel *et al.*, 1985) have been favoured. Such granular formulations have a number of advantages over foliar-applied liquid formulations. Amongst these are: the ability to place them precisely in the top of the seed furrow, leaving weeds between the rows to be removed using mechanical methods; and the residual activity that they exhibit can enhance efficacy. Most recently, the development of 'Pesta' granules (Connick *et al.*, 1991), in which the fungal agent is encapsulated in a wheat gluten matrix, has shown some promising results. The nutrient content of these granules is said to aid the effect of the fungus. Unfortunately, it can also have undesirable results and 'Pesta' granules containing no fungus will sometimes impair control of *Amaranthus retroflexus* by promoting the growth of soil-borne fungi (J. Lawrie and M.P. Greaves, unpublished data). This growth, which is usually of rapidly growing fungi, such as *Penicillium* spp., can overgrow the agent included in the granule and prevent its infection of the target weed. On the other hand, it is certain that the soil environment is buffered against those environmental perturbations, especially desiccation, which can make the leaf surface such a hostile environment for foliar-applied fungi. There is a clear need for more research into granule development and performance so as to capitalize on the potential advantages and minimize the problems. The experience gained in the encapsulation in materials, such as gelatinized starch (Schisler *et al.*, 1996), may offer further opportunities for better development of mycoherbicides. Similarly, exploitation of BCAs encapsulated in granules of solid substrates, coated with oil and an absorbent, suggests that this may be a way forward. Quimby *et al.* (1994) patented this system for granules consisting of alginate, starch or wheat gluten with a coating of oil that forms an invert emulsion with water. The oil is absorbed into a material such as hydrated silica. On re-wetting, these granules retain water for as long as 12 h and so may preclude the need for dew to promote and sustain fungal growth on and infection of the host weed. Much of the work to develop granular or encapsulated formulations of mycoherbicides duplicates work that was done previously to develop formulations of entomopathogenic fungi. As yet, however, it has not matched the extent, depth or achievement of that work. Dialogue between these two aspects of the same problem should therefore be encouraged. At the same time, the needs for appropriate and properly understood spraying systems must be recognized and addressed. In this way it is possible that real progress in developing effective and reliable formulations of mycoherbicides will be achieved.

It is imperative that the predominantly piecemeal development that has characterized mycoherbicide formulation to the present is replaced with a more organized and sustained effort. Otherwise, the scene will continue to be dogged by formulations that function adequately within the confines of a defined experimental programme but which are of unreliable efficacy in practice.

Potential

The use of indigenous, naturally occurring weed pathogens, formulated and applied like chemical herbicides, has potential to reduce chemical inputs and to provide viable, economic and effective weed-control components within integrated weed management programmes, such as in rice and other tropical crops. Much of the research effort elsewhere is shifting away from discovering new mycoherbicides to solving production, storage and efficacy problems of existing ones. There is clearly a need to better understand the biochemical and physiological aspects of pathogenesis by the selected fungal BCA so that weak links in host defence can be exploited. As detailed above, many mycoherbicides still need the augmentation of formulants, as well as chemical and/or biological synergists, to provide the lethality to weeds that the consumer desires. The key to the successful development of mycoherbicides, especially in developing countries, may lie in the involvement of 'small market' business enterprises or producer cooperatives, each supplying their immediate area, using local labour and thereby adding to the economic viability of rural areas.

Thus far, agrochemical companies or the 'industry partners' have not invested any serious money in mycoherbicide research and development and they have tended to 'sit on the fence', awaiting any significant advance or breakthrough from the many and diverse, small, public-funded projects (Charudattan, 1991). These have not been forthcoming and it is considered doubtful that a commercially viable mycoherbicide will ever be marketed against the relatively narrow range of priority weeds targeted by the industry. None the less, there are recent encouraging signs that mycoherbicides can satisfy an important niche market in situations where chemical herbicides are either ineffective or environmentally undesirable. Coincidentally, two of these products incorporate non-specific pathogens which have adapted to and are highly pathogenic against exotic, invasive, woody weeds. In South Africa, Stumpout™, based on an indigenous white rot fungus, *Cylindrobasidium laeve* (Pers.: Fr.) Chamois, and produced at the cottage-industry level, is currently sold and used as a mycoherbicide to prevent regrowth of Australian wattle species (Morris *et al.*, 1998). As well as invading native ecosystems, such as the fynbos, these alien trees (*Acacia mearnsii* De Wildemann and *Acacia pycnantha* Benth.) disrupt water flow and have a direct and indirect impact on diminishing water resources. Thus, this mycoherbicide is now playing an important role in an ambitious clearance programme (Moran *et al.*, 1999). Similarly, Biochon™ is also based on a white rot fungus, *Chondrostereum purpureum* (Pers. ex Fr.) Pouzar, and is marketed in the Netherlands by a small biocontrol company (Koppert Biological Systems) for suppression of an introduced North American tree species (*Prunus serotina* Ehr.), which is invading both natural forests and plantation tree crops (Ravensburg, 1998). Elegant modelling and epidemiological studies were undertaken, initially as part of a pest risk analysis, to assess the threat posed by this plurivorous pathogen to commercial fruit-tree crops (de Jong *et al.*, 1990, 1991). This has stimulated further work in Canada on the same BCA (Prasad, 1994), and a commercial product (Ecoclear™) has recently been registered in North America for use in conifer plantations and public rights of way (Shamoun and Hintz, 1998). The Canadian Forestry Service is under tremendous public pressure to minimize or eliminate completely the reliance on chemical pesticides in forest management and therefore it would seem that the increasing use of biological alternatives, such as mycoherbicides, is both necessary and inevitable. Indeed, in Ontario, a government initiative was proposed to reduce pesticide inputs by 50% by 2002 (Swanton *et al.*, 1993). If similar pressures are directed against pesticides in agricultural ecosystems, the agrochemical companies may need to revisit and reassess the inundative approach, using fungal BCAs, for the long-term and sustainable management of weeds.

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